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MINI REVIEW

Regulation of Respiration and ATP Synthesis in Higher Organisms: Hypothesis[1]

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Abstract

The present view on the regulation of respiration and ATP synthesis in higher organisms implies only Michaelis–Menten type kinetics and respiratory control as regulatory principles. Recent experimental observations, suggesting further regulatory mechanisms at respiratory chain complexes, are reviewed. A new hypothesis is presented implying regulation of respiration and ATP synthesis in higher organisms mainly via allosteric modification of respiratory chain complexes, in particular of cytochrome *c* oxidase. The allosteric effectors, e.g., metabolites, cofactors, ions, hormones, and the membrane potential are suggested to change the activity and the coupling degree of cytochrome *c* oxidase by binding to specific sites at nuclear coded subunits. Recent results on the structure and activity of cytochrome *c* oxidase, supporting the hypothesis, are reviewed.

Key Words: Regulation; cytochrome *c* oxidase; respiration; ATP synthesis; hypothesis; proton translocation; H^+/e^- stoichiometry; isozymes; allosteric modification.

Introduction

In aerobic cells ATP is mainly synthesized in mitochondria by oxidative phosphorylation. The reaction can be described by the general equation

$$NADH + H^+ + \tfrac{1}{2}O_2 + 3ADP + 3P_i \rightarrow NAD^+ + 4H_2O + 3ATP$$

Within the cell, e.g., under physiological substrate concentrations, the reaction proceeds far from equilibrium. The free energy change of this reaction

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was estimated in various cells to about -55 kJ/mol, corresponding to a thermodynamic efficiency of 74% (Erecinska and Wilson, 1982). It is generally believed that the rate of ATP synthesis can be expressed by the rate of respiration, if a tight coupling between respiration and phosphorylation is assumed. Since the energetic needs of animal cells are variable, and may change by an order of magnitude within seconds, it is of particular interest to understand the factors which regulate the rate of respiration and by that the rate of ATP synthesis.

From measurements of the [NADH]/[NAD^+] and [ATP]/[ADP] $\cdot$ [P_i] ratios as well as the redox state of cytochrome *c* in intact cells, Erecinska and Wilson concluded that the respiratory chain between NADH and cytochrome *c*, e.g., the first two sites of oxidative phosphorylation, are at near-equilibrium (Erecinska and Wilson, 1978, 1982; Erecinska *et al.*, 1974). They suggest that only the third site, the cytochrome *c* oxidase oxygen reaction, is responsible for the control of respiration rates. Wilson and co-workers derived a model on the control of mitochondrial respiration in intact cells implying only three parameters controlling the rate: (1) the activity (amount) of cytochrome *c* oxidase, (2) the level of reduction of cytochrome *c*, and (3) the extramitochondrial [ATP]/[ADP] $\cdot$ [P_i] ratio (Erecinska and Wilson, 1978, 1982; Wilson *et al.*, 1977). This model involved no rate-limiting step at the translocation of adenine nucleotides across the mitochondrial inner membrane (Erecinska and Wilson, 1982). Instead a strong influence of intracellular [P_i] on the respiration rate was found (Erecinska *et al.*, 1977; van der Meer *et al.*, 1980; van Dam *et al.*, 1980; Stucki, 1980). An opposite view, however, was presented by other groups, who concluded that the extramitochondrial [ATP]/[ADP] ratio and not the [ATP]/[ADP] $\cdot$ [P_i] ratio represents the rate-limiting step in active respiration [Davis and Lumeng, 1975; Küster *et al.*, 1976; Letko *et al.*, 1980; Brawand *et al.*, 1980; Kunz *et al.*, 1981; Williamson *et al.*, 1981). Whereas the energy dependence of adenine nucleotide exchange in mitochondria has been clearly demonstrated in isolated mitochondria (Davis and Lumeng, 1975; Letko *et al.*, 1980; Brawand *et al.*, 1980; Heldt *et al.*, 1972; Slater *et al.*, 1973) and with the reconstituted ADP/ATP carrier (Krämer and Klingenberg, 1980, 1982), recent reports argue against a regulatory function of the ADP/ATP carrier on the respiration rate of cells *in vivo* (Forman and Wilson, 1983; Erecinska and Wilson, 1984).

