Biochemistry (Moscow), Vol. 70, No. 11, 2005, pp. 1199-1211. Translated from Biokhimiya, Vol. 70, No. 11, 2005, pp. 1459-1473.

REVIEW

Glutathione in Bacteria

G. V. Smirnova* and O. N. Oktyabrsky

Institute of Ecology and Genetics of Microorganisms, Ural Division of the Russian Academy of Sciences, ul. Goleva 13, 614081 Perm, Russia; fax: (3422) 446-711; E-mail: smirnova@iegm.ru

Received December 1, 2004
Revision received January 11, 2005

Abstract—Glutathione metabolism and its role in vital functions of bacterial cells are considered, as well as common features and differences between the functions of glutathione in prokaryotic and eukaryotic cells. Particular attention is given to the recent data for the role of glutathione in bacterial redox-regulation and adaptation to stresses.

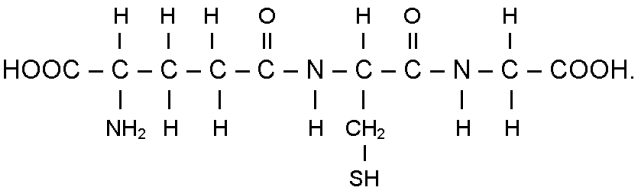
Key words: glutathione, bacteria, redox-regulation, stresses

PROPERTIES AND METABOLISM OF GLUTATHIONE

Interest in the study of the functions of glutathione (GSH), a compound discovered at the end of XIXth century, remains high. In scientific and popular-science publications glutathione is often called the most potent natural antioxidant, the best instrument of cellular defense guaranteeing health for an organism, and achievements of the last two decades in the study of medical and clinical aspects of GSH amount to a virtual "glutathione revolution". These successes are related mainly to the role of glutathione in eukaryotic cells. Physiological and biochemical aspects and the role of GSH in bacteria are studied to a considerably lesser degree. Here we attempt to summarize the data on GSH metabolism and functions in bacteria and to reveal common features and differences typical for the role of GSH in prokaryotic and eukaryotic cells. Particular attention is given to recent information on the role of GSH in bacterial adaptation to various stresses.

Glutathione is present in relatively high concentrations in all eukaryotic cells. In prokaryotic cells, it is found chiefly in gram-negative bacteria including *Escherichia coli*. In most gram-positive bacteria, with the exception of some *Streptococcus* and *Enterococcus* species, GSH is not found [1, 2]. However, some gram-positive bacteria are able to synthesize GSH or consume GSH from the growth medium [3, 4]. Facultative anaerobic and aerobic prokaryotes, which are characterized by absence of glutathione, produce other low molecular weight thiols which possibly fulfill the same functions as GSH [2, 5].

Glutathione is a tripeptide (L-γ-glutamyl-L-cysteinyl-glycine, molecular mass 307 daltons) that has two negatively charged carboxyl groups and a positively charged amino group at physiological pH values:



The presence of the γ-glutamyl bond protects the tripeptide from degradation by intracellular peptidases, and the sulfhydryl group of cysteine can serve as an electron donor, endowing glutathione with reducing properties and ability to remove free radicals. One-electron reaction of GSH with free radicals results in the formation of a thiyl radical, GS˙, which reacts with another GS˙ radical to form glutathione disulfide (GSSG). A second type of redox reaction in which glutathione participates is thiol–disulfide exchange. This reaction plays a key role in the formation of protein disulfides (GSSR) and may be an important element in regulation of biological processes [6]. A third type of redox reaction involves two-electron oxidation of GSH with the formation of an intermediate reacting with a second identical or nonidentical molecule to form glutathione disulfide GSSG or mixed disulfide, respectively.

Abbreviations: GSH) reduced glutathione; GSSG) oxidized glutathione; GOR) glutathione oxidoreductase; CoA) coenzyme A; Grx) glutaredoxin; Dsb) periplasmic redox proteins; NEM) N-ethylmaleimide; GST) glutathione S-transferase; Msc) mechanosensitive ion channels; PcpC) tetrachloro-*p*-hydroquinone dehydrogenase; ROS) reactive oxygen species.

* To whom correspondence should be addressed.

0006-2979/05/7011-1199

GSH is synthesized in all cell types via two sequential reactions catalyzed by γ-glutamyl-cysteine synthetase (γ-GCS) (EC 6.3.2.2) and GSH-synthetase (GS) (EC 6.3.2.3). γ-GCS catalyzes the formation of a peptide bond between the γ-carboxyl group of glutamate and the α-amino group of cysteine. Glutathione synthetase forms the peptide bond between the α-carboxyl of cysteine in γ-glutamyl-cysteine and the α-amino group of glycine. Both reactions depend on ATP, and their mechanisms are similar: both involve the formation of an acylphosphate intermediate [7]. γ-GCS isolated from *E. coli* cells differs from eukaryotic enzymes and consists of one polypeptide with molecular mass of 55 kD. The enzyme displays maximum activity at pH 8.5 and temperature of 45°C. GSH-synthetase from *E. coli* cells consists of four identical subunits of 38 kD and displays maximum activity at high pH values (8.0-8.5) and temperature of 45°C [8, 9]. The genes *gshA* and *gshB* encoding γ-GCS and GSH-synthetase have been sequenced, and their crystal structures have been studied [9-11].

Escherichia coli mutants deficient in both enzymes of glutathione biosynthesis have normal growth rate in minimal medium, but they are substantially more sensitive to a number of chemical substances and oxygen than wild type cells [9, 12, 13]. In bacteria, as in other organisms, GSH synthesis is limited by the bioavailability of L-cysteine and is stimulated by the addition of cysteine or its precursors into the medium [14].

γ-Glutamyl transpeptidase (GGT) (EC 2.3.2.2) is the key enzyme in the reaction chain leading to GSH degradation. In the first stage GSH is transported from the cell into the medium and interacts with GGT, the active center of which is localized on the outer side of the plasmatic membrane, to form a γ-glutamyl residue bound with the enzyme and L-cysteinyl-glycine. Then cysteinyl-glycine undergoes cleavage by dipeptidase (EC 3.4.13.6) to cysteine and glycine, which are absorbed by the cell. The larger portion of intracellular cysteine is re-incorporated in GSH, whereas another portion, depending on cell requirement, enters protein synthesis [7]. The γ-glutamyl residue of glutathione can either be transferred onto an amino acid or interact with water or another glutathione molecule and return to the cell. So, glutathione can take part in the transport of amino acids, particularly cysteine.

When the intracellular concentration of cysteine increases as a result of metabolic imbalance, this amino acid can be involved in reduction of trivalent iron due to catalysis by the Fenton reaction in the presence of H_2O_2 to form the toxic hydroxyl radical, which damages cellular macromolecules, particularly DNA. So, under normal conditions bacteria and other cells maintain a very low intracellular pool of cysteine (<200 μM) [15] and require a nontoxic reserve form of cysteine, such as GSH.

In *E. coli* GSH can really be used as a reserve form of reduced sulfur, and an interrelation exists between the GSH and cysteine pools [14]. Like many other bacteria, *E. coli* is also able to transport GSH into the medium and has the enzymes γ-glutamyl transpeptidase and cysteinyl-glycinase. Any of the peptidases A, B, and N or dipeptidase D can catalyze decomposition of cysteinyl-glycine and provide for its utilization as a source of cysteine [16]. Thus, through the function of GGT, glutathione can play two roles: a reserve form of cysteine and a component of the transporting system for exogenous cysteine.

GGT isolated from *E. coli* cells is well characterized [17]. The gene encoding *E. coli* GGT has been mapped and sequenced. Bacterial GGT is essentially homologous to the enzyme from human and rat cells [18, 19]. The only phenotypic difference of *ggt* mutants from wild-type cells is elevated excretion of glutathione into the medium [18].

Activity of *E. coli* K12 GGT depends on growth temperature with a maximum at 20°C. It is seven-fold decreased at 37°C and disappears at 45°C. The biological significance of enhanced GGT activity at low temperature is not clear, because mutation in the *ggt* gene does not influence bacterial growth, nutritional needs, and sensitivity to cold [18].

It is interesting that some bacteria cannot synthesize glutathione, but possess γ-glutamyl transpeptidase activity. This allows them to use glutathione as a source of cysteine [20].

Apart from GGT, the product of the *ybiK* gene (the expression of which is regulated by the transcription activator of the cysteine regulon *cysB*) was recently found to participate in glutathione transport into *E. coli* cells. Cells with mutations in the genes *ybiK* and *cysA* could not use glutathione as the sole source of sulfur, thus suggesting an important role of *ybiK* in glutathione transport [21].

In general, despite great recent achievements in studies of glutathione transport in eukaryotic cells, GSH transport in bacteria remains poorly studied. Note that the transport of glutathione is a constituent part of its metabolic cycle including intracellular synthesis, egress into the extracellular medium, with following reentry into the cell in the form of the entire molecule or its fragments. All these processes are energy-dependent and, in general, resemble a futile cycle. Its biological role has long been intriguing, the more so because the whole cycle or its elements are found in all organisms containing glutathione.

