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REVIEW

Glycation, Glycolysis, and Neurodegenerative Diseases: Is There Any Connection?

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Abstract—This review considers the interrelation between different types of protein glycation, glycolysis, and the development of amyloid neurodegenerative diseases. The primary focus is on the role of the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase in changing the concentration of carbonyl compounds – first and foremost, glyceraldehyde-3-phosphate and methylglyoxal. It has been suggested that various modifications of the enzyme – from the oxidation of the sulfhydryl groups of the active site to glycation with sugars – can lead to its inactivation, which causes a direct increase in glyceraldehyde-3-phosphate concentration and an indirect increase in the content of other aldehydes. This "primary inactivation" of glyceraldehyde-3-phosphate dehydrogenase promotes its glycation with aldehydes, including its own substrate, and a further irreversible decrease in its activity. Such a cycle can lead to numerous consequences – from the induction of apoptosis, which is activated by modified forms of the enzyme, to glycation of amyloidogenic proteins by glycolytic aldehydes. Of particular importance during the inhibition of glyceraldehyde-3-phosphate dehydrogenase is an increase in the content of the glycating compound methylglyoxal, which is much more active than reducing sugars (glucose, fructose, and others). In addition, methylglyoxal is formed by two pathways – in the cascade of reactions during glycation and from glycolytic aldehydes. The ability of methylglyoxal to glycate proteins makes it the main participant in this protein modification. We consider the effect of glycation on the pathological transformation of amyloidogenic proteins and peptides – β-amyloid peptide, α-synuclein, and prions. Our primary focus is on the glycation of monomeric forms of these proteins with methylglyoxal, although most works are dedicated to the analysis of the presence of "advanced glycation end products" in the already formed aggregates and fibrils of amyloid proteins. In our opinion, the modification of aggregates and fibrils is secondary in nature and does not play an important role in the development of neurodegenerative diseases. The glycation of amyloid proteins with carbonyl compounds can be one of the triggers of their transformation into toxic forms. The possible role of glycation of amyloidogenic proteins in the prevention of their modification by ubiquitin and the SUMO proteins due to a disruption of their degradation is separately considered.

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Many works have been devoted to protein glycation, including reports and reviews on the possible implications of this process in the development of neurodegenerative diseases [1-5]. It is impossible to discuss all such works in one review, and it would be hardly practical. We will focus on two aspects that are close to our own line of research. First, we will consider how glycation can affect glycolysis, in the first instance by inhibiting glyceraldehyde-3-phosphate dehydrogenase, and how change in the rate of glycolysis can alter the effectiveness of glycation of various proteins. Hence, we will mainly focus on glycation by carbonyl compounds, which are characterized by rapid and effective modification of proteins, in contrast to reducing sugars (glucose, fructose, and others). Second, we will discuss the possible influence of this type of glycation of some amyloidogenic proteins on their pathological transformation.

Glycation is a nonenzymatic modification of proteins by various sugars. The most widely known is glycation of proteins by reducing sugars. However, it should be noted that glycation of proteins with sugars (glucose, ribose, fructose, and their derivatives) is not very efficient and requires a prolonged incubation time, preferably at higher temperatures. In the case of food industry, cooking, etc.,

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this type of glycation is certainly important, especially if one considers the possible influence of glycated proteins on proteins and microorganisms of the gastrointestinal tract [6-10]. However, for intracellular processes, glycation by reducing six- and five-carbon sugars is of less importance, since it requires a long time or high temperatures. It is of course possible to find quite a few studies in which, in diabetes, it was possible to detect advanced glycation end products in proteins, including amyloid inclusions characteristic of various neurodegenerative diseases. However, it is difficult to say unambiguously whether the observed modifications are the cause of neurodegenerative diseases, or the modification of amyloid inclusions occurs after their formation and is not associated with the development of the disease. In our opinion, it is much more important to study protein glycation by such active carbonyl compounds as methylglyoxal, glyceraldehyde-3-phosphate, and other aldehydes whose modification efficiency is several orders of magnitude higher than that of reducing sugars. Of importance is the study of protein glycation by glyceraldehyde-3-phosphate, which is an intermediate product of glycolysis always present in cells and able to modify proteins efficiently. Despite this, for several subjective reasons, its participation in the glycation of proteins is practically unstudied.

BASIC MECHANISMS OF GLYCATION

Before switching to the overview of the role of glycation in the regulation of glycolysis and in the modification of amyloid proteins, we will briefly discuss various types of glycation. Glycation of proteins with reducing sugars, for example, glucose, is the most widely known type. Although this process is called a reaction, it is more like a complex cascade of transformations. To date, the generally accepted scheme of the reaction is the John Hodge diagram (Fig. 1), with some subsequent additions [11] in which the process is divided into seven main stages.

During the first stage, a nonenzymatic condensation reaction occurs between the α-amino or N-terminal protein group and the carbonyl group of a reducing sugar (stage 1 in Fig. 1). Then, in a reversible nucleophilic addition reaction, a Schiff's base is quickly formed. Further, over a period of weeks, a slow rearrangement of the Schiff base leads to the formation of a stable ketoamine, the Amadori product (stage 2 in Fig. 1). Finally, after dehydration and rearrangement, as well as the formation of bonds with adjacent proteins, "advanced glycation end products" (AGEs) are formed. The term "glycation" is used in a broad sense: it can refer both to the formation of Schiff bases (N-substituted imines) with an amino acid residue in a protein and/or to the formation of Amadori products, to the formation of a bond between amino acids and dicarbonyl degradation products, to the synthesis of advanced glycation end products.

Under oxidizing conditions in the presence of free radicals, decay products such as hydroxyacetone and diacetyl can be formed from the Schiff base obtained in the first step (stage 5 in Fig. 1). The Amadori product is further decomposed or auto-oxidized along three possible routes: furfurals are formed under acidic conditions; under alkaline conditions, reductones (compounds with dienol and neighboring carbonyl groups) are formed; compounds with carbonyl and hydroxycarbonyl groups similar to stage 5 products are formed in stage 4. The latter is mainly realized under oxidizing conditions and leads to the formation of highly reactive intermediate dicarbonyl compounds (glyoxal, methylglyoxal, glycolaldehyde, etc.).