Using the control theory of Kacser and Burns (1973) and Heinrich and Rapoport (1974), different groups determined the "control strength" of different steps in mitochondrial oxidative phosphorylation. In addition to the ADP/ATP carrier a certain amount of control was also found at the dicarboxylate carrier (when succinate was used as substrate) and the cytochrome *c* oxidase (Groen *et al.*, 1982; Tager *et al.*, 1983), the magnitude

of which, however, was found to depend on the respiration rate and the complexity of the metabolic system (Gellerich *et al.*, 1983). Clearly there are several steps involved in the control of respiration rates.

Apart from different views as to the sites of regulation, so far it was generally assumed that respiration and ATP synthesis are only regulated by Michaelis–Menten type kinetics, e.g., by substrate concentrations, and by respiratory control, which however, according to the above equation, can also be understood as substrate-dependent regulation. However, in a number of recent publications strong indications accumulated that in cells of higher organisms respiration and ATP synthesis may also be regulated by "allosteric effectors," in addition to regulation by "isosteric effectors," e.g., substrates.

Modern knowledge on the regulation of metabolic flow is in accordance with this view, as presented in a textbook: "The flow of molecules in most metabolic pathways is determined primarily by the amounts and activities of certain enzymes rather than by the availability of substrates. Essentially irreversible reactions are potential control sites" (Streyer, 1981). Cytochrome *c* oxidase catalyzes the main irreversible step of energy metabolism. Although at present no specific mechanism for allosteric modification of respiratory chain complexes are known, the following observations strongly suggest a regulation of electron transport and proton translocation independent from regulation by Michaelis–Menten type kinetics, in particular at the third coupling site of the respiratory chain.

The Long-Lasting Discussion on Proton Translocation and H^+/O Stoichiometry of Cytochrome c Oxidase

Peter Mitchell's chemiosmotic hypothesis implied in its original version (Mitchell, 1966) alternating hydrogen and electron carriers along the respiratory chain of mitochondria. Since cytochrome *c* oxidase is no hydrogen carrier, the enzyme was not assumed to generate a thermodynamic proton potential difference (Moyle and Mitchell, 1978). This view was supported by studies of Papa *et al.* (1980). An opposite view, however, was presented by Wikström, who found in isolated mitochondria (Wikström and Saari, 1977; Wikström and Krab, 1978) and in reconstituted cytochrome *c* oxidase (Krab and Wikström, 1978) a vectorial proton translocation coupled to electron transport with a stoichiometrry of $H^+/O = 2$. The value was corroborated by others with the reconstituted cytochrome *c* oxidase system (Casey *et al.*, 1979; Siegel and Carafoli, 1980; Prochaska *et al.*, 1981; Proteau *et al.*, 1983). Substantially higher H^+/O ratios of up to 4, however, were measured for the cytochrome *c* oxidase reaction in intact mitochondria or mitoplasts (Azzone *et al.*, 1979; Reynafarje *et al.*, 1982; Lemasters *et al.*, 1984).

Nevertheless the proton pumping activity of cytochrome *c* oxidase was principally doubted by Papa *et al.*, who ascribed the observed acidification to scalar protons produced by reaction of reduced cytochrome *c* with cytochrome *c* oxidase (Papa *et al.*, 1983a, b). On the other hand, the high H^+/O ratios above 2, measured in mitoplasts by the group of Lehninger using a fast-responding oxygen electrode (Reynafarje *et al.*, 1982; Costa *et al.*, 1984), were recently criticized by Wikström and coworkers, who argued that the authors underestimated the time constant of their oxygen electrode (Krab *et al.*, 1984). Lehninger *et al.* (1985) could demonstrate, however, that in mitoplasts two types of proton translocation by cytochrome *c* oxidase can be measured, differing in their sensitivity to dicyclohexylcarbodiimide. The authors suggest: "that the H^+/O ratio of cytochrome oxidase may be under biological regulation, so that its H^+ stoichiometry may be either 2 or 4 per O reduced, depending upon intracellular conditions" (Lehninger *et al.*, 1985).