Glutathione disulfide (GSSG) produced in the course of cell life is highly toxic because it easily reacts with free sulfhydryl groups. Reduction of GSSG to GSH is catalyzed by glutathione reductase (GOR) (EC 1.6.4.2), which seems to play a crucial role in evolutionary adaptation of organisms to atmospheric oxygen. GOR is a member of the flavin-containing enzymes, pyridine nucleotide disulfide oxidoreductases, and catalyzes the transfer of reduced equivalents from NADPH to GSSG:

$$NADPH + GSSG + H^+ \rightarrow 2GSH + NADP.$$

GOR is widespread among bacteria, fungi, plants, protozoa, and animals. All glutathione reductases isolated from different sources are very homologous, showing high evolutionary conservativeness of this protein.

Escherichia coli with mutations in the gene *gor* encoding glutathione reductase retain normal growth and can maintain a high pool of reduced glutathione, thus suggesting the possible existence of an alternative GOR-independent pathway of GSSG reduction [22]. This function may be executed by glutaredoxin or thioredoxin systems [23]. GOR isolated from *E. coli* cells is a dimer composed by two identical subunits with molecular mass of 55 kD; it is highly homologous to the enzyme from human cells [24, 25].

In *S. typhimurium* and *E. coli* expression of *gor* is controlled by the transcription factor OxyR regulating adaptation to peroxide stress [26, 27]. Moreover, the *E. coli* gene *gor* is under the OxyR-independent control of an alternative σ^s subunit of RNA-polymerase (RpoS) [28], which is a regulator of general response to various stresses. Thus, GOR is involved in a global regulatory circuit providing bacterial adaptation to various stress conditions.

GLUTATHIONE STATUS

The glutathione status is defined as the concentration of total glutathione and the ratio between its different forms in the cell [6]. The most important forms of glutathione are reduced GSH, GSSG, and mixed disulfides, mainly GSS-protein, and mixed disulfides with low molecular weight SH-compounds, such as cysteine, α-pantetheine, and coenzyme A (CoA).

In *E. coli* cells, 99.5% of glutathione exists in the reduced form. The concentration of GSSG is 0.17-0.33% of the total intracellular glutathione, and the ratio GSH/GSSG = 300-600. The level of mixed disulfides does not exceed 1% (1.5-2% of total glutathione is excreted into the medium) [1, 29, 30].

Glutathionylspermidine is another form of glutathione in *E. coli*. In stationary culture, virtually all intracellular spermidine and a significant portion of the glutathione are converted into glutathionylspermidine. When the culture is diluted with fresh medium, glutathionylspermidine rapidly decomposes to glutathione and spermidine [31]. Interconversion of glutathionylspermidine is supposed to play an important role in metabolic integration of glutathione and spermidine. In stationary growth phase and transition to anaerobiosis the level of CoASSG, the mixed disulfide of glutathione and CoA, in *E. coli* cells significantly increases. Its intracellular level also increases in response to fluoride, cyanide, and antibiotics [32, 33]. Changes in both glutathione status and pools of spermidine and CoA are reversible and controlled by the cell itself due to the activity of enzymes decomposing glutathione conjugates.

Glutathione status depends on the dynamic balance between its synthesis, decomposition, transport, oxidation, and reduction, so it can vary depending on what reaction prevails, which, in turn, depends on cellular state and environmental conditions. Changes in glutathione status might be observed under both normal physiological situations and stresses or result from genetic defects or the action of some chemicals. Significant changes in intracellular level of GSH in *E. coli* are observed upon transition of the culture from logarithmic to stationary phase, ammonium depletion, transition to anaerobiosis, and exposure to rifampicin and acetate [1, 14, 34]. The most significant changes in GSH status are observed in *E. coli* mutants in genes encoding enzymes of glutathione metabolism. The absence of glutathione reductase results in increased fraction of GSSG: although the level of GSH remains almost unchanged, the ratio GSH/GSSG decreases almost twofold. The most appreciable changes in glutathione status are observed in cells deficient in glutathione reductase, catalase, and GSH synthesis, in which the concentration of GSSG and mixed disulfides more than tenfold increases [29].

Oxidation of GSH to GSSG and mixed disulfides is reversible, and subsequent reduction is catalyzed by glutathione reductase and glutaredoxins. Irreversible changes in glutathione status appear resulting from inhibition of synthesis, covalent binding, or irreversible efflux of glutathione. Most existing data concern changes in the level of reduced glutathione, whereas the status of other glutathione forms in the cell under various influences remains far less studied.

Diamide was suggested as an agent oxidizing GSH to GSSG [35]. Taken at concentration 0.1 to 5 mM, it was supposed to be inactive towards protein sulfhydryl groups and only exhibit its effect on cell metabolism via oxidation of low molecular weight thiols. However, it was later shown that diamide is not so specific for glutathione oxidation [36].

To exhaust the intracellular pool of glutathione, some electrophilic agents are used, such as diethylmaleimide, *N*-ethylmaleimide (NEM), and bromobenzene, which form conjugates with GSH. However, these substances are toxic and have nonspecific effects on proteins and other components of cells. An inhibitor of γ-glutamyl-cysteine synthetase, L-buthionine-[S,R]-sulfoximine (BSO), is widely used for inhibition of GSH synthesis in eukaryotes. However, this inhibitor has no apparent effect on *E. coli* [37]. The best way allowing complete absence of glutathione in bacterial cells is selection of mutants in genes encoding the GSH biosynthesis enzymes.

The common way to increase the intracellular concentration of GSH is to supply cells with cysteine by addition of either cysteine or its oxidized form (cystine) or alternative sources of cysteine into the medium [14, 37, 38]. As mentioned above, the disadvantage of cysteine is its toxicity. The most widely used alternative sources of

cysteine are L-2-oxo-4-thiazolidinecarboxylate (OXO), cysteamine, N-acetylcysteine, and ethyl and methyl esters of GSH. The latter easily penetrate the cellular membrane and hydrolyze to release GSH. Another way to increase the intracellular glutathione concentration is to create gene-engineered strains carrying additional copies of genes encoding the glutathione biosynthesis enzymes [9].

Some bacteria including *E. coli* can accumulate micromolar levels of glutathione in the medium [39, 40]. A little is known on the role of extracellular glutathione in physiology of bacteria. One of its possible functions is to protect the cells against toxic substances through their neutralization on "far boundaries" [41]. In *E. coli*, the transport of cysteine from the medium includes its reduction with the participation of extracellular glutathione [42].

The extracellular concentration of glutathione changes depending on growth conditions and phase [18, 40]. The available data suggest that GGT is an important but not the only component of the system controlling the level of extracellular glutathione [18]. It is worthy of note that in *E. coli* culture, even in aerobiosis, the level of GSH in the medium 20-30-fold exceeds that of GSSG, and the GSH/GSSG ratio in the medium remains rather high despite being an order of magnitude lower than in the cytoplasm [30]. Probably, *E. coli* cells growing under aerobic conditions maintain homeostasis (both the level and redox status) of extracellular glutathione, because very high concentrations of oxidants are necessary to oxidize most of the extracellular glutathione. When the level of oxidants is moderate, one can only observe a transient decrease in both GSH level and the $GSH_{out}/GSSG_{out}$ ratio [30].

Stress factors often cause appreciable changes in the level of extracellular glutathione. In particular, hyperosmotic shock caused a transient decrease in the concentration of extracellular glutathione in *E. coli* [43], whereas gramicidin increased it [34]. Increased level of glutathione was observed in the medium upon heat and cold shock and in cells adapted to increased or decreased temperature [18, 44, 45].

Changes in the levels of glutathione and other low molecular weight thiols in the extracellular space are so great under certain conditions that they can significantly influence redox potential (Eh) of the microbial culture. It was shown that in aerobic cultures the redox potential jumps (by 150 mV) may accompany various stresses, such as heat and osmotic shocks, starvation, UV-irradiation, and treatment with antibiotics. These Eh jumps were not directly connected with changes in parameters that commonly determine the redox potential of microbial culture (pH, pO_2, etc.) [46-49]. Subsequent studies demonstrated that in both *E. coli* and *B. subtilis* the most significant contribution to Eh under these circumstances comes from changes in the level of extracellular thiols including glutathione, both dissolved in the culture medium and associated with the outer surface of cells [50, 51]. The above described Eh jumps were only observed when bacteria grew on synthetic mineral media. When the medium formula was more complex and containing protein hydrolyzates, the Eh changes in stresses did not correlate with changes in the concentration of extracellular thiols.

GLUTATHIONE AS AN INTEGRAL PART OF CELLULAR REDOX SYSTEM

A great number of reactions proceeding in cells are coupled with transfer of redox equivalents. So, maintenance of a particular redox state in cytoplasm is an important condition for the normal life of the cell.