In the next stage of the reaction, in the absence of amino acids, an aldol condensation of furfurals, reductones, and aldehydes occurs, by which aldols and nitrogen-free polymers are synthesized (stage 7 in Fig. 1). With the addition of amino acids, intermediate products such as α-amino ketones and aldehydes are formed (Strecker degradation).

Finally, due to the interaction of amino acids with the intermediate compounds from stages 3, 4, and 5, "advanced glycation end products" are formed (stage 8 in Fig. 1).

The main sites for protein glycation are the side chains of arginine and lysine residues, the amino groups of N-terminal amino acid residues, as well as the thiol groups of cysteine residues. The reaction proceeds differently depending on the concentration and reactivity of the glycating agent. All reducing sugars can take part in glycation, but the activities of D-ribose and reactive carbonyl compounds are much higher [13]. The reaction flow is also influenced by degree of saturation of the medium with oxygen, pH, temperature, presence of metal ions, and the degree of protein unfolding [14, 15]. The availability of residues undergoing glycation for modification and the pK of neighboring amino acids are other influencing factors [16]. We especially note that during the complex cascade of reactions during glycation, methylglyoxal is formed, the compound that is most often used to modify proteins to reproduce processes occurring *in vivo*.

INFLUENCE OF CARBONYL COMPOUNDS ON GLYCOLYSIS

As noted in the previous section, when glycation takes place, carbonyl compounds are formed that are characterized by high reactivity and ability to modify proteins effectively. To start with, these include methylglyoxal, as well as glycolaldehyde. It is worth noting that this is not the only route of their formation. Glycolysis can be another source of these aldehydes, which can be seen from the diagram shown in Fig. 2. Moreover, during gly-

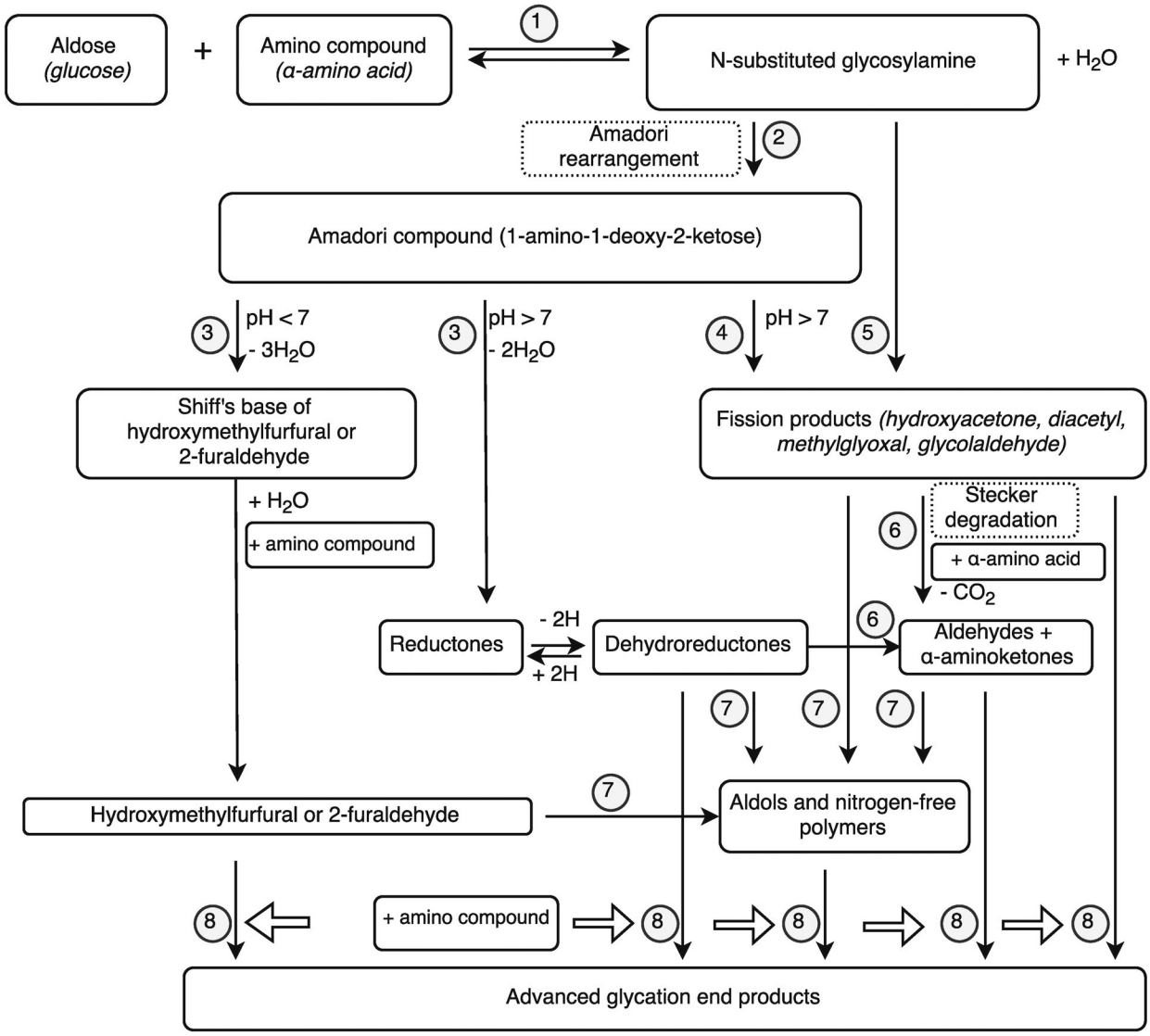

Fig. 1. Step-by-step scheme of the course of glycation reactions based on the Hodge diagram. Adapted from [11, 12]. Numbers in circles denote the main stages of the reaction.

colysis, glyceraldehyde-3-phosphate and dihydroxyacetone phosphate are formed, which not only can be converted into methylglyoxal, but can also independently glycate proteins. It is obvious that an increase in methylglyoxal content, as well as glycolytic aldehydes, may be due to inhibition of glucose flux downstream of triose phosphates, for example, the subsequent glycolysis reactions or the tricarboxylic acid cycle. However, the most likely cause of accumulation of aldehydes is the inhibition of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which uses glyceraldehyde-3-phosphate as the substrate.