The Unexpected Linear Relationship Between Flow and Force for the Steady State Far from Equilibrium

Respiring mitochondria operate far from thermodynamic equilibrium. Therefore nonequilibrium thermodynamics (Prigogine, 1961; Katchalsky and Curran, 1965) have been applied first by Rottenberg *et al.* (1967, 1970) to describe oxidative phosphorylation on the basis of linear flow–force relations. Since from kinetic considerations flows rise exponentially with increasing forces (Hill, 1977), additional assumptions were required to explain the observed linear relationship between flow and forces for a reaction proceeding far from equilibrium (Rottenberg, 1979), A nontight coupling of electron transport and phosphorylation (Rottenberg, 1979), a molecular slipping in redox and ATPase proton pumps (Pietrobon *et al.*, 1983), or a leak conductance for redox processes, ion transport, and phosphorylation (van Dam *et al.*, 1980) were suggested in order to explain the linear relationship between flow and forces, which results in a much higher efficiency as compared to the nonlinear relationship. Pietrobon *et al.* (1982) concluded that the extension of the linear relationship, pertinent to the domain close to equilibrium, to the domain far from equilibrium implies "complex regulatory mechanisms on the molecular level of the pumps," and speculate that "nature has prolonged the linear domain of the phenomenological equations into a regime far from equilibrium in order to optimize efficiency."

The Variable Degree of Coupling between Respiration and Phosphorylation

A further indication for regulatory properties of mitochondrial energy converters came from theoretical studies on the efficiency of oxidative

phosphorylation. The formalism of nonequilibrium thermodynamics, first applied by Kedem and Caplan (1965) for the definition of efficiency, led Stucki to the calculation of optimal values for the degree of coupling between respiration and phosphorylation according to the energetic needs of the cell (Stucki, 1980). It was found that a fully coupled energy converter is incompatible with optimal efficiency unless all net flows vanish (Stucki, 1980). In a recent study Soboll and Stucki measured the cytosolic and mitochondrial adenine nucleotide contents in rat livers under glucose and oleate perfused conditions. They concluded that fatty acids change the degree of coupling (Soboll and Stucki, 1985). O'Shea and Chappell (1984) measured the protonmotive force and the respiration rate of isolated rat liver mitochondria under various coupled and uncoupled conditions and made the interesting observation that the ratio $\Delta O/\Delta p$ does not have a unique value. Instead they observed that the rate of respiration and the magnitude of the Δp are more sensitive to the agents used for the titration than to each other. This result indicates a regulation of respiration by other factors than Michaelis–Menten type kinetics. It suggests a variable coupling degree of oxidative phosphorylation depending on the substrate.

The Short-Term Effect of Thyroid Hormones on ATP Synthesis in Mitochondria

Thyroid hormones act on the metabolism of most tissues (except, for example, nervous tissue and spleen) mainly by increasing the amount of catabolic enzymes and decreasing the amount of anabolic enzymes (Kadenbach *et al.*, 1964; Kadenbach, 1966a). Beside these long-term effects which are exerted via transcription and translation (Tata, 1970), short-term effects of thyroid hormones have also been described more recently (Sterling *et al.*, 1977; Palacios-Romero and Mowbray, 1979; Corrigall *et al.*, 1984). An increase of the P/O ratio of isolated mitochondria from hypothyroid rats 15 min after triiodothyronine (T_3) injection *in vivo* (Palacios-Romero and Mowbray, 1979), a cycloheximide-insensitive stimulation of hepatocyte oxygen consumption by T_3 (Kaminski, 1980), an increase of oligomycin-sensitive oxygen consumption of perfused livers from hypothyroid rats by T_3 within one hour (Müller and Seitz, 1981), and a concomitant decrease of mitochondrial ATP/ADP ratio at unchanged cytosolic ATP/ADP ratio (Seitz *et al.*, 1985) have been described. *In vivo* hyperthyroidism caused almost doubling of cytosolic, and reduction to about one-third of mitochondrial ATP/ADP ratios (Seitz *et al.*, 1985). Although these data suggest a regulatory effect of thyroid hormones on the adenine nucleotide carrier, measurements of the control strength (Groen *et al.*, 1982) of the translocator in liver mitochondria from normal and thyroidectomized rats indicate another

step of the respiratory chain controlling the rate of respiration and ATP synthesis (Holness *et al.*, 1984).