Both redox activity of glutathione with its resistance to autooxidation and high concentration and its ability to maintain its reduced state make GSH the most important intracellular redox buffer. The intracellular concentration of glutathione is 500-1000 times higher than that of NADPH and other intracellular redox systems. So, changes in the redox state of glutathione can directly reflect changes in the redox status of the cell [52]. The redox system of glutathione encompasses glutathione itself, glutaredoxins, and glutathione reductase. The functions of this system in many respects overlap those of the thioredoxin redox system, the components of which are thioredoxins and thioredoxin reductase. The properties of the components of these systems and their interaction with glutathione are briefly described below.

Glutaredoxins catalyze reduction of disulfides or mixed disulfides using GSH. The glutathione disulfide produced undergoes reduction by glutathione reductase [53]. The multiplicity of glutaredoxin isoforms in different organisms possibly reflects multiplicity of their functions. *Escherichia coli* cells contain glutaredoxins Grx1, Grx2, and Grx3 encoded by the genes *grxA*, *grxB*, and *grxC*, respectively [54].

The active center of Grx1 contains two cysteines separated by two amino acids and is represented by the sequence -Cys-Pro-Tyr-Cys-. An important function of glutaredoxins is their participation in transduction of intracellular regulatory signals and redox regulation of transcription factors. Thus, in *E. coli* cells Grx1 is involved in reduction of the transcription factor OxyR [55, 56]. *Escherichia coli* mutants in each glutaredoxin separately or in all three glutaredoxins together retain ability to grow on rich and minimal media [57-59]. However, triple glutaredoxin mutants carrying an additional mutation in glutathione reductase cannot grow on minimal medium without a source of reduced sulfur.

Thioredoxin is a small (12 kD) and widely distributed protein whose structure changes minimally from archaebacteria through humans. A characteristic feature of all thioredoxins is a conservative active center, Cys-Gly-Pro-Cys. Thioredoxin oxidized due to the transfer of

reductive equivalents to its substrates is reduced by thioredoxin reductase [60]. Thioredoxin 1 can fulfill the function of electron donor for ribonucleotide reductase, 3′-phosphoadenylylsulfate reductase (PAPS), and methionine sulfoxide reductase [61]. Despite the central role of thioredoxin in many metabolic processes in *E. coli*, deletion of the gene encoding thioredoxin 1 (*trxA*) does not lead to essential changes in phenotype [62]. Although the redox systems of thioredoxin and glutathione do not exchange their reductive equivalents, both these systems are functionally overlapping and double each other to a great extent [63, 64]. For this reason, the lack of components of one of these systems often remains phenotypically inconspicuous; only multiple mutations affecting both systems lead to significant disturbance of metabolism.

Escherichia coli cells maintain a balance between the levels of ribonucleotide reductase, thioredoxin, glutaredoxin 1, and glutathione. Mutations in *gshA* and/or *trxA* result in increased induction of both ribonucleotide reductase and Grx1. Double mutants *gshA trxA* retain viability, but they contain extremely high level of Grx1 (55-fold higher than that in wild-type cells). At the same time, *E. coli* mutants in *gshA* have significantly increased levels of thioredoxin and glutaredoxin [65].

Unlike cytoplasm with reductive conditions, the periplasm of *E. coli* cells is a compartment with more oxidative conditions. Most of all protein SH-groups in cytoplasm are in the reduced state, whereas many, but not all periplasmic proteins require formation of disulfide bonds to form the proper tertiary structure necessary to fulfill their functions. In *E. coli* cell periplasm, disulfide bond formation in proteins is catalyzed by several redox proteins called Dsb (DsbA, DsbB, DsbC, and DsbD) [66]. There is still no data on the functional link between the glutathione system and Dsb proteins.

Thus, the cytoplasm and periplasm of *E. coli* have redox pathways maintaining the thiol–disulfide balance. The main components of these pathways have been identified. Their importance for cell life is suggested by the fact that although functions of many of them are rather specific, they can overlap and compensate each other under certain circumstances.

GLUTATHIONYLATION OF PROTEINS AND REGULATION OF CELL FUNCTIONS

In recent years, redox-sensitive regulators of transcription have been found in prokaryotic cells whose oxidative modification is a signal for induction of controlled genes or other regulatory events. Activation of these regulatory proteins occurs via different mechanisms. In particular, the single cysteine residue of OhrR forms the stable derivative of sulfenic acid (C_{15}-SOH), the repressors PpsR/CrtJ form a convertible disulfide bond, and oxidation of Hsp33 is accompanied by formation of two disulfide bonds with release of zinc followed by dimerization of the protein [67]. The effect of the "essential" SH-groups in these proteins makes them implicit targets for redox regulation with participation of thiol-containing substances. One of the distinct molecular mechanisms of this kind of regulation is formation of mixed disulfides between thiol groups of proteins and glutathione. This process is called glutathionylation versus the broader conception of "S-thiolation", which involves formation of mixed disulfides of proteins with glutathione, cysteine, and other thiols (including non-physiological ones) [68]. A hypothesis was proposed that glutathionylation is a posttranslational mechanism of protein regulation in response to changes in intracellular redox potential, which is analogous to other regulatory mechanisms (phosphorylation, methylation, carboxylation, and ADP-ribosylation) associated with covalent modification of proteins. The formation of mixed disulfides was hypothesized to be a result of thiol–disulfide exchange between protein SH-groups and glutathione disulfide. A shift in the ratio GSH/GSSG to disulfide is therein accompanied by glutathionylation, whereas decrease in concentration of GSSG leads to cleavage of disulfide bond to form a free SH-group in the protein molecule (dethiolation).

Since glutathionylation of essential thiol groups leads to changes in enzymatic activity and shift in metabolic processes, biological disulfides might play a role of messengers in transmission of cellular signals [69]. Experiments *in vitro* have shown that GSSG can modulate activities of many enzymes including enzymes of glycolysis and gluconeogenesis, adenylate cyclase, protein kinases, etc. [70, 71]. Nevertheless, formation of mixed disulfides of glutathione with distinct proteins has not been demonstrated in *in vivo* experiments. Moreover, the ratios GSH/GSSG are so strongly shifted to GSH in cells even in oxidative stress that protein SH-groups should to be in the reduced state; hence, the existence of such regulatory mechanisms *in vivo* is hard to suppose [70, 71].

Interest in the problem of glutathionylation has been revived in recent years in connection with intensive studies on processes of redox regulation and appearance of sensitive methods allowing detection of glutathionylated proteins in cells [68]. It was found that in eukaryotic cells, mixed disulfides of GSH with proteins are accumulated in certain situations, and this process can be under strict control.

Along with the previously proposed mechanism of formation of mixed disulfides with the participation of GSSG, an alternative mechanism was proposed in which mixed disulfides are produced from a reaction between a protein thiol modified via oxidation and GSH with no detectable shift in GSH/GSSG ratio [72]. Dethiolation of glutathionylated proteins may occur either non-enzymatically or enzymatically with involvement of thioredoxin, glutaredoxin, or protein disulfide isomerase [73].

Regulation of enzymatic activity and metabolism via glutathionylation is also possible in prokaryotic cells, in which the portion of mixed disulfides of proteins with glutathione significantly increases when the intracellular GSH/GSSG ratio decreases due to exposure of cells to diamide or damage of cellular redox systems [29, 36, 65]. One of a few prokaryotic proteins whose regulation was found to be possible via formation of its mixed disulfide with glutathione is 3′-phosphoadenylylsulfate reductase (PAPS-reductase) of *E. coli*. This enzyme is inactivated on formation of a mixed disulfide between glutathione and cysteine 239 in the active center, and its dethiolation is catalyzed by glutaredoxins via a mono-thiol mechanism [59, 74]. Activation of the transcription regulator OxyR can also be associated with glutathionylation of this protein [75].

GLUTATHIONE AND REGULATION OF INTRACELLULAR POTASSIUM CONCENTRATION

One of important functions of glutathione in gram-negative bacteria is regulation of intracellular level of K^+, whose transport and accumulation plays a pivotal role in maintenance of cell turgor and homeostasis of intracellular pH. The intracellular concentration of K^+ in *E. coli* cells is a result of a dynamic balance between the processes of potassium uptake by cells and its release from the cells. In *E. coli* cells, the genes *kefB* and *kefC* were identified whose mutations cause a fast efflux of potassium from the cells [76]. The same effect was achieved when bacteria were treated with NEM or by other thiol reagents. It is also known that *E. coli* cells deficient in glutathione synthesis rapidly lose K^+ when transferred into a K^+-free medium and cannot grow without exogenous GSH in a medium with low potassium level [77]. These data led to the following hypothesis: the products of *kefB* and *kefC* form potassium channels, whereas glutathione participates in their regulation because it is a component of a gate closing the channel [77].