Obviously, any inhibition of glycolysis on the level of glycolytic oxidoreduction catalyzed by GAPDH should result in the accumulation of glyceraldehyde-3-phosphate, methylglyoxal, and other aldehydes that can glycate various proteins, including GAPDH. The main triggering mechanism for reducing GAPDH activity is oxidative stress. Active oxygen forms oxidize the sulfhydryl groups of cysteine residues in GAPDH active site, which leads to reversible inactivation of the enzyme during early stages. Many studies have been devoted to investigation of inactivation of GAPDH by active oxygen forms and other compounds reacting with the sulfhydryl groups of the active site. Nevertheless, usually these processes have not yet been connected with their possible influence on the formation of carbonyl compounds. However, if we assume that GAPDH glycation leads to its inactivation, a "vicious cycle" is formed. Thus, the more GAPDH activity decreases, the higher the concentrations of glyceraldehyde-3-phosphate and methylglyoxal, which glycate GAPDH, further reducing its activity. There is no doubt that GAPDH activation is also possible, primarily by

small-molecule thiols (glutathione and cysteine), which should lead to a decrease in the concentration of various aldehydes. Yet there is no specific information on the decrease in the concentration of glyceraldehyde-3-phosphate and methylglyoxal due to the activation of the enzyme.

In summary, formation of carbonyl compounds not only occurs as a result of various transformations during protein glycation, but also in glycolysis. A special role can be played by glyceraldehyde-3-phosphate formed in the course of glycolysis. Since GAPDH is directly involved in the regulation of the content of this compound, it makes sense to consider its glycation in greater detail.

GLYCATION OF GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE

There are several reasons to consider the glycation of GAPDH with particular attention. First, as noted in the previous section, it is this glycolytic enzyme that participates either directly or indirectly in the formation of carbonyl compounds, especially glyceraldehyde-3-phosphate and methylglyoxal. Consequently, along with the reasons described above, the change in GAPDH activity as a result of glycation should change the content of the aforementioned aldehydes in a cell. Assuming that glycation leads to inactivation of GAPDH, it will result in a direct increase in the concentration of glyceraldehyde-3-phosphate, and in the increase in methylglyoxal content through a chain of reactions. The second reason is connected to the fact that GAPDH concentration in the cytoplasm is extremely high and reaches 5-15% of the total soluble protein content, which makes this enzyme one of the main targets for glycation. Finally, the third reason resides in the fact that there is more and more information about the additional functions of this unusual enzyme involved in literally all vital activities, including their pathology. Therefore, the change in GAPDH properties as a result of its glycation should also affect such additional functions.

Obviously, since glycation processes considered in this review are nonenzymatic, it cannot be specific, and all proteins containing amino acid residues that are suitable for modification should be subjected to glycation to some extent. Naturally, GAPDH can be easily glycated *in vitro* by different compounds. Therefore, the first objective that arose in the study of GAPDH glycation was to