Stimulation of Respiration by Fatty Acid Oxidation

The stimulatory effect of fatty acids on hepatic oxygen consumption is well known for many years (Berry, 1974; Debeer *et al.*, 1974). The increased formation of acetyl-CoA from fatty acids was found to be independent of the phosphorylation potential (Erecinska *et al.*, 1977; Klingenberg, 1963; Berry *et al.*, 1983). Since fatty acid oxidation does not induce additional ATP-consuming reactions, the increased respiration should be explained by additional energy-requiring processes, if oxygen uptake is strongly coupled to ATP synthesis. In an extensive study with isolated hepatocytes Berry *et al.* (1983) found a 30% stimulation of oxygen uptake but no change of the ATP/ADP ratio by fatty acids. The authors concluded that oxidation of fatty acids to acetyl-CoA is obligatorily associated with a reversed electron transfer in the respiratory chain, originally described by Chance and Hollunger (1961) and by Klingenberg and Schollmeyer (1961) with isolated mitochondria. A reversed electron transfer, however, must not necessarily be assumed, since the increased formation of β-hydroxybutyrate from acetoacetate can easily be explained by the abundant formation of NADH by the hydroxyacyl-CoA dehydrogenase. A much simpler explanation could be given by a partial uncoupling of proton translocation by cytochrome *c* oxidase via allosteric interaction with acyl-CoA.

Hypothesis

We suggest that respiration and ATP synthesis in higher organisms are mainly regulated by allosteric modification of respiratory chain complexes in addition and independent of regulation by isosteric effectors, e.g., substrates (in case of cytochrome *c* oxidase, the reduction state of cytochrome *c*) and respiratory control. The allosteric effectors (ligands) such as substrates, metabolites, cofactors, ions, hormones, and the membrane potential are suggested to bind at specific binding sites on subunits of the respiratory chain complexes, in particular of cytochrome *c* oxidase. Binding of allosteric ligands results in a conformational change of the subunit which is transmitted to the catalytic center of the complex due to tight association of the subunits. Two catalytic activities of cytochrome *c* oxidase are suggested to be affected by allosteric modification: (i) the rate of respiration, e.g., electron transfer from ferrocytochrome *c* to oxygen, and (ii) the H^+/e^- stoichiometry. An additional regulatory principle for respiration and ATP synthesis is suggested

to be realized in higher organisms by expression of tissue-specific, developmental-specific, and hormone-specific isozymes of cytochrome *c* oxidase, based on different nuclear coded but identical mitochondrial coded subunits.

Recent results on the structure and activity of cytochrome *c* oxidase strongly support the hypothesis.

The Molecular Structure of Cytochrome c Oxidase from Different Organisms

The term cytochrome *c* oxidase denotes an enzyme which transfers electrons from mammalian type cytochrome *c* to dioxygen accompanied by electrochemical conservation of energy. Aerobic respiration of all higher organisms and also of some bacteria is indispensably connected with this enzyme, containing two heme *a* and two copper ions as essential prosthetic groups. In contrast to the uniform catalytic features of the enzyme from different organisms, a large variety of protein subunits was found tightly associated to the membrane-bound enzyme complex. Cytochrome *c* oxidase from prokaryotes contains two or three subunits (Poole, 1983); from *dictyostelium discoideum*, six (Bisson *et al.*, 1984); from yeast, nine (Power *et al.*, 1984); and from mammalian sources, thirteen different subunits (Kadenbach *et al.*, 1983a; Kuhn-Nentwig and Kadenbach, 1984), which occur in stoichiometric amounts (Merle and Kadenbach, 1980a). Thus the number of polypeptides in the enzyme complex increases with the evolutionary stage of the organism. Only two subunits were found in cytochrome *c* oxidase of *Paracoccus denitrificans*, which are related to subunits I and II of the enzyme from yeast and bovine heart, as demonstrated by amino acid sequence homology (Steffens *et al.*, 1983). The four redox centers are liganded by subunits I and II in all enzymes (Kadenbach, 1983; Capaldi *et al.*, 1983; but also see Buse *et al.*, 1984), and subunit II was shown to bind the substrate cytochrome *c* (Millett *et al.*, 1982; Bisson *et al.*, 1978). In eukaryotes the three large subunits I–III are coded on the mitochondrial, and the ten smaller subunits on the nuclear genome (Kadenbach, 1983; Kadenbach and Merle, 1981).