Patch clamp-analysis has confirmed the presence of glutathione-regulated K^+-channels in membranes of *E. coli*. GSH promotes closing of these channels, whereas their maximum activation is observed in the presence of both GSH and NEM [78]. The KefB- and KefC-systems of K^+ efflux are not unique for *E. coli*. Their homologs are found in many other gram-negative bacteria, thus suggesting importance of their functions [78]. In particular, these systems might participate in protection of cells from toxic effect of electrophilic reagents [79, 80]. It has been demonstrated that glutathione S-conjugates of electrophilic substances can interact with KefB and KefC channels to cause their activation and fast efflux of K^+ from the cells [79, 81]. So, the channels KefB and KefC are under negative control of GSH and positive control of glutathione S-conjugates. Moreover, glutathione S-conjugate-dependent efflux of K^+ from the *E. coli* cells is accompanied by acidification of the cytoplasm, which in some way facilitates survival of bacteria [79, 80, 82].

Mechanosensitive (MS) ion channels play an important role in regulation of turgor pressure in bacteria [78]. These channels are membrane proteins, which react to membrane tension by increased ability to be in the open state and to pass ions along their electrochemical gradient. In *E. coli*, gene products have been identified that form independent mechanosensitive channels MscL, MscS, and MscK (KefA) involved in adaptation to hypoosmotic shock [83]. It was recently found that GSH decreases the ability of these channels to open in response to membrane tension [84]. At physiological concentrations, GSH inhibits activity of MS-channels by 70-75%. It has been hypothesized that glutathione can act as reductive reagent altering the redox state of a disulfide bond in either an MS-channel molecule or an associated regulatory protein [84].

DEACTIVATION OF TOXIC SUBSTANCES

Because of its nucleophilic properties, GSH reacts with a great variety of electrophilic components to produce GSH conjugates [6, 7]. The reaction of conjugate formation can develop either spontaneously or under catalysis by enzymes called glutathione S-transferases (GST). The following stage is degradation of the conjugates to relatively harmless mercapturic acids through a pathway that is one of the most important detoxification processes. Along with this, glutathione conjugates of electrophilic and lipophilic substances can be transported into the medium through ATP-dependent pumps transporting substances from cells and belonging to the family of multidrug resistance proteins.

Glutathione S-transferases are found in both eukaryotic and prokaryotic cells and comprise a large family of multifunctional proteins. GSTs are subdivided into classes α, μ, π, and θ depending on their substrate specificity and amino acid sequence. All known bacterial GSTs fall into the class θ. *Escherichia coli* contains nine GST-like genes in its genome [85]. GST present in bacterial cells was found to heighten their resistance to some antibiotics [86]. Moreover, some of these enzymes possess alkylperoxidase activity, which might play a role in protection from oxidative stress [87]. Bacterial glutathione S-transferases are involved in detoxification of epoxides as well as pesticides and herbicides [85]. Detoxification of NEM in *E. coli* cells also passes through the stage of formation of a conjugate with glutathione, although GST has not yet been proven to take part in this process. The adduct of glutathione with NEM undergoes hydrolysis into a nontoxic (for *E. coli*) substance and glutathione [88].

Escherichia coli cells are able to transport glutathione S-conjugates into the medium, and their transport is inhibited by the same substances as is the transport of GS-conjugates in mammalian cells, thus indicating resemblance of these processes in eu- and prokaryotes [89].

Unlike eukaryotic GSTs specializing in processes of detoxification, some bacterial GSTs, such as dichloromethane dehalogenase (DCMD) and dehalogenase of aromatic compounds (PcpC), are involved in basal metabolism supplying bacterial cells with sources of carbon and energy [85].

Glutathione also participates in detoxification of some reactive aldehydes that can be produced from oxidative processes in cells. Two enzymatic systems use glutathione as a cofactor in formation of a thioester intermediate with subsequent regeneration of glutathione in thiol form. The glyoxalase system catalyzes conversion of 2-oxoaldehydes, such as methylglyoxal and phenylglyoxal, into corresponding 2-hydroxy acids [90]. The second system includes formaldehyde dehydrogenase oxidizing formaldehyde to formic acid in the presence of NAD.

The first stage of glyoxalase system functioning is formation of S-D-lactoylglutathione from glutathione and the semimercaptal adduct of methylglyoxal catalyzed by glyoxalase I. In the second stage, the thioester product is hydrolyzed into D-lactate and GSH by glyoxalase II [90, 91]. Glyoxalases I and II are widely distributed enzymes: they have been isolated from cells of mammals, yeasts, plants, and prokaryotes. Wide distribution of glyoxalases is not surprising in connection with their role in detoxification of their physiological substrate, methylglyoxal, which possesses mutagenic activity. Bacteria lacked GSH are more sensitive to methylglyoxal than wild-type cells and rapidly lose viability in its presence [12, 85]. An *E. coli* mutant resistant to methylglyoxal was selected that possesses elevated activities of glyoxalase and glutathione synthesis enzymes [92].

In the case of formaldehyde, the first stage is transformation of formaldehyde to S-formylglutathione by formaldehyde dehydrogenase PHDH. In the second stage, S-formylglutathione hydrolase catalyzes hydrolysis of the thioester [90]. Like glyoxalase I, formaldehyde dehydrogenase is a widely distributed enzyme that is present in cells of animals, plants, and microorganisms.

Exposure of cells to toxic metals is often accompanied by decrease in the level of thiols, and the ability of bacterial cells to maintain a high level of glutathione largely determines their resistance to toxic metals and tellurite [93, 94]. Bacteria resistant to mercuric chloride twofold increase both activity of glutathione reductase and the rate of glutathione synthesis in response to addition of $HgCl_2$ [93]. Conversely, *E. coli* strains deficient in biosynthesis of glutathione have elevated sensitivity to salts of mercury, silver, cadmium, and arsenic [12, 41, 95].

It has been reported that the treatment of *E. coli* with chlorine compounds led to decrease in the level of intracellular GSH to a very low value [96]. Glutathione synthesis-deficient mutants compared to wild-type cells are more sensitive to these substances [96, 97].

In the great majority of cases conjugation of various xenobiotics with GSH results in their detoxification; however, in some instances the interaction of a substance with glutathione with following metabolism is accompanied by appearance of toxic and mutagenic derivatives. In particular, alkylnitrosoguanidines, such as *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) and its analog *N*-ethyl-*N*′-nitro-*N*-nitrosoguanidine (ENNG) can be activated by GSH. As a result, products may be produced possessing increased DNA-alkylating ability and, hence, high mutagenic activity [98]. So, glutathione-deficient mutants of *E. coli* and *S. typhimurium* compared to parental strains have elevated resistance to the mutagenic effect of these substances [99, 100]. Pretreatment of bacteria with high concentrations of acetate at acidic pH of the medium also diminished the mutagenic effect of MNNG [51]. Yet in this situation a decrease in GSH level might contribute to the observed effect, because the treatment of *E. coli* with acetate results in diminution of intracellular glutathione [34, 51]. The following mechanism can be proposed for decrease in the level of intracellular glutathione under the influence of acetate. Under the conditions described, the treatment of cells with acetate should lead to acidification of cytoplasm and, hence, inhibition of glutathione synthesis, because activities of both γ-GCS and GSH-synthetase linearly decrease with decrease in pH below physiological values [8, 9]. Moreover, the treatment of cells growing on synthetic mineral medium with acetate leads to partial efflux of low molecular weight thiols from the cells to the medium [50].

As in eukaryotes, glutathione-dependent elevation of mutagenic and carcinogenic effects of some xenobiotics, such as haloalkanes and haloalkenes, in bacteria may occur involving glutathione S-transferases [101].

ADAPTATION TO STRESSES

Oxidative stress. Oxidative stress results from the effect of reactive oxygen species (ROS)—such as superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\cdot}$), and hydroperoxides (ROOH)—on the organism. Because of their high reactivity, ROS can damage all biological macromolecules and are an implicit threat for the cell. During evolution, bacteria and other organisms have developed mechanisms protecting them from various types of oxidative stress [102, 103].

In eukaryotes glutathione as a cofactor of selenium-dependent and independent glutathione peroxidases plays a key role in defense against oxidative stress. A powerful GSH oxidation occurs under oxidative stress, and decrease in the ratio GSH/GSSG is one of general fea-

tures of oxidative stress in eukaryotic cells. Unlike eukaryotes, *E. coli*, as well as other bacteria, does not contain glutathione peroxidase [5], so variations in the status of glutathione and its role in response to oxidative stress significantly differ in eukaryotes and bacteria [7, 63]. In particular, only lethal doses of H_2O_2 cause significant increase in the level of intracellular GSSG and decrease in the ratio GSH/GSSG in *E. coli* [13, 30]. It has been found that *E. coli* mutants deficient in glutathione synthesis do not show increased sensitivity to H_2O_2 and cumene hydroperoxide in exponential growth phase [13]. Nonetheless, non-growing *gshA* mutants compared to wild-type cells become appreciably more sensitive to hydrogen peroxide [96].

