EXTENDED ABSTRACT

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Glycation of glyceraldehyde-3-phosphate dehydrogenase in the presence of glucose and glyceraldehyde-3-phosphate

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Abstract

Glycation of proteins is significantly accelerated with the increase in the blood glucose level in patients with diabetes. One of the potential targets of glycation is the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Glycation may result in the inactivation of GAPDH, leading to accumulation of its substrate (glyceraldehyde-3-phosphate), dixydroxyacetone phosphate, and methylglyoxal, which may result in further glycation of proteins. We investigated the effect of glycation of GAPDH on the catalytic properties of the enzyme and on its stability. The enzyme was glycated in the presence of 20 mM glucose or 1 mM glyceraldehyde-3-phosphate (G-3-P). The enzymatic activity of GAPDH decreased by 90% and 50% after 24 h of incubation in the presence of G-3-P and glucose, respectively. The addition of β-mercaptoethanol or glutathione partially protected the enzyme from inactivation. The glycation decreased thermal stability of GAPDH and increased its propensity to aggregate. Thus, it was demonstrated that the substrate of the reaction catalyzed by GAPDH, glyceraldehyde-3-phosphate, inactivates the enzyme and promotes its denaturation and aggregation. We suppose that the inactivation of GAPDH caused by glycation of the enzyme could inhibit the ATP-producing stages of glycolysis. Besides, considering the involvement of GAPDH in the induction and development of neurodegenerative diseases, the modification of this protein by sugars and aldehydes may affect its ability to produce amyloid structures with different amyloidogenic proteins, such as beta-amyloid peptide and alpha-synuclein.

Introduction

A large number of research papers are devoted to protein glycation and to the role of this process in normal cell functioning and in the development of different pathological states. A special attention is paid to investigation of the role of glycation in diabetes (Bucala *et al*, 2014, Beisswenger, 2014, Hu et al, 2015) and in some neurodegenerative diseases (Takeuchi and Yamagishi, 2009, Ramasamy *et al*, 2015, Juranek *et al*, 2015, Allaman *et al*, 2015). Non-enzymatic glycation results in the reversible condensation of aldehyde groups of reducing sugars with amino groups of proteins yielding Schiff bases with subsequent irreversible rearrangement to Amadori intermediates. The subsequent reactions lead to the formation of highly reactive dicarbonyl compounds that are capable of reacting with amino, sulfhydryl, and guanidine groups of proteins. Besides, proteins can be modified with dicarbonyl compounds and aldehydes formed during different metabolic processes

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(Allaman *et al*, 2015). One of the most important glycating agents is methylglyoxal, a dicarbonyl compound that is formed from dihydroxyacetone phosphate (product of aldolase reaction of glycolysis) by the non-enzymatic hydrolysis of the phosphate group. Presumably, the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) could play a crucial role in glycation of proteins, since inactivation of this enzyme results in the accumulation of its substrate (glyceraldehyde-3-phosphate), dihydroxyacetone phosphate and, as a result, methylglyoxal.

In the present work, we investigated the effect of reducing sugars and glyceraldehyde-3-phosphate on the catalytic activity of GAPDH, its stability and propensity to aggregate. In our previous reports, we demonstrated that misfolded forms of GAPDH are capable of blocking chaperonin GroEL (Polyakova *et al*, 2005) and directly interact with amyloid-beta peptide 1-42 (Naletova *et al*, 2008). Considering the information on the glycation of GAPDH by methylglyoxal (Lee *et al*, 2005), we assume that non-enzymatic glycation of GAPDH may contribute to different pathological states including Alzheimer's and Parkinson's diseases.