The function of the third mitochondrial coded subunit III, which is absent in the *P. denitrificans* enzyme, remained controversial. Because of its extreme hydrophobicity (Thalenfeld and Tzagoloff, 1980; Anderson *et al.*, 1982) the protein tends to aggregate during SDS-gel electrophoresis (Hundt and Kadenbach, 1977) and may not be detected. Some authors have claimed that subunit III may not represent an intrinsic component of cytochrome *c* oxidase (Penttilä *et al.*, 1979; Thomson and Ferguson-Miller, 1983; Saraste *et al.*, 1980). Later Penttilä and Wikström observed, however, that a subunit III-depleted enzyme from beef heart, when reconstituted in liposomes, does

not pump protons under conditions where the unmodified enzyme showed vectorial proton translocation with a stoichiometry of $H^+/e^- = 0.9$. The participation of subunit III in proton translocation was also concluded from the inhibitory effect of dicyclohexylcarbodiimide, which binds covalently to subunit III (Prochaska *et al.*, 1981; Casey *et al.*, 1980). The vectorial proton translocation of cytochrome *c* oxidase appears, however, much more complex, since the isolated enzyme of *P. denitrificans*, which does not have subunit III, can also translocate protons with a stoichiometry of $H^+/e^- = 0.6$ in the reconstituted system (Solioz *et al.*, 1982). This proton translocation is insensitive to dicyclohexylcarbodiimide (Püttner *et al.*, 1983).

The nuclear coded subunits of cytochrome *c* oxidase have not yet been ascribed a specific function, but were suggested to have a regulatory role (Kadenbach, 1983; Kadenbach and Merle, 1981). The occurrence of ten different nuclear coded subunits in mammalian cytochrome *c* oxidase has been clearly demonstrated by *N*-terminal amino acid sequence analysis (Kadenbach *et al.*, 1983b) and by immunological distinction with subunit-specific antisera (Kuhn-Nentwig and Kadenbach, 1984; Kuhn-Nentwig and Kadenbach, 1985a). Nevertheless controversial views as to the number of subunits of mammalian cytochrome *c* oxidase can still be found in the literature (Thompson and Ferguson-Miller, 1983; Capaldi *et al.*, 1983; Saraste, 1983), partly due to the difficulty in separating all 13 subunits by SDS-gel electrophoresis (Kadenbach *et al.*, 1983a).

The nuclear coded subunits are tightly associated within the enzyme complex as demonstrated by immunoprecipitation of all thirteen subunits from a total soluble Triton X-100 extract of rat liver mitochondria with a monospecific antiserum against subunit IV (Merle *et al.*, 1981). Using cross-linking reagents, Jarausch and Kadenbach (1985a) analyzed the structural arrangement of subunits within the rat liver enzyme complex. From these data a tight association of nuclear coded subunits with the catalytic subunits I–III was concluded. The orientation of subunits in the membrane was investigated with mitoplasts by proteolytic digestion and immunoblotting (Jarausch and Kadenbach, 1985b; Kuhn-Nentwig and Kadenbach, 1985b). Most nuclear coded subunits were found to be oriented to the cytosolic face of the inner membrane, surrounding the binding site for cytochrome *c* at subunit II.