Sensitivity of mutants in *gshA* and *gor* to oxidants significantly depends on not only growth phase, but on the strain studied, medium composition, and growth conditions [59]. This is one possible reason for inconsistency of data on the role of glutathione in the response of bacteria to oxidative stress.

Interestingly, the bacteria *Haemophilus influenzae* and *Lactococcus lactis*, which cannot synthesize glutathione, can take it up from the medium, thus increasing their resistance to H_2O_2 [4, 104].

One possible function of the glutathione system in antioxidant defense of bacteria is connected with its involvement in regulation of activity of the transcription factor OxyR. This regulator plays a pivotal role in adaptation of *E. coli* and *S. typhimurium* to peroxide stress. When bacteria are treated with low doses of H_2O_2, OxyR activates expression of several genes, such as *katG*, *ahpCF*, *gorA*, *dps*, *grxA*, and *trxC*, encoding hydroperoxidase I, alkylhydroperoxide reductase, glutathione reductase, nonspecific DNA-binding protein, glutaredoxin 1, and thioredoxin 2, respectively [26, 105].

Genetic and biochemical studies have demonstrated intramolecular disulfide bond formation between Cys199 and Cys208 under the action of H_2O_2 [55], leading to conformational change in the regulatory domain of OxyR [106]. Reduction of disulfide the bond by glutaredoxin 1 in the presence of GSH results in inactivation of OxyR *in vitro* and *in vivo* [55, 56]. Mutations of genes encoding glutaredoxin 1 (*grxA*) or glutathione reductase (*gor*) decrease the rate of OxyR reduction. Double mutations *gor trxA* and *gshA trxA* partially activate OxyR in the absence of added H_2O_2, but mutations *grxA trxA* have only a minor effect. The data suggests that OxyR is sensitive to both H_2O_2 and intracellular thiol-disulfide status change [56]. Interestingly, *gor* and *grxA* are the members of the OxyR regulon. This endows the system with the ability for autoregulation under oxidative stress.

Results published recently by other investigators challenge the model described above [67, 75]. In these studies, no formation of intramolecular disulfide bonding between Cys199 and Cys208 in native activated OxyR was found, suggesting that such binding has no role in its regulation. The authors proved that the activity of OxyR is controlled via various modifications of Cys199 in response to various stresses: a stable derivative of sulfenic acid (C_{199}-SOH) is formed under oxidative stress, C_{199}-SNO is formed under the action of nitrosothiols, and glutathionylation takes place under disulfide stress, i.e. mixed disulfide with glutathione (C_{199}-S-S-G) is formed [75, 107]. These modifications of Cys199 are supposed to activate OxyR in different ways, enabling initiation of various transcriptional responses under the action of various stresses. Thus, a model of multiple active states was proposed instead of the model of thiol-disulfide switching. It is important that both models suppose the involvement of glutathione and other thiol-containing redox systems in the adaptive regulation of *E. coli* to oxidative stress induced by hydrogen peroxide. This supposition is supported by the fact that the activation of OxyR and elevation of basal expression of OxyR-regulated genes occur in *E. coli* cells deficient in glutathione (*gshA*) and thioredoxin (*trxA*) [108, 109]. The absence of Trx1 and GSH results in elevation of Trx2 and thioredoxin reductase transcription, but it does not alter the expression of glutaredoxins 2 and 3 [108].

As in peroxide stress, the data are ambiguous on the role of glutathione in defense against oxidative stress induced by superoxide anion generating compounds. Sensitivity of *gshA* mutants to superoxide generators depends on strain, cultivation conditions, and growth phase [59].

The treatment of *E. coli* cells with the superoxide generator methyl viologen (paraquat) substantially decreases GSH level but not the intracellular concentrations of GSSG or mixed disulfides of glutathione with proteins [37], while menadione, another generator of superoxide anions, induced dose-dependent decrease in total glutathione pool, elevation of $GSSG_{in}$, and decrease in $GSH_{in}/GSSG_{in}$ ratio [30]. Interestingly, the treatment of *E. coli sodAsodB* cells deficient in two superoxide dismutases with menadione was not accompanied by GSH decrease and GSSG accumulation, and $GSH_{in}/GSSG_{in}$ ratio was three times higher than in wild-type parent strain [30]. One possible cause of low GSSG level in *E. coli sodAsodB* cells treated with menadione may be the decreased rate of superoxide dismutation and, hence, intracellular H_2O_2 production. It is this H_2O_2 produced by superoxide dismutation that may be responsible for GSH oxidation in cytoplasm, because the inhibition of GSSG formation in the reaction of menadione with GSH by catalase, but not superoxide dismutase, was demonstrated in the experiments *in vitro* earlier [110]. It should be noted that the treatment of cells with generators of superoxide anion results in the decrease in NADPH level [103], which is a donor of reductive equivalents in the reaction of glutathione reductase. The deficit of NADPH may be an indirect cause of GSSG accumulation.

Treatment of *E. coli* cells with compounds generating superoxide results in an adaptive response accompanied by the induction of a large number of proteins, some of which are positively regulated by SoxRS proteins. The list of genes in the *soxRS* regulon has been widened to 60 by the use of new genetic techniques, and the total number of genes responding to superoxide stress is at least 112 [111]. SoxR protein consisting of two subunits each containing a [2Fe-2S]-cluster plays the role of sensory element in the response to superoxide stress. These clusters can be oxidized and reduced reversibly, but only the oxidized SoxR form is a potent transcription activator. A rapid reversible oxidation of SoxR occurs under its treatment with substances generating superoxide. GSH may influence the regulation of redox-sensitive transcription factor SoxR in some way, because GSH favored the destruction of [2Fe-2S]-clusters of SoxR in *in vitro* experiments [112], and paraquat-induced *soxS* expression was decreased in *E. coli* double mutants *gor trxA* [113].

Note the interpretation of the results of studies on the role of glutathione in oxidative stress in bacteria is complex due to overlapping functions of different components of antioxidant systems and the ability of cells to activate other systems instead of ones lost as a result of mutation. It is obvious, however, that redox systems of glutathione and other thiols comprise an integral part of the response to oxidative stress.

Despite common mechanisms of antioxidant defense and the role of glutathione in pro- and eukaryotes, a difference exists due to more complex regulation system of antioxidant response in eukaryotic cells. Unlike eukaryotic cells, the GSH synthesis enzymes in *E. coli* are not regulated by transcription factors and are not expressed in response to H_2O_2 or superoxide generators [105, 111]. Nonetheless, glutathione biosynthesis seems to be indirectly regulated by H_2O_2 independently from OxyR by expression of *cysK* and other genes controlling the biosynthetic pathway of cysteine, the amino acid limiting GSH synthesis during normal growth [105].

GSH catabolism as a source of reactive oxygen species. It is paradoxical that glutathione, possessing antioxidant properties, can simultaneously act as a "prooxidant", whose catabolism can be a source reactive oxygen species. Under certain conditions, GSH at physiological concentrations exhibits a mutagenic effect on bacterial cells, and this effect is associated with γ-glutamyl transpeptidase activity [114, 115]. Unlike the initial substrate (GSH), cysteinyl-glycine produced from the reaction catalyzed by GGT can reduce free or chelated Fe^{3+}, which, in turn, initiates (in presence of O_2) a reaction cascade leading to formation of superoxide, hydrogen peroxide, and hydroxyl radical.

Some doubts have been cast upon the possible physiological role of these processes, because the active center of GGT is exposed to the external membrane surface and, hence, under normal conditions contacts with very low micromolar GSH concentrations (not the millimolar ones typical for inner medium of the cell and usually employed in most experiments). Besides, when growing in cysteinyl-glycine medium, *E. coli* CM86 strain is characterized by the same mutation frequency as the wild-type cells [16]. This effect may apparently be associated with the high level of antioxidant defense in the studied strain. Low doses of L-cysteine, cysteinyl-glycine, and GSH have been demonstrated to be mutagenic for *E. coli* WP2 strain deficient in OxyR (regulating the response to peroxide stress), whereas high doses of these substances are necessary to observe minor mutagenicity in *oxyR*(+) [116]. This fact suggests suppression of thiol-mediated mutagenesis by OxyR-regulated antioxidant defense and confirms the oxidative character of the mutagenesis. Only high doses of reactive thiols had mutagenic effect in the presence of catalase and glutathione peroxidase [116]. Thus, effects associated with the prooxidant role of GSH in the presence of GGT *in vivo* largely depend on the activity of cellular antioxidant systems. Nonetheless, a weak production of ROS in metabolism of extracellular GSH seems to be of certain importance in regulation of cellular processes. A hypothesis has been propounded that in eukaryotic cells low-level hydrogen peroxide generated by GGT with involvement of extracellular glutathione can act as a continuous "signal of life" to support cell proliferation and to protect from apoptosis by the influence on ROS-sensitive regulatory signaling pathways [117].