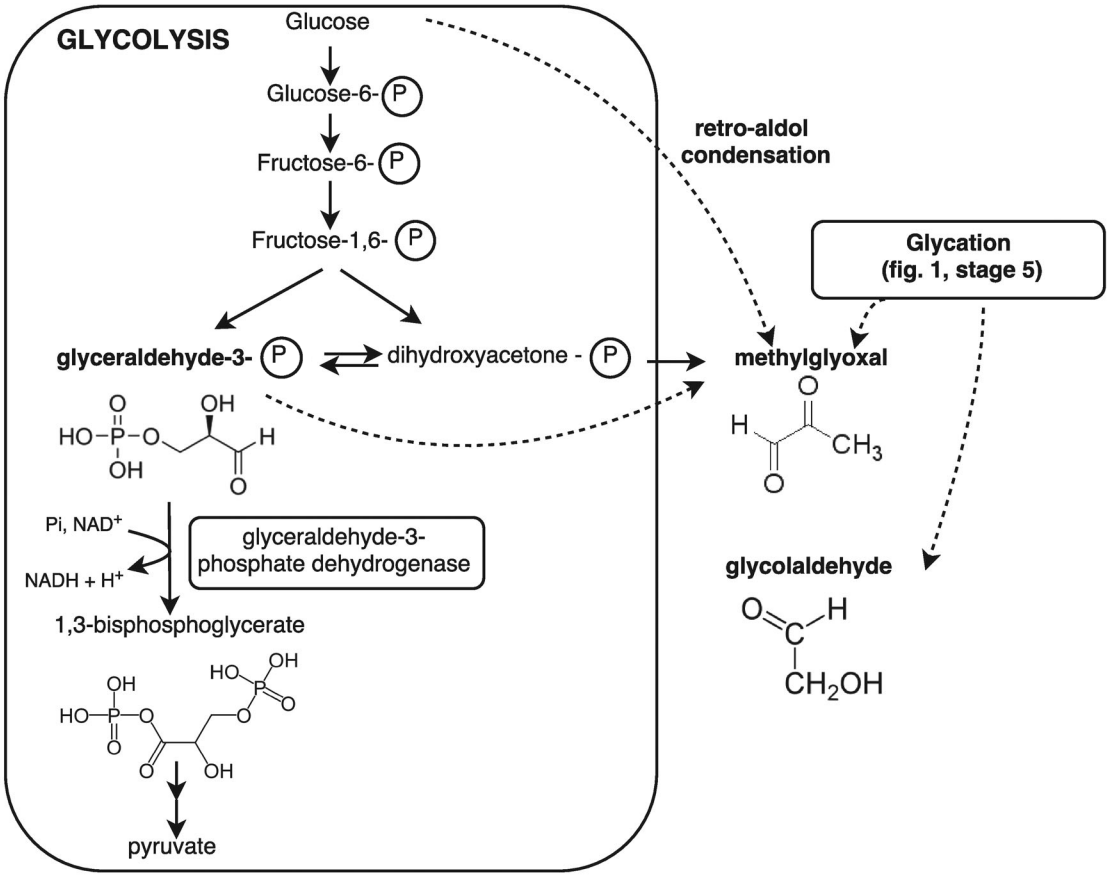

Fig. 2. Pathways of formation of highly reactive carbonyl compounds in the cell. The original scheme includes common knowledge on the stages of glycolysis and data on the formation of methylglyoxal from triose phosphates [17].

prove the presence of glycated enzyme forms in the cell. These studies, conducted about 20 years ago, demonstrated that along with the usual unmodified GAPDH, a glycated form of the enzyme can be isolated from rabbit skeletal muscles and human erythrocytes. Glycated GAPDH contained about two sugar residues per tetramer of the enzyme and had significantly lower catalytic activity. Glycation did not affect the amino acid residues taking direct part in the catalytic act. However, the glycated enzyme was less stable and had increased sensitivity of the active site to various exposures. It was suggested that the amino acid residues located near the active site undergo changes that alter the conformation of the active site, thereby reducing the catalytic activity of the enzyme [18, 19].

So, what kinds of compounds can glycate GAPDH? In principle, reducing sugars can modify the enzyme, but the effectiveness of these processes is not great. In the first reports, it was observed that prolonged incubation in the presence of 1 M glucose had practically no effect on GAPDH activity either in isolated preparations of the enzyme or in cell lysates [20]. However, the absence of any effects at very high glucose concentrations, hundreds of times higher than physiological, raises doubts about the correctness of these results. In subsequent studies, the influence of glucose was investigated under conditions close to physiological. In this way, it was shown that when growing rat embryos in the presence of 30 mM glucose, GAPDH activity was decreased by 40-50% compared to the control, with a significant increase in embryonal developmental defects. Addition of acetyl-cysteine together with glucose increased GAPDH activity and reduced the level of developmental defects to values typical for normal glucose concentration. Perhaps a decrease in GAPDH activity as a result of glycation can occur not only due to amino group modification, but also due to oxidation of the SH-groups of the enzyme with active oxygen forms that are formed during glycation [21]. Similar efficiency of GAPDH glycation by glucose was confirmed in our work [22].

GAPDH is more efficiently glycated by fructose [23], as well as by fructose-6-phosphate and glucose-6-phosphate, which are natural products of glucose metabolism [24].

Thus, GAPDH glycation by different sugars takes place and affects the activity of this enzyme. However, the effectiveness of GAPDH modification by carbonyl compounds methylglyoxal, glycolaldehyde, and dihydroxyacetone, causing enzyme inactivation, is substantially higher than that of reducing sugars. The possibility of GAPDH modification by methylglyoxal over a wide range of concentrations was studied in significant detail. It was demonstrated that at very high (millimolar) concentrations of methylglyoxal, up to 50 a.a. of the enzyme are modified. This modification is accompanied by an increase in the molecular weight of the protein (up to 40 kDa instead of 36 kDa for GAPDH monomer), a decrease in the isoelectric point (from 8.5 to 7.5), and, of course, almost complete loss of enzymatic activity [25]. At the same time, even low concentrations of methylglyoxal, characteristic of *in vivo* as well, have a similar effect on GAPDH, albeit on a smaller scale [25]. GAPDH is very sensitive to chemical modification by methylglyoxal due to the high reactivity of the Cys149 residue as well as of the arginine and lysine that are located within the active site in the immediate vicinity of Cys149. Various small molecule aldehydes and carbonyl compounds selectively react with these amino acid residues [20, 26, 27]. It is believed that glycation by methylglyoxal can lead to a change in GAPDH structure due to the modification of lysine and arginine residues (and possibly cysteine) of the active site, which causes inhibition of the enzyme.

As previously noted, methylglyoxal can be formed during glycolysis in the process of dephosphorylation of dihydroxyacetone phosphate and glyceraldehyde-3-phosphate. Along with aldolase and triosephosphate isomerase, the content of these metabolites is regulated by GAPDH activity, which converts glyceraldehyde-3-phosphate to 1,3-diphosphoglycerate. It has been shown that a decrease in GAPDH activity leads to an increase in the production of methylglyoxal both *in vitro* and *in vivo* [28, 29].

Hence, glycation of proteins, including GAPDH, by methylglyoxal is quite well researched, but glycation of proteins by glyceraldehyde-3-phosphate has remained practically unexplored until our works. The lack of interest in glycation of proteins by glyceraldehyde-3-phosphate was due to practical reasons – its high cost and instability. It is obvious that the change in GAPDH activity should primarily influence the concentration of this metabolite, glyceraldehyde-3-phosphate being its substrate. In addition, the probability of modification of this particular dehydrogenase by glyceraldehyde-3-phosphate is increased due to the compartmentalization of glycolytic enzymes. The existence of various glycolytic enzyme complexes, including complexes between aldolase supplying glyceraldehyde-3-phosphate, and GAPDH, is still under question. However, there is no doubt that these two enzymes are not evenly distributed in the cell, but are brought closer together, primarily due to adsorption on various structural elements. Consequently, the formation of glyceraldehyde-3-phosphate in the aldolase reaction can lead to its involvement in GAPDH glycation, especially when it is impossible to use it as a substrate due to inhibition of the activity of this enzyme. At the same time, based on a comparison of the chemical properties of two aldehydes, efficiency of glycation by glyceraldehyde-3-phosphate should not be lower than that of methylglyoxal. In our work, it was shown that incubation of GAPDH for one day in the presence of 1 mM glyceraldehyde-3-phosphate caused a 90% decrease in the activity of the enzyme. The addition of small molecule thiols (β-

mercaptoethanol or reduced glutathione) protected the enzyme from inactivation only partially. Glycation led to a significant decrease in thermal stability of the enzyme and stimulated its aggregation [22].