The Occurrence of Multiple, Tissue-Specific Forms of Mammalian Cytochrome c Oxidase

An indication for a regulatory role of nuclear coded subunits was derived from the discovery of different, tissue-specific forms of these proteins (Merle and Kadenbach, 1980b; Kadenbach *et al.*, 1981) which were assumed

to be encoded on different genes (Kadenbach *et al.*, 1982). Between five corresponding nuclear coded subunits of cytochrome *c* oxidase from pig liver and heart, different *N*-terminal amino acid sequences were found (Kadenbach *et al.*, 1983b). The differences were corroborated by immunological methods (Jarausch and Kadenbach, 1982). With a quantitative nitrocellulose ELISA, for nine nuclear coded subunits differences were detected between various tissues of adult rats, and also between the same tissue of adult and fetal rats (liver, heart, skeletal muscle) (Kuhn-Nentwig and Kadenbach, 1985a).

A functional implication of tissue-specific isozymes of cytochrome *c* oxidase was derived from studies on mapping of the binding domain for cytochrome *c*. Cytochrome *c* oxidase from pig liver, heart, diaphragm, and kidney was labelled with radioactive glycine ethyl ester and the water-soluble 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide in the presence and absence of cytochrome *c*. In addition to subunit II, the known binding site for cytochrome *c* (Millett *et al.*, 1982; Bisson *et al.*, 1978), a cytochrome *c*-protected labeling of nuclear coded subunits was found (Kadenbach and Stroh, 1984; Kadenbach *et al.*, 1985). This cytochrome *c*-protected labeling was different in liver or kidney (subunits II, VIa, VIc, VIIa, and VIII) and heart or diaphragm (II and VIc). It was concluded that the binding domain for cytochrome *c* at subunit II of cytochrome *c* oxidase from different tissues is modified by tissue-specific nuclear coded subunits.

Direct kinetic differences between cytochrome *c* oxidase from bovine liver and heart were demonstrated with the membrane-bound, dissolved, and isolated enzymes (Merle and Kadenbach, 1982). These results were corroborated with the reconstituted bovine liver and heart enzymes (Büge and Kadenbach, unpublished results). In addition, the involvement of nuclear coded subunits in the catalytic activity was demonstrated by changed kinetic properties of reconstituted bovine heart cytochrome *c* oxidase after trypsin treatment, which specifically cleaved only subunit VIa and half of subunit IV (Büge and Kadenbach, 1985).

The Variable Catalytic Activity of Isolated Cytochrome c Oxidase

It is well known that the activity of isolated cytochrome *c* oxidase varies to a large extent depending on the detergent used for solubilization, the concentration and type of applied buffer ions, the method of activity measurement, the presence of lipids, and the isolation method. In Fig. 1 is presented the effect of increasing concentrations of nucleotides, phosphate, and KCl on the activity of isolated pig heart cytochrome *c* oxidase, measured polarographically in the presence of lauryl maltoside. Although for all anions a maximal activity is observed at about the same ionic strength, phosphate

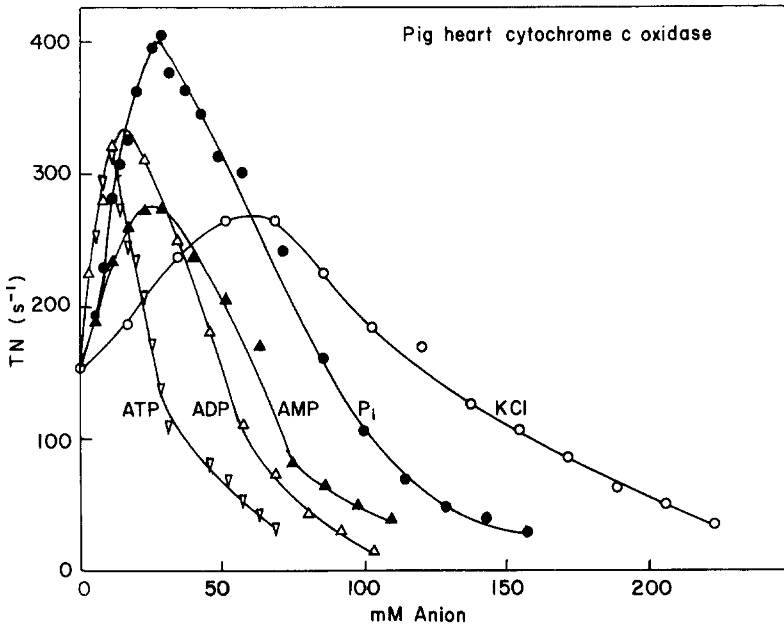

Fig. 1. Effect of various anions on the activity of isolated pig heart cytochrome *c* oxidase. The activity was measured polarographically at 25°C in 25 mmol Tris acetate, pH 7.6, with the given concentration of the indicated anion, 7 mmol Tris ascorbate, 0.7 mmol TMPD, 0.014 mmol EDTA, 1 mmol lauryl maltoside, 40 μmol cytochrome *c*, and 20 nmol cytochrome *c* oxidase.