ROS production leading to death of foreign microorganisms due to oxidative damage is known to be one of the elements of immune response. Microorganisms can apparently also use ROS to attack host cells. In particular, *Pseudomonas aeruginosa* use pyocyanine they produce and glutathione from human epithelial cells to form free pyocyanine radicals and $O_2^{\cdot-}$. Thus GSH is presumed to enhance pyocyanine-induced cytotoxicity [118].

Temperature stresses. Studies on eukaryotic cells suggest a possible protective role of glutathione in cell response to temperature stresses. Increase in the level of intracellular glutathione in response to heat shock, as well as increase in thermal sensitivity of cells on depletion of intracellular glutathione under the influence of SH-reagents or glutathione synthesis inhibitors or in mutants deficient in GSH synthesis, are considered as proof of implication of glutathione in thermoresistance of eukaryotic cells [119, 120]. A positive role of glutathione in resistance to heat shock resistance is connected to its antioxidant properties, because in various cell types elevation of temperature is accompanied by increased production of free radicals and peroxides [121, 122].

Available data do not give an unambiguous answer to the question of the role of glutathione in adaptation of bacteria to heat shock. In particular, in growing *E. coli* the fast increase in growth temperature from 30 to 42°C led to inhibition of glutathione reductase activity, decrease in intracellular concentration of glutathione, and increase

in its concentration in the medium. Interestingly, mutants *gshA* deficient in glutathione synthesis were more resistant to the fast elevation of temperature than were the parental cells [45]. The levels of both intra- and extracellular glutathione were appreciably higher in cells adapted to elevated temperature compared to those growing at optimum temperature [18, 45]. These data suggest that both the role and dynamics of glutathione in response to heat shock may significantly differ in eukaryotic cells and bacteria.

Unlike heat shock, fast decrease in temperature from 37 to 20°C had more pronounced inhibitory effect on the growth of *E. coli* cells deficient in glutathione synthesis compared with the parental cells. The cold shock was not accompanied by visible changes in the status of intracellular glutathione. However, both the concentration of intracellular GSH and the ratio $GSH_{in}/GSSG_{in}$ were lower in the cells adapted to lower temperature and growing at 20°C than in the cells growing at 37°C [44]. Increase in the level of extracellular glutathione observed at low temperatures [18, 44] suggests that GSH release into the medium may be one of possible causes of decrease in the level of GSH_{in}. In various eukaryotic cell types, the effect of low temperatures is accompanied by induction of antioxidant response in which glutathione plays an important role [123]. Some features of oxidative stress are found under cold stress and bacterial growth at lower temperature [44]. The question still remains whether glutathione is involved in antioxidant defense of bacteria under cold shock or whether it performs other functions.

Osmotic stress. In the course of evolution, bacteria have developed turgor pressure regulating systems with the main function of accumulation of intracellular osmolytes that do not inhibit enzymatic activities and enable bacterial growth in media with high osmolarity [124]. So, *E. coli* cells restore turgor tension via accumulation of large amounts of K^+ ions in the initial stage of the response to hyperosmotic stress. However, intracellular K^+ in abundance can inhibit a number of enzymes, so the replacement of K^+ by betaine, proline, and trehalose occurs at later stages of cellular response to osmotic stress [125, 126]. Glutathione is one of the *E. coli* metabolites whose cytoplasmic level becomes elevated under hyperosmotic stress [127, 128]. The change in level of extracellular glutathione in response to osmolarity elevation is different: rapid decrease in glutathione level at the first stage is replaced by its elevation at the second one [43]. It is likely that glutathione uptake from the medium can contribute to the accumulation of intracellular glutathione in the initial stage of osmotic adaptation.

Glutathione obviously plays a positive role in osmoadaptation of *E. coli* cells, because *E. coli gshA* and *gor* mutants grow more poorly than parental cells in medium with elevated osmolarity. The negative influence of *gshA* mutation on osmoadaptation is more obvious in a medium with high osmolarity and at low extracellular K^+ concentrations [43, 128].

The elevated level of intracellular glutathione under osmotic stress might suggest its direct participation in the maintenance of turgor tension. Calculation suggests, however, that glutathione concentration is relatively low and, even being at the highest level of its accumulation, glutathione cannot contribute substantially into the maintenance of turgor tension under osmotic stress [127, 128]. Taking into account that GSH functioning is retentive for cytoplasmic potassium [77], and change in transmembrane flows of K^+ is an essential element of *E. coli* response to osmotic stress, a possibility was tested of glutathione participation in osmotic adaptation as a factor controlling potassium retention [128]. However, the authors came to the conclusion that the role of glutathione in osmotic adaptation is not directly associated with its effect on K^+ retention. Another work showed that the wild-type bacteria export potassium at a higher rate than *gshA* mutants in the second stage of the response to hyperosmotic stress [43]. The retarded K^+ efflux in the strain deficient in glutathione can be either associated with the disturbance of normal functioning of K^+-exit channels in the absence of GSH or due to change in rate of trehalose synthesis substituting for potassium. However, glutathione probably does not affect trehalose synthesis, because *gshA* and *otsA* (the gene encoding trehalose-6-phosphate synthetase) mutations have independent and additive effect on *E. coli* osmotic adaptation [128]. It is just the influence of GSH on changes in the K^+ pool that possibly determines the higher ability to osmotic adaptation in cells with normal GSH level.

Unlike *gshA* and *gor* mutants, *E. coli* cells deficient in γ-glutamyl transpeptidase display higher ability for osmotic adaptation and elevate their intracellular glutathione concentration more rapidly than do the wild-type cells in response to osmotic stress [43]. These data give additional confirmation that cytoplasmic glutathione accumulation under osmotic stress can be a positive factor for osmotic adaptation.

It should be borne in mind that the GSH molecule is negatively charged at physiological pH values, and the elevation of intracellular glutathione can facilitate maintenance of ion balance, when the intracellular accumulation of positively charged potassium ions occurs under hyperosmotic stress. This is supported by the fact that a positive correlation exists between changes in intracellular potassium and glutathione pools at the initial stages of *E. coli* response to osmotic stress, which is especially obvious when bacteria are grown on mineral media [43, 129]. Note that the osmoprotector betaine added into the *E. coli* culture growing in the medium with high osmolarity induces immediate efflux of potassium ions as well as low molecular weight thiols, the major part of which is glutathione [130]. However, the contribution of glutathione into the ion balance maintenance is not deter-

mining, because from 70 to 90% of the potassium positive charge is compensated by glutamate anions [128, 131].

Osmotic stress in *E. coli* was shown to be accompanied by reactions typical for oxidative stress [132]. This is supported by decreased ability in *E. coli* cells deficient in SodA and SodB superoxide dismutase synthesis to grow in media with high osmolarity as well as enhanced *sodA*, *soxS*, and *katE* expression in response to osmotic stress. One cannot exclude an involvement of glutathione as an antioxidant in cellular defense against oxidative damage under osmotic stress.

The treatment of *E. coli* cells with compounds inducing disturbance of intracellular pH homeostasis and ion balance also resulted in changes in glutathione status inside and outside the cells. The presence of glutathione endowed the cells more resistance to these exposures, because the growth of mutants deficient in glutathione synthesis was inhibited more than the growth of the parental cells [34].

This work was supported by the Russian Foundation for Basic Research (grants 01-04-48129 and 03-04-49137) and by the Presidium of Russian Academy of Sciences (Program "Molecular and Cellular Biology").