Materials and methods

Chemicals and reagents

We used the following reagents: glucose (Merck), glyceraldehyde-3-phosphate diethyl acetal (barium salt) and sodium azide (Sigma-Aldrich); glycine and NAD^+ (MP Biomedicals), potassium dihydrogen phosphate (PanReac), nitro blue tetrazolium (Chemapol). Glyceraldehyde-3-phosphate dehydrogenase was isolated from rabbit muscles as indicated in (Scheek and Slater, 1982). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH, 1 mg/ml) was incubated in 10 mM potassium phosphate buffer, pH 7.4, containing 0.01% sodium azide in the presence of 20 mM glucose or 1 mM glyceraldehyde-3-phosphate (G-3-P). During the incubation, aliquots were taken from the reaction mixture to determine the enzymatic activity of GAPDH and Amadori products. The enzymatic activity of GAPDH was determined by accumulation of NADH (absorption at 340 nm) during the reaction of G-3-P oxidation in the assay mixture containing 50 mM glycine, 50 mM potassium phosphate, pH 9.0, 1 mM NAD^+, 1 mM G-3-P and 1-2 μg of the enzyme. To determine Amadori products, 100μl of the tested solution were added to 900μl of buffer containing 100 mM sodium carbonate, pH 8.3, and 0.25 mM nitro blue tetrazolium. After 30 min of incubation at 37 °C, absorption of the sample at 530 nm was determined. The blank sample contained 100 μl of GAPDH solution without sugars and was treated in the same way. Differential scanning calorimetry (DSC) experiments were performed using a DASM-4 adiabatic microcalorimeter (Biopribor, Russia) in a 0.47-ml cell at a heating rate of 1 °C/min. Kinetics of thermal aggregation of GAPDH was monitored by an increase in the turbidity at 320 nm using a SIM Aminco DW-2000 spectrophotometer (USA).

Results and discussion

The data presented in Fig. 1 A demonstrate that prolonged incubation of GAPDH in the presence of glucose or glyceraldehyde-3-phosphate (G-3-P) results in a decrease in the GAPDH activity compared to the sample without additions. After 24 h of incubation in the presence of 20 mM glucose, GAPDH activity decreased by approximately 50%, while in the presence of 1 mM G-3-P, GAPDH activity was inhibited by 90% (Fig. 1A, curves 2 and 3, respectively). The accumulation of Amadori products was much more pronounced in the presence of G-3-P than in the presence of glucose (Fig. 1 B, curves 3 and 2, respectively). The addition of beta-mercaptoethanol (final concentration, 0.1 mM) completely prevented inactivation of GAPDH in the presence of G-3-P for the first 18 h of incubation. Reduced glutathione (0.1 mM) also protected GAPDH from inactivation, but with less efficiency. Glycation of GAPDH by G-3-P does not affect the Tm value of the protein

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(58.5±0.5 °C, Fig. 2A). However, broadening of the peak of the modified GAPDH (Fig. 2A, curve 2) points to a less cooperativity of its melting, which suggests partial unfolding of the protein during glycation with G-3-P. The propensity to aggregate was more pronounced in the case of GAPDH glycated with G-3-P (Fig. 2 B, curve *2*). Thus, we demonstrated that GAPDH can be efficiently glycated by its natural substrate, glyceraldehyde-3-phosphate. Glycation resulted in the inactivation and partial unfolding of the enzyme and stimulated its aggregation. The protective effect of low-molecular-weight thiols points to the involvement of the sulfhydryl groups in the glycation process. We assume that reactive oxygen species that are known to be produced during the glycation of protein amino groups are responsible for the modification of SH-groups of the GAPDH active site and contribute to the inactivation of the enzyme. The scheme presented in Fig.3 demonstrates direct or indirect (through interaction with chaperones) participation of glycated GAPDH in the formation of amyloid-like structures. It should be noted that the inhibition of GAPDH during glycation in the presence of sugars and dicarbonyl reagents must result in further accumulation of glyceraldehyde-3-phosphate, dihydroxyacetone phosphate and methylglyoxal leading to glycation and inactivation of GAPDH.