It should be noted that the effectiveness of glycation is directly related to the conformational state of the proteins being modified. This was exemplified in the case of GAPDH and aspartate aminotransferase when they were modified with methylglyoxal and glyceraldehyde. The efficiency of advanced glycation end products formation was significantly higher in the case of denatured rather than native proteins [30].

It can be assumed based on a comparison of the effectiveness of glycation by different compounds that an increase in sugar concentration, for example in diabetes mellitus, serves as a trigger mechanism for glycation. There are two possible pathways. On one hand, glucose and other sugars can directly glycate GAPDH, albeit in minimal capacity, and inhibit its activity, thereby increasing the concentrations of various aldehydes participating in or linked to glycolysis. On the other hand, similar outcome can be caused by an increase in the concentration of sugars taking part in the first stages of glycolysis. Such an increase should cause an upsurge in the concentration of glycolytic aldehydes, which can modify and inactivate GAPDH.

MODIFICATION OF AMYLOIDOGENIC PROTEINS BY GLYCATING ALDEHYDES

The development and progression of neurodegenerative diseases can clearly be associated with various types of protein glycation. Most information has accumulated about the presence of advanced glycation end products in various amyloid structures. For example, there are many works that used immunochemical approaches devoted to the colocalization of advanced glycation end products and various structures formed by amyloidogenic proteins (α-synuclein, β-amyloid peptide, tau protein, prions, etc.). Such colocalization is characteristic, for example, of senile plaques, fibrils, and Lewy bodies. It was also found that as a result of glycation and subsequent transformations of the formed products, the properties of the amyloid structures significantly change. First of all, this is due to the fact that highly reactive glycation products form crosslinks between polypeptide chains of amyloid proteins.

As a matter of course, the accumulation of glycated amyloid proteins is facilitated by the fact that amyloid structures exist in an organism for a very long time. Moreover, the stability of these structures removes them from the usual protein life cycle since they are practically inaccessible to conventional protein degradation systems, including chaperones and proteasomes. In our opinion, glycation of already formed amyloid structures does not deserve the attention that is given to it in the literature. This in fact resembles a postmortem modification of already nonfunctional structures, which has little significance for the development, and, even more so, for the onset of the disease. This view is consistent with current ideas about the status of fibrillar amyloid structures in the pathogenesis of neurodegenerative diseases. Many researchers believe that the formation of large amyloid structures (for example, amyloid plaques) allows concentrating toxic amyloid proteins within them, thus eliminating their possible harmful effects on nerve cells. Given such a view of these events, modification by advanced glycation end products may even have a positive effect on the development of the disease, stabilizing amyloid aggregates.

GLYCATION OF α-SYNUCLEIN AND PARKINSON'S DISEASE

It is now generally accepted that glycation of alpha-synuclein is an important factor that stimulates its aggregation, the subsequent formation of Lewy bodies, and the development of Parkinson's disease [31]. In most of the studies, the presence of advanced glycation end products in various structures of nerve tissues characteristic of neurodegenerative diseases was investigated. The first study of the relationship between glycation and neurodegenerative diseases was conducted on brain tissue from patients with Parkinson's disease. The presence of advanced glycation end products in Lewy bodies, especially in their peripheral part, was revealed [32, 33]. Later it was shown that in the cerebral cortex, tonsils, and substantia nigra of patients with early Parkinson's disease the content of advanced glycation end products was increased, although no α-synuclein aggregates were found [34]. At the same time, glycated proteins are also present in the substantia nigra of the cerebral cortex of healthy people, but the glycation level is higher in patients with Parkinson's disease.

Rather many works are devoted to α-synuclein glycation *in vitro*. α-Synuclein contains 15 lysine residues, which are all potential sites for glycation. Due to the absence of cysteine residues in the α-synuclein molecule, glycation by cysteine residues, which plays an important role in the transformation of a number of proteins, cannot occur. It has been shown that α-synuclein glycation affects the nucleation of protein aggregates and causes oligomerization of α-synuclein, stabilizing oligomers [35]. In addition, in the presence of advanced glycation end products, the aggregation of recombinant human α-synuclein *in vitro* was accelerated because of crosslinks formation, while the concentration of reactive oxygen species increased [36]. Advanced glycation end products may participate in the formation of crosslinks and elevate the resistance of α-synuclein to proteolysis [32].

α-Synuclein also undergoes glycation in the presence of D-ribose ("ribosylation") leading to the formation of aggregates that resemble globules. These aggre-

gates were found to be highly toxic and significantly slow the growth of SH-SY5Y cells [37].

The influence of intermediate glycation products on the development of synucleopathies is best studied by the example of methylglyoxal and glyoxal. Several studies have shown that in the presence of dicarbonyl compounds, mixtures of monomers, oligomers, and fibrils of α-synuclein are formed [38]. Moreover, although the addition of methylglyoxal and glyoxal stimulated the aggregation of α-synuclein, it slowed the formation of fibrils [38]. In the presence of dicarbonyl compounds, the aggregates were spherical or formed atypical short fibrils. Perhaps such a structure of aggregates hinders further aggregation and makes the formation of mature fibrils difficult.

The fact that α-synuclein glycation leads to the formation of stable oligomers and inhibits fibrillation can be very important for the study of synucleinopathies. Recent *in vivo* studies indicate that α-synuclein oligomers are more toxic than larger aggregates, and possibly play a major role in the development of synucleinopathies [31, 39, 40].