REFERENCES

1. Fahey, R. C., Brown, W. C., Adams, W. B., and Worsham, M. B. (1978) *J. Bacteriol.*, **133**, 1126-1129.
2. Newton, G. L., Arnold, K., Price, M. S., Sherrill, C., delCardayre, S. B., Aharonowitz, Y., Cohen, G., Davies, J., Fahey, R. C., and Davis, C. (1996) *J. Bacteriol.*, **178**, 1990-1995.
3. Sherrill, C., and Fahey, R. C. (1998) *J. Bacteriol.*, **180**, 1454-1459.
4. Vergauwen, B., Pauwels, F., Vaneechoutte, M., and van Beeumen, J. J. (2003) *J. Bacteriol.*, **185**, 1572-1581.
5. Sundquist, A. R., and Fahey, R. C. (1989) *J. Mol. Evol.*, **29**, 429-435.
6. Kosower, N. S., and Kosower, E. M. (1978) *Int. Rev. Cytol.*, **54**, 109-160.
7. Meister, A., and Anderson, M. E. (1983) *Ann. Rev. Biochem.*, **52**, 711-760.
8. Gushima, H., Miya, T., Murata, K., and Kimura, A. (1983) *J. Appl. Biochem.*, **5**, 210-218.
9. Murata, K., and Kimura, A. (1990) *Biotech. Adv.*, **8**, 5-96.
10. Matsuda, K., Mizuguchi, K., Nishioka, T., Kato, H., Go, N., and Oda, J. (1996) *Protein Eng.*, **9**, 1083-1092.
11. Hibi, T., Hisada, H., Nakatsu, T., Kato, H., and Oda, J. (2002) *Acta Crystallogr. Sec. D- Biol. Crystallogr.*, **58**, 316-318.
12. Apontoweil, P., and Berends, W. (1975) *Biochim. Biophys. Acta*, **399**, 10-22.
13. Greenberg, J. T., and Demple, B. (1986) *J. Bacteriol.*, **168**, 1026-1029.
14. Loewen, P. C. (1979) *Can. J. Biochem.*, **57**, 107-111.
15. Park, S., and Imlay, J. A. (2003) *J. Bacteriol.*, **185**, 1942-1950.
16. Suzuki, H., Kamatani, S., Kim, E. S., and Kumagai, H. (2001) *J. Bacteriol.*, **183**, 1489-1490.
17. Suzuki, H., Kumagai, H., and Tochikura, T. (1986) *J. Bacteriol.*, **168**, 1325-1331.
18. Suzuki, H., Kumagai, H., and Tochikura, T. (1987) *J. Bacteriol.*, **169**, 3926-3931.
19. Suzuki, H., Kumagai, H., Echigo, T., and Tochikura, T. (1989) *J. Bacteriol.*, **171**, 5169-5172.
20. Xu, K., and Strauch, M. A. (1996) *J. Bacteriol.*, **178**, 4319-4322.
21. Parry, J., and Clark, D. P. (2002) *FEMS Microbiol. Lett.*, **209**, 81-85.
22. Davis, N. K., Greer, S., Jones-Mortimer, M. C., and Perham, R. N. (1982) *J. Gen. Microbiol.*, **128**, 1631-1634.
23. Russel, M., and Holmgren, A. (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 990-994.
24. Mata, A. M., Pinto, M. C., and Lopez-Barea, J. (1984) *Z. Naturforsch.*, **39**, 908-915.
25. Greer, S., and Perham, R. N. (1986) *Biochemistry*, **25**, 2736-2742.
26. Christman, M. F., Morgan, R. W., Jacobson, F. S., and Ames, B. N. (1985) *Cell*, **41**, 753-762.
27. Michan, C., Manchado, M., Dorado, G., and Pueyo, C. (1999) *J. Bacteriol.*, **181**, 2759-2764.
28. Becker-Hapak, M., and Eisenstark, A. (1995) *FEMS Microbiol. Lett.*, **134**, 39-44.
29. Alonso-Moraga, A., Bocanegra, A., Torres, J. M., Lopez-Barea, J., and Pueyo, C. (1987) *Mol. Cell. Biochem.*, **73**, 61-68.
30. Smirnova, G. V., Muzyka, N. G., Glukhovchenko, M. N., and Oktyabrsky, O. N. (2000) *Free Rad. Biol. Med.*, **28**, 1009-1016.
31. Tabor, H. C., and Tabor, W. (1975) *J. Biol. Chem.*, **250**, 2648-2654.
32. Loewen, P. (1977) *Can. J. Biochem.*, **55**, 1019-1027.
33. Loewen, P. C. (1978) *Can. J. Biochem.*, **56**, 753-759.
34. Smirnova, G. V., Torkhova, O. A., and Oktyabrsky, O. N. (2003) *Mikrobiologiya*, **72**, 542-547.
35. Kosower, E. M., Correa, W., Kinon, B. J., and Kosower, N. S. (1971) *Biochim. Biophys. Acta*, **264**, 39-44.
36. Hibberd, K. A., Berget, P. B., Warner, H. R., and Fuchs, J. A. (1978) *J. Bacteriol.*, **133**, 1150-1155.
37. Romero, M. J., and Canada, A. T. (1991) *Toxicol. Appl. Pharmacol.*, **111**, 485-495.
38. Meister, A. **(**1988) *J. Biol. Chem.*, **263**, 17205-17208.
39. Nakayama, R., Kumagai, H., and Tochikura, T. (1984) *Appl. Environ. Microbiol.*, **47**, 653-657.
40. Owens, R. A., and Hartman, P. E. (1986) *J. Bacteriol.*, **168**, 109-114.
41. Owens, R. A., and Hartman, P. E. (1986) *Environ. Mutagen.*, **8**, 659-673.
42. Smirnova, G. V., Muzyka, N. G., and Oktyabrsky, O. N. (2005) *Biochemistry (Moscow)*, **70**, 926-934.
43. Smirnova, G. V., Krasnykh, T. A., and Oktyabrsky, O. N. (2001) *Biochemistry (Moscow)*, **66**, 973-978.
44. Smirnova, G. V., Zakirova, O. N., and Oktyabrsky, O. N. (2001) *Mikrobiologiya*, **70**, 45-50.
45. Smirnova, G. V., Zakirova, O. N., and Oktyabrsky, O. N. (2001) *Mikrobiologiya*, **70**, 512-518.
46. Oktyabrsky, O. N., Pshenichnov, R. A., Tkachenko, A. G., Zelenin, E. N., and Smirnova, G. V. (1981) *Mikrobiologiya*, **50**, 467-470.