stimulates the enzyme to the largest extent, whereas AMP and KCl have the smallest stimulatory effect. Since half-maximal stimulation of activity occurs at about physiological concentrations of phosphate and the nucleotides, a regulation of activity by these anions cannot be excluded.

Regulation of enzyme activity requires that the normal activity be far below the maximal activity. This may be concluded for cytochrome *c* oxidase from Fig. 2, where the activity was measured with ascorbate, TMPD, and cytochrome *c* in uncoupled rat liver mitoplasts before and after the addition of increasing amounts of lauryl maltoside. Addition of 1 mmol detergent stimulates the activity fivefold, leading to a molecular turnover of more than 1000 per second at 30°C. The parallelism between "denaturation" of the physiological environment of cytochrome *c* oxidase and stimulation of activity is also evident from Table I, where the molecular activity of the liver enzyme *in situ*, in the perfused organ, in isolated hepatocytes and in isolated mitochondria is compared. The about hundredfold turnover number of the detergent-solubilized enzyme as compared to the enzyme *in vivo* indicates the large regulatory capacity of cytochrome *c* oxidase.

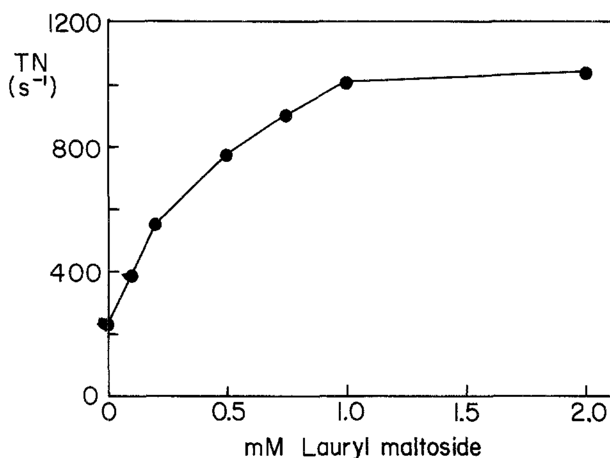

Fig. 2. Increase of cytochrome *c* oxidase activity of isolated mitoplasts by addition of lauryl maltoside. Cytochrome *c* oxidase activity of rat liver mitoplasts, isolated by swelling shrinking (Jarausch and Kadenbach, 1985b), was measured at 30°C polarographically in 250 mmol sucrose, 40 mmol KCl, 10 mmol KP_i, 10 mmol Hepes, pH 7.2, 4 mmol $MgCl_2$ with 7 mmol ascorbate, 0.7 mmol TMPD, and 40 μmol cytochrome *c* as substrate, in the presence of uncoupler (0.8 μmol valinomycin and 9 μmol FCCP) and inhibitors (0.1 μmol antimycin A, 1.0 μmol rotenone, 1 μg/ml oligomycin, 40 μmol mersalyl, and 5 μmol carboxyatractyloside). The indicated amount of lauryl maltoside was added after about 2 min preincubation. The turnover number (TN, mole cytochrome *c* · mole cytochrome *c* oxidase^{-1} · s^{-1}) was calculated based on a heme *a* content of 0.25 nmol · mg protein^{-1} in mitoplasts.