Does diabetes mellitus affect the development of Parkinson's disease? According to some studies, diabetes can be a risk factor for the development of Parkinson's disease [41-43], in some cases leading to a more severe course of the disease [44, 45]. The concentration of methylglyoxal in the blood is increased in diabetes patients [44, 45]. In addition, patients with Parkinson's often suffer from diabetes [46]. However, the results of meta-analysis indicate an insufficient amount of data for an unambiguous conclusion [47-49].

To study the possible role of diabetes in the development of Parkinson's disease, animal models with hyperglycemia due to a high-fat diet were proposed [50]. With this model, a sharp 34-fold increase in the formation of advanced glycation end products in the brain (mainly in the *substantia nigra*) was found [50]. In the modeling of Parkinson's disease in rats by administering various chemicals (6-hydroxydopamine and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), a high-fat diet increased the loss of dopaminergic neurons in the *substantia nigra* and the striatum [51, 52]. It is likely that a diet high in fat can increase the risk of Parkinson's disease due to the development of hyperglycemia. As mentioned above, GAPDH plays an important role in regulating the concentration of methylglyoxal. Perhaps different types of single nucleotide polymorphisms in the GAPDH gene leading either to an increase or to a decrease in the risk of developing Parkinson's disease are due to this function of GAPDH [53].

GLYCATION AND ALZHEIMER'S DISEASE

Hypotheses about the participation of advanced glycation end products in the development of Alzheimer's disease were first suggested in the mid-1990s [54]. As stated above, the stability of protein structures, of which long-lived intracellular neurofibrillary tangles and extracellular amyloid plaques consist, make them ideal substrates for glycation. Although glycation takes a long time, even at normal glucose concentrations it eventually leads to the synthesis of advanced glycation end products. Most of the studies on the relationship between glycation and the development of Alzheimer's disease have been devoted to identifying advanced glycation end products in various nerve tissues [55]. Intracellular deposits of advanced glycation end products with perinuclear localization in the pyramidal neurons of the human brain were found. Starting from the second decade of life, vesicles containing advanced glycation end products are selectively accumulated in the pyramidal neurons. According to the data of high-resolution immunohistochemistry, advanced glycation end products accumulate in endosomes or lysosomes and are a component of the lipofuscin pigment [56]. Almost all neurons that show a diffuse cytoplasmic positive staining for advanced glycation end products also contain a hyperphosphorylated tau protein [57]. Tau protein glycation is involved in the process of neurofibrillary tangles formation, and because of cross-linking as a result of glycation the stability of protein deposits in these structures increases [58-60]. Extracellular deposits of advanced glycation end products were also detected. However, although such deposits were detected in the early stages of the formation of senile plaques in Alzheimer's disease, they disappeared with the aging of the plaque or after the capturing of amyloid deposits by microglia. A definite, albeit not clarified, role in the pathology of Alzheimer's disease is played by receptors for advanced glycation end products that recognize glycated β-amyloid peptide [61]. Perhaps these receptors recognize glycated β-amyloid peptide and send it to the lysosomal degradation path, thereby preventing its toxic effect [62]. On the other hand, there is direct evidence of increased toxicity of glycated β-amyloid peptide [63].

A few studies on the effect of glycation on amyloid transformation of β-amyloid peptide *in vitro* have been reported. For instance, it has been revealed that methylglyoxal glycates β-amyloid peptide, thereby forming larger oligomeric forms containing β-sheets [64]. In other studies, it was found that the formation of advanced glycation end products accelerates the transition from monomeric β-amyloid peptide to its oligomeric form and then to even larger aggregates [65].

It should also be taken into account that an increase in glucose concentration in diabetes contributes to the formation of advanced glycation end products in different tissues. This may explain the increased level of advanced glycation end products and cross-linked proteins in the brain of patients with neurological diseases, including Alzheimer's disease. On the other hand, disturbances in

energy metabolism due to the development of Alzheimer's and Parkinson's diseases stimulate the accumulation of carbonyl compounds, which further enhance glycation of both glycolytic enzymes and amyloidogenic proteins. In particular, the following factors characteristic of Alzheimer's disease (and some other neurodegenerative diseases) can contribute to the formation of advanced glycation end products: (i) intracellular increase in the concentration of reactive carbonyl compounds (methylglyoxal and glyceraldehyde-3-phosphate) as a result of the accumulation of triose phosphates during glucose metabolism disruption, caused, for example, by inhibition of GAPDH (see above) and/or mitochondrial respiration; (ii) increase in concentration of non-chelated transition metal ions – copper and iron – that are weakly bound to amyloid plaques and accelerate the oxidation of glycated proteins and synthesis of highly reactive glycation products [57]; (iii) depletion of the pool of compounds protecting proteins from glycation, for example, carnosine and anserine [66]; (iv) disturbance of β-amyloid peptide degradation, which increases the half-life of the peptide and increases the likelihood of its glycation [65].

GLYCATION OF PRION PROTEIN

Relatively little is known about the role of glycation in the development of prion diseases, which are of genetic or infectious origin. In general, studies have been devoted to the identification of advanced glycation end products in already formed amyloid inclusions containing prion proteins [67-71]. Thus, in Creutzfeldt–Jakob disease a colocalization of the prion protein with advanced glycation end products as well as with their receptors was found [68]. A similar situation was observed in the transmission form of spongiform encephalopathy. In this case, a very specific prion protein glycation was found: of the 21 residues of arginine and lysine, only lysine residues 23, 24, and 27 and arginine 37 were glycated [69]. The molecular mechanisms of the influence of glycation on the formation of amyloid structures of the prion protein were not completely elucidated in subsequent studies [72, 73]. Perhaps glycation occurs when the prion protein is transformed into its pathological form. However, glycation also certainly occurs after amyloid fibrils of the prion are formed, which provides additional resistance of the fibrils to degradation. To clarify the role of glycation in the pathological transformation of a prion protein, it is necessary to modify it with the most reactive compounds, such as methylglyoxal and glyceraldehyde-3-phosphate. This approach will accelerate glycation, allowing avoidance of spontaneous aggregation of prion protein that occurs during its long incubation in the presence of reducing sugars.