47. Oktyabrsky, O. N., and Pshenichnov, R. A. (1982) *Mikrobiologiya*, **51**, 515-517.
48. Oktyabrsky, O. N., and Smirnova, G. V. (1989) *Acta Biotechnol.*, **9**, 203-209.
49. Oktyabrsky, O. N., and Smirnova, G. V. (1993) *Bioelectrochem. Bioenerg.*, **32**, 287-294.
50. Oktiabrsky, O. N., and Smirnova, G. V. (1988) *Biokhimiya*, **53**, 2042-2050.
51. Oktyabrsky, O. N., Golyasnaya, N. V., Smirnova, G. V., Demakov, V. A., Posokhina, N. Kh., and Kholstova, T. A. (1993) *Mutat. Res., DNA Repair*, **293**, 197-204.
52. Schafer, F. Q., and Buettner, G. R. (2001) *Free Rad. Biol. Med.*, **30**, 1191-1212.
53. Holmgren, A., and Aslund, F. (1995) *Meth. Enzymol.*, **252**, 283-292.
54. Aslund, F., Ehn, B., Miranda-Vizuete, A., Pueyo, C., and Holmgren, A. (1994) *Proc. Natl. Acad. Sci. USA*, **91**, 9813-9817.
55. Zheng, M., Aslund, F., and Storz, G. (1998) *Science*, **279**, 1718-1721.
56. Aslund, F., Zheng, M., Beckwith, J., and Storz, G. (1999) *Proc. Natl. Acad. Sci. USA*, **96**, 6161-6165.
57. Russel, M., and Holmgren, A. (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 990-994.
58. Prinz, W. A., Aslund, F., Holmgren, A., and Beckwith, J. (1997) *J. Biol. Chem.*, **272**, 15661-15667.
59. Vlamis-Gardikas, A., Potamitou, A., Zarivach, R., Hochman, A., and Holmgren, A. (2002) *J. Biol. Chem.*, **277**, 10861-10868.
60. Arner, E. S. J., and Holmgren, A. (2000) *Eur. J. Biochem.*, **267**, 6102-6109.
61. Gleason, F. K., and Holmgren, A. (1988) *FEMS Microbiol. Rev.*, **54**, 271-298.
62. Russel, M., and Model, P. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 29-33.
63. Carmel-Harel, O., and Storz, G. (2000) *Annu. Rev. Microbiol.*, **54**, 439-461.
64. Potamitou, A., Holmgren, A., and Vlamis-Gardikas, A. (2002) *J. Biol. Chem.*, **277**, 18561-18567.
65. Miranda-Vizuete, A., Rodriguez-Ariza, A., Toribio, F., Holmgren, A., Lopez-Barea, J., and Pueyo, C. (1996) *J. Biol. Chem.*, **271**, 19099-19103.
66. Ritz, D., and Beckwith, J. (2001) *Annu. Rev. Microbiol.*, **55**, 21-48.
67. Paget, M. S. B., and Buttner, M. J. (2003) *Annu. Rev. Genet.*, **37**, 91-121.
68. Klatt, P., and Lamas, S. (2000) *Eur. J. Biochem.*, **267**, 4928-4944.
69. Gilbert, H. F. (1982) *J. Biol. Chem.*, **257**, 12086-12091.
70. Ziegler, D. M. (1985) *Ann. Rev. Biochem.*, **54**, 305-329.
71. Ondarza, R. N. (1989) *Biosci. Rep.*, **9**, 593-604.
72. Thomas, J. A., Poland, B., and Honzatko, R. (1995) *Arch. Biochem. Biophys.*, **319**, 1-9.
73. Jung, C. H., and Thomas, J. A. (1996) *Arch. Biochem. Biophys.*, **335**, 61-72.
74. Lillig, C. H., Potamitou, A., Schwenn, J. D., Vlamis-Gardikas, A., and Holmgren, A. (2003) *J. Biol. Chem.*, **278**, 22325-22330.
75. Kim, S. O., Merchant, K., Nudelman, R., Beyer, W. F., Jr., and Keng, T. (2002) *Cell*, **109**, 383-396.
76. Epstein, W., Buurman, E., McLaggan, D., and Naprstek, J. (1993) *Biochem. Trans.*, **21**, 1006-1610.
77. Meury, J., and Kepes, A. (1982) *EMBO J.*, **1**, 339-343.
78. Booth, I. R., Jones, M. A., McLaggan, D., Nikolaev, Y., Ness, L. S., Wood, C. M., Miller, S., Totemeyer, S., and Ferguson, G. P. (1996) in *Handbook of Biological Physics*, Vol. 2 (Konings, W. N., Kabac, H. R., and Lolkema, J. S., eds.) Elsevier Science, pp. 693-729.
79. Ferguson, G. P., McLaggan, D., and Booth, I. R. (1995) *Mol. Microbiol.*, **17**, 1025-1033.
80. Ness, L. S., Ferguson, G. P., Nikolaev, Y., and Booth, I. R. (1997) *Appl. Environ. Microbiol.*, **63**, 4083-4086.
81. Elmore, M. J., Lamb, A. J., Ritchie, G. Y., Douglas, R. M., Munro, A., Gajewska, A., and Booth, I. R. (1990) *Mol. Microbiol.*, **4**, 405-412.
82. Ferguson, G. P., and Booth, I. R. (1998) *J. Bacteriol.*, **180**, 4314-4318.
83. McLaggan, D., Jones, M. A., Gouesbet, G., Levina, N., Lindey, S., Epstein, W., and Booth, I. R. (2002) *Mol. Microbiol.*, **43**, 521-536.
84. Koprowski, P., and Kubalski, A. (1999) *Eur. J. Physiol.*, **438**, 361-364.
85. Vuilleumier, S. (1997) *J. Bacteriol.*, **179**, 1431-1441.
86. Perito, B., Allocati, N., Casalone, E., Masulli, M., Dragani, B., Polsinelli, M., Aceto, A., and Ilio, C. D. (1996) *Biochem. J.*, **318**, 157-162.
87. Nishida, M., Kong, K. H., Inoue, H., and Takahashi, K. (1994) *J. Biol. Chem.*, **269**, 32536-32541.
88. McLaggan, D., Rufino, H., Jaspars, M., and Booth, I. R. (2000) *Appl. Environ. Microbiol.*, **66**, 1393-1399.
89. Kaluzna, A., and Bartosz, G. (1997) *Biochem. Mol. Biol. Int.*, **43**, 161-171.
90. Mannervik, B., Carlberg, I., and Larson, K. (1989) in *Glutathione: Chemical, Biochemical and Medical Aspects*, Pt. A, B (Dolphin, D., Poulson, R., and Avramovic, D., eds.) N. Y., pp. 476-516.
91. Cooper, R. A. (1984) *Annu. Rev. Microbiol.*, **38**, 49-68.
92. Murata, K., Tani, K., Kato, J., and Chibata, I. (1980) *J. Gen. Microbiol.*, **120**, 515-517.
93. Gachhui, R., Pahan, K., Ray, S., Chaudhuri, J., and Mandal, A. (1991) *Bull. Environ. Contam. Toxicol.*, **46**, 336-342.
94. Turner, R. J., Aharonowitz, Y., Weiner, J. H., and Taylor, D. E. (2001) *Can. J. Microbiol.*, **47**, 33-40.
95. Latinwo, L. M., Donald, C., Ikediobi, C., and Silver, S. (1998) *Biochem. Biophys. Res. Commun.*, **242**, 67-70.
96. Chesney, J. A., Eaton, J. W., and Mahoney, J. R., Jr. (1996) *J. Bacteriol.*, **178**, 2131-2135.
97. Saby, S., Leroy, P., and Block, J. C. (1999) *Appl. Environ. Microbiol.*, **65**, 5600-5603.
98. Lawley, P. D., and Thatcher, C. J. (1970) *Biochem. J.*, **116**, 693-707.
99. Sedgwick, B., and Robins, P. (1980) *Mol. Gen. Genet.*, **180**, 85-90.
100. Mohn, G. R., de Knijff P., and Baars, A. (1988) *Mutat. Res.*, **11**, 25-31.
101. Perham, R. A., Andersen, M. E., Warren, S. H., Ross, T. M., and Claxton, L. D. (1997) *Toxicol. Appl. Pharmacol.*, **144**, 183-188.
102. Storz, G., and Imlay, J. A. (1999) *Curr. Opin. Microbiol.*, **2**, 188-194.
103. Pomposiello, P. J., and Demple, B. (2002) *Adv. Microb. Physiol.*, **46**, 319-341.
104. Li, Y., Hugenholtz, J., Abee, T., and Molenaar, D. (2003) *Appl. Environ. Microbiol.*, **69**, 5739-5745.

105. Zheng, M., Wang, X., Templeton, L. J., Smulski, D. R., LaRossa, R. A., and Storz, G. (2001) *J. Bacteriol.*, **183**, 4562-4570.
106. Choi, S. J., Kim, H. J., Mukhopadhyay, P., Cho, S., Woo, J. R., Storz, G., and Ryu, S. E. (2001) *Cell*, **105**, 103-113.
107. Hausladen, A., Privalle, C. T., Keng, T., DeAngelo, J., and Stamler, J. S. (1996) *Cell*, **86**, 719-729.
108. Prieto-Alamo, M. J., Jurado, J., Gallardo-Madueno, R., Monje-Casas, F., Holmgren, A., and Pueyo, C. (2000) *J. Biol. Chem.*, **275**, 13398-13405.
109. Oktyabrsky, O. N., Smirnova, G. V., and Muzyka, N. G. (2001) *Free Rad. Biol. Med.*, **31**, 250-255.
110. Ross, D., Thor, H., Orrenius, S., and Moldeus, P. (1985) *Chem. Biol. Interact.*, **55**, 177-184.
111. Pomposiello, P. J., Bennik, M. H. J., and Demple, B. (2001) *J. Bacteriol.*, **183**, 3890-3902.
112. Ding, H., and Demple, B. (1996) *Proc. Natl. Acad. Sci. USA*, **93**, 9449-9453.
113. Ding, H., and Demple, B. (1998) *Biochemistry*, **37**, 17280-17286.
114. Stark, A. A., Zeiger, E., and Pagano, D. A. (1988) *Carcinogenesis*, **9**, 771-777.
115. Glatt, H. (1989) *Mutagenesis*, **4**, 221-227.
116. Martinez, A., Urios, A., and Blanco, M. (1999) *Mutat. Res.*, **446**, 205-213.
117. Paolicchi, A., Dominici, S., Pieri, L., Maellaro, E., and Pompella, A. (2002) *Biochem. Pharmacol.*, **64**, 1027-1035.
118. O'Malley, Y. Q., Reszka, K. J., Spitz, D. R., Denning, G. M., and Britigan, B. E. (2004) *Amer. J. Physiol. Lung Cell Mol. Physiol.*, **287**, L94-L103.
119. Mitchell, J. B., and Russo, A. (1983) *Radiat. Res.*, **95**, 471-485.
120. Sugiyama, K., Kawamura, A., Izawa, S., and Inoue, Y. (2000) *Biochem. J.*, **352**, 71-78.
121. Privalle, C. T., and Fridovich, I. (1987) *Proc. Natl. Acad. Sci. USA*, **84**, 2723-2726.
122. Davidson, J. F., Whyte, B., Bissinger, P. H., and Schiestl, R. H. (1996) *Proc. Natl. Acad. Sci. USA*, **93**, 5116-5121.
123. Zhang, L., Onda, K., Imai, R., Horiuchi, H., and Ohta, A. (2003) *Biochem. Biophys. Res. Commun.*, **307**, 308-314.
124. Wood, J. M. (1999) *Microbiol. Mol. Biol. Rev.*, **63**, 230-262.
125. Larsen, P. I., Sydnes, L. K., Landfald, B., and Strom, A. R. (1987) *Arch. Microbiol.*, **147**, 1-7.
126. Dinnbier, U., Limpinsel, E., Schmid, R., and Bakker, E. P. (1988) *Arch. Microbiol.*, **150**, 348-357.
127. Munro, G. F., Hercules, K., Morgan, J., and Sauerbier, W. (1972) *J. Biol. Chem.*, **247**, 1272-1280.
128. McLaggan, D., Logan, T. M., Lynn, D. G., and Epstein, W. (1990). *J. Bacteriol.*, **172**, 3631-3636.
129. Oktyabrsky, O. N., and Smirnova, G. V. (1993) *Biochem. Mol. Biol. Int.*, **30**, 377-383.
130. Smirnova, G. V., and Oktyabrsky, O. N. (1995) *Arch. Microbiol.*, **163**, 76-78.
131. McLaggan, D., Naprstek, J., Buurman, E. T., and Epstein, W. (1994) *J. Biol. Chem.*, **269**, 1911-1917.
132. Smirnova, G. V., Muzyka, N. G., and Oktyabrsky, O. N. (2000) *FEMS Microbiol. Lett.*, **186**, 209-213.