Regulation of Cytochrome c Oxidase by Allosteric Modification

Regulation of energy metabolism by allosteric modulation of key enzymes of glycogen degradation, glycolysis, and citric acid cycle is well established (Cohen, 1983). Allosteric regulation of respiratory chain enzymes has not yet been demonstrated due to the difficulty of isolation and reconstituting the physiological conditions for asymmetric oriented membrane proteins. We suggest that evolution and differentiation of organisms was accompanied by an increase in the regulatory capacity of cytochrome *c* oxidase (Kadenbach, 1983) and explain the increase in the number of nuclear coded subunits from none in prokaryotes over three (*D. discoideum*) and six (yeast) to ten in mammals by acquisition of specific binding sites (receptors) for metabolites, hormones, second messengers, and other intracellular signals. These subunits are suggested to function by transmitting the signal of allosteric ligands to the catalytic center of the complex via conformational change. Therefore a tight association of the regulatory with the catalytic subunits is essential. This view is supported by the unexpected finding that the sequence of mammalian mitochondrial genomes evolve very fast, more than twice the rate for silent substitutions in mammalian globin genes

Table I. Molecular Turnover of Rat Liver Cytochrome *c* Oxidase under Various Conditions[a]

State of integration	Method	Conditions	Temperature (°C)	Turnover number ($mol\, e^- \cdot s^{-1} \cdot mol\, aa_3^{-1}$)	Reference
Liver, in situ	Reflectance spectrophotometric	Pressed liver	37	20–26	Sato *et al.* (1979)
Liver, perfused	Oxygen electrode	Fed liver, noncirculating perfusion, cyanide-sensitive O_2 uptake	37	13	Kimmig *et al.* (1983)
Hepatocytes	Oxygen electrode	10 mmol lactate, 10 mmol NH_4Cl	37	86	Stubbs *et al.* (1978)
		10 nmol dihydroxyacetone	37	36	Stubbs *et al.* (1978)
Mitochondria	Oxygen electrode	Oxoglutarate, state 3	30	11	Yamasaki (1975)
		Succinate, state 3	30	38	Yamazaki (1975)
		Ascorbate + TMPD state 3	30	56	Yamazaki (1975)
		Ascorbate + TMPD + CCCP	30	65	Yamazaki (1975)
Mitoplasts	Oxygen electrode	Ascorbate + TMPD + FCCP + valinomycin	30	220	This paper
		Ascorbate + TMPD + 1 mmol lauryl maltoside + FCCP + valinomycin	30	1015	This paper

[a] The data were calculated assuming 60 mg of mitochondrial protein per gram wet weight (Scholz, R., and Bücher, Th., 1965) and a mitochondrial cytochrome aa_3 content of 0.125 nmol per milligram protein.

(Anderson *et al.*, 1982), despite the fact that evolution of some regions of subunits I and II has remained extremely conservative (Prochaska *et al.*, 1981). Conformational interaction of regulatory with catalytic proteins requires an optimal fit, which is maintained during evolution only by coevolution.

We postulate that allosteric effectors of cytochrome *c* oxidase have only two ultimate effects: variation of the rate of respiration and the degree of coupling. We assume that the variations are rather small, and therefore have not yet been described with the isolated enzyme, although they have profound physiological consequences. Stucki (1980) calculated that the optimal values for the degree of coupling (q values between 0 and 1) are $q = 0.972$ for maximal output power of oxidative phosphorylation at optimal efficiency and minimal energy costs, and $q = 0.953$ for maximal net flow of ATP synthesis at optimal efficiency and minimal energy costs. Accordingly changes of 20–50% in the rate of respiration will largely influence the overall metabolism of a cell. As discussed above (Table I) the isolated cytochrome *c* oxidase can have a hundredfold molecular turnover than *in situ*. Therefore our presently available methods did not allow us to detect the regulatory properties of cytochrome *c* oxidase.

The tissue-specific occurrence of most nuclear coded subunits can be explained by (a) the occurrence of some signals (metabolites) only in certain tissues, and (b) the necessity to have in different tissues a different response by the same signal. Nature has assured the uniform catalytic activity of cytochrome *c* oxidase by coding subunits I–III on mitochondrial DNA, which is assumed to be identical in all cells of an organism (Hayashi *et al.*, 1978; Giles *et al.*, 1980). The diversity of regulatory properties of the enzyme is achieved by coding the regulatory subunits in the nucleus, which is amenable to hormonal, developmental and other kinds of regulation of protein expression.

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