INTERRELATION BETWEEN GLYCATION AND OTHER POSTTRANSLATIONAL MODIFICATIONS OF LYSINE RESIDUES OF AMYLOIDOGENIC PROTEINS

Glycation of amyloidogenic proteins may be important for the pathogenesis of neurodegenerative diseases, not only because of its direct effect on the structure of proteins and subsequent aggregation. It is known that glycation mainly affects the lysine residues of proteins. However, the posttranslational modifications of proteins, such as SUMOylation and ubiquitinylation, occurs precisely at lysine residues. These processes are rather well studied for α-synuclein. It is known that ubiquitinylation of proteins that is *per se* the addition of the small protein ubiquitin to lysine residues promotes their proteasomal degradation [74]. Ubiquitinylated α-synuclein was found in Lewy bodies [75-81]. α-Synuclein can be ubiquitinated only on 9 of 15 lysine residues [82, 83]. The most frequent site for ubiquitylation of soluble α-synuclein is the Lys23 residue, while the protein in fibrils is less efficiently ubiquitylated, mainly on lysine residues 6, 10, and 12. Ubiquitinylation affects the aggregation, fibrillation, α-synuclein toxicity [80, 83-88], and, naturally, its degradation [88, 89]. Despite the inconsistency of the available data, on the whole it can be considered that, in one way or another, ubiquitinylation inhibits the accumulation of toxic α-synuclein aggregates in neurons and the development of synucleinopathies. Similar information exists on the SUMOylation of α-synuclein, which is a modification with the small ubiquitin-like protein SUMO [90, 91]. With this modification, an isopeptide bond is formed between the C-terminus of a glycine residue in the SUMO protein and the ε-amino group of a lysine residue in the protein substrate [90]. α-Synuclein contains one classical conserved SUMO-acceptor site ($VK^{96}KD$) and another very similar site ($GK^{102}NE$), which was shown to interact with SUMO both *in vitro* and *in vivo* [92, 93]. The effect of SUMOylation on the aggregation of α-synuclein has been studied by many authors. In general, it can be considered that SUMOylation reduces or eliminates the toxicity of α-synuclein and prevents the formation of fibrils [87, 92, 94], as well as accelerating the degradation of this protein.

It should be noted that since ubiquitinylation and SUMOylation occur on different lysine residues [95], these two posttranslational modifications probably do not affect each other. Therefore, if α-synuclein glycation occurs prior to its modification by the SUMO protein and/or ubiquitin, then both the utilization of α-synuclein by the proteasome systems and the transport of this protein to the nucleus are fundamentally changed. Perhaps this explains the contradictory information about the role of glycation in the development of amyloid diseases. According to some data, glycation promotes the development of neurodegenerative diseases, for example

Parkinson's disease, while in other cases glycation prevents the amyloid transformation of this protein. Probably, in systems close to *in vivo*, glycation disrupts the utilization of α-synuclein due to the prevention of ubiquitinylation and SUMOylation, which leads to the development of synucleinopathies. This being said, the enhancing effect of glycation on the formation of toxic forms of α-synuclein observed with isolated proteins may have a minimal effect on the course of the disease in substantially more complex *in vivo* systems.

The answer to the question asked in the title of the article – "Glycation, Glycolysis, and Neurodegenerative Diseases: Is There Any Connection?" – is certainly positive. This interrelation exists, but data on specific mechanisms for its realization are insufficient and contradictory. In this review, we would like to emphasize the role played by GAPDH in the considered processes. Figure 3 shows the possible role of glyceraldehyde-3-phosphate dehydrogenase in glycation. It is known that during glycolysis active carbonyl compounds such as glyceraldehyde-3-phosphate and methylglyoxal are formed. Their excessive accumulation may be due to inhibition of glycolysis downstream of triose phosphates, due to glycation reactions in the cell, and also glycolic enzyme, GAPDH, glycation by these products. As shown in the figure, the main targets of carbonyl compounds may be the residues of cysteine, arginine, and lysine in the active site of GAPDH. The more GAPDH activity decreases, the more the concentrations of glyceraldehyde-3-phosphate and methylglyoxal increase, which again glycate GAPDH, further reducing its activity. Accumulation of highly reactive glycating agents in the cell leads to their interaction with proteins involved in the development of various neurodegenerative diseases: prion protein, β-amyloid, and α-synuclein. At the same time, there are many data on the glycation of these proteins by methylglyoxal, but very little is known about the effects of protein glycation by glyceraldehyde-3-phosphate. Glycation of amyloidogenic proteins by carbonyl compounds can lead to the stabilization of transition states (oligomers), acceleration of the aggregation process, disruption of the degradation system, and ultimately to the development of neurodegenerative diseases. It should also be noted that since GAPDH itself can be subjected to glycation and subsequent inactivation, such a chain of events, in princi-

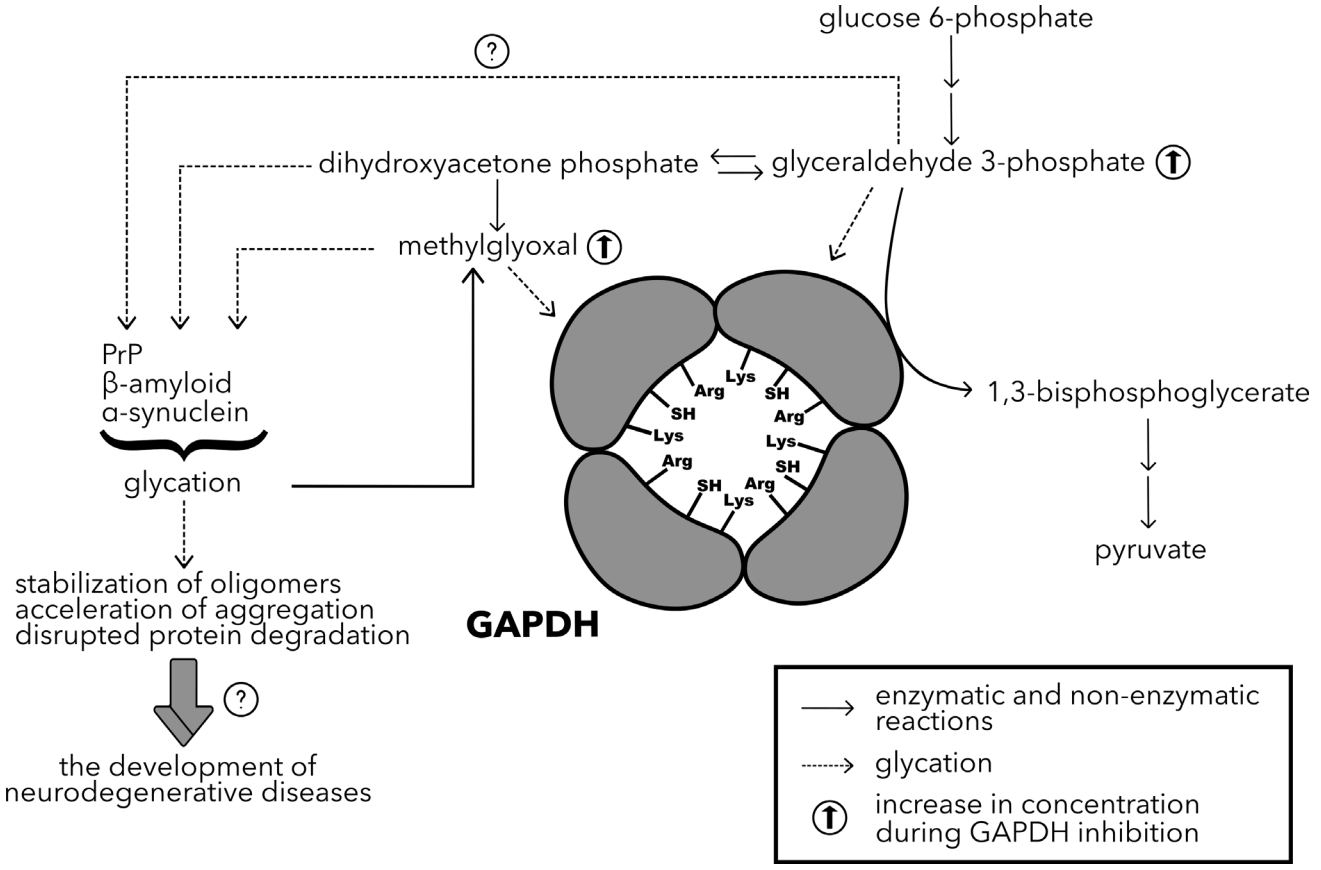

Fig. 3. Diagram illustrating the effect of GAPDH glycation on its activity and an increase in the concentrations of glyceraldehyde-3-phosphate and methylglyoxal, as well as the possible role of amyloidogenic glycation of proteins by these aldehydes in the development of neurodegenerative diseases.

ple, could lead to the blocking of further glycolytic reactions and the accumulation of carbonyl compounds. However, there are practically no studies even of the isolated enzyme glycation by glyceraldehyde-3-phosphate, and these aspects have not yet been studied in cellular models and especially in living organisms. It is possible that a very high content of GAPDH in the cytoplasm will not allow the realization of all the described events, and remaining in the active state dehydrogenase would be able to fully support its glycolytic functions. However, it is undoubtedly necessary to test these assumptions on systems as close to *in vivo* as possible.

The influence of GAPDH glycation on its various non-glycolytic functions, including participation in apoptosis, DNA repair, etc., requires further study [3, 96-99]. While there are at least limited data about the influence of glycation on the catalytic functions of GAPDH, there is nothing on changes in its non-glycolytic functions. Undoubtedly, the study of protein glycation by glyceraldehyde-3-phosphate, as effective as methylglyoxal, deserves attention. The two aldehydes should have a similar effect, but the glyceraldehyde-3-phosphate level is not associated with the cascade of glycation reactions and is directly regulated by GAPDH. Perhaps it is glyceraldehyde-3-phosphate that is the link between the processes of glycolysis and amyloidogenic transformations of proteins.

The role of amyloidogenic glycation of proteins in the pathogenesis of amyloidosis has been recognized. However, the main attention was given to the correlation between the accumulation of advanced glycation end products in amyloid structures and the development of neurodegenerative diseases. Although such a correlation exists, in our opinion, this is a secondary process, due to the fact that long-lasting amyloid structures undergo various types of glycation, which also proceeds rather slowly. A more important type of glycation is the effective modification of monomeric forms of amyloidogenic proteins by carbonyl compounds, including methylglyoxal and glyceraldehyde-3-phosphate. As for methylglyoxal, a direct effect of this modification on the amyloid transformation of α-synuclein and β-amyloid peptide was established. However, there are still no data on the modification of amyloidogenic proteins by glyceraldehyde-3-phosphate. It is worth noting that there are data on the relationship between energy metabolism and neurodegenerative diseases of amyloid nature (for example, Alzheimer's and Parkinson's diseases). Moreover, there are works indicating the involvement of GAPDH in the pathogenesis of Parkinson's disease. However, there have so far been no attempts to link the processes of GAPDH glycation with its participation in the regulation the contents of glyceraldehyde-3-phosphate, methylglyoxal, and other carbonyl compounds and the development of amyloid neurodegenerative diseases. In this review, we drew attention to another aspect that had not previously been considered, namely, the possible role of glycation of amyloidogenic proteins in the disruption of their degradation. It is known that modification with ubiquitin and SUMO protein occurs on lysine residues and, consequently, their preliminary glycation excludes such a modification and prevents the degradation of the proteins.

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