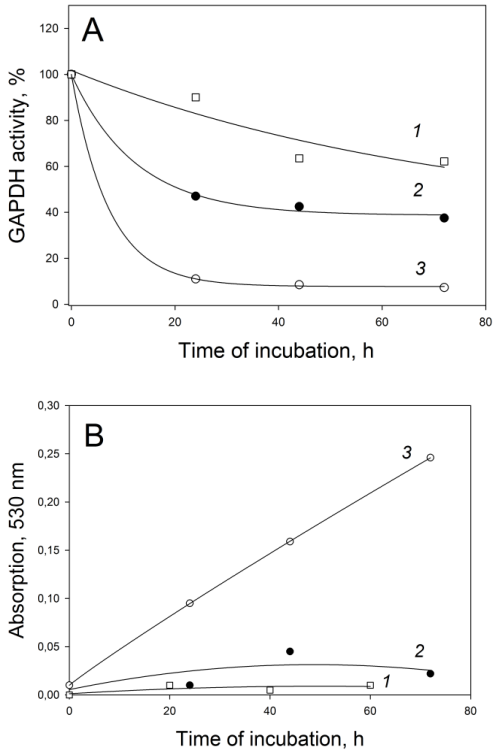

Figure 1. GAPDH activity (**A**) and Amadori products formation (**B**) during incubation of GAPDH with glucose and glyceraldehyde-3-phosphate at 20°C. GAPDH was incubated in 10 mM potassium phosphate buffer, pH 7.5, without additions (1), in the presence of 20 mM glucose (2) or 1 mM G-3-P (3).

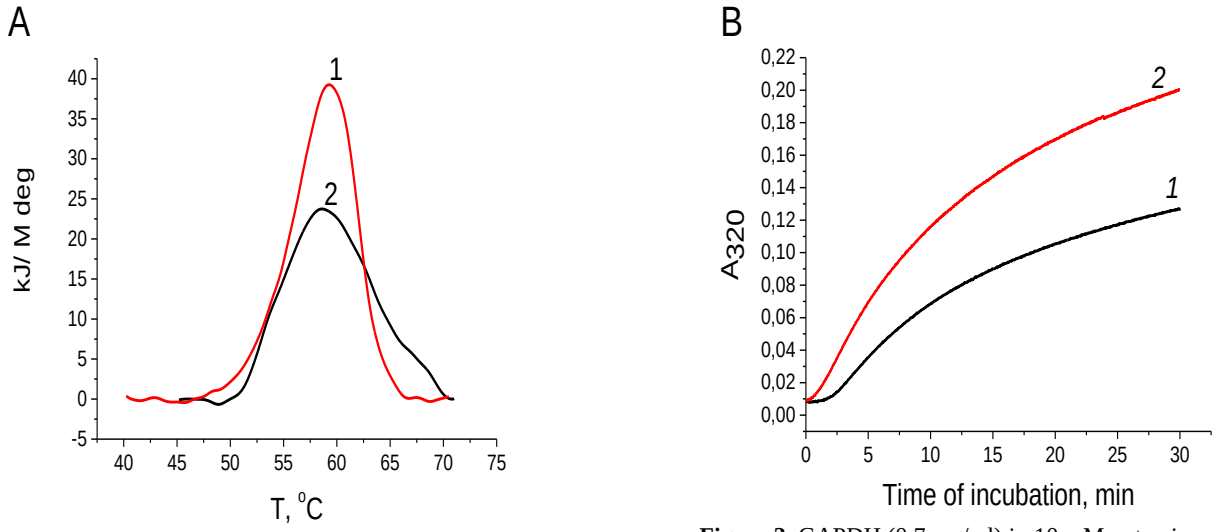

Figure 2. GAPDH (0.7 mg/ml) in 10 mM potassium phosphate buffer, pH 7.5, containing 0.01% sodium azide, was incubated

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for 4 days at 20 °C alone (*1*) or with 1 mM G-3-P (*2*). **A**) DSC curves for native (*1*) and glycated (*2*) GAPDH. **B**) Thermal aggregation of native (*1*) and glycated (*2*) GAPDH at 50 °C. Aliquots of 100 µl from samples 1 and 2 were taken and added into a preheated to 50 °C cuvettes containing 900 µl of the same buffer, and the growth in the turbidity was monitored at 320 nm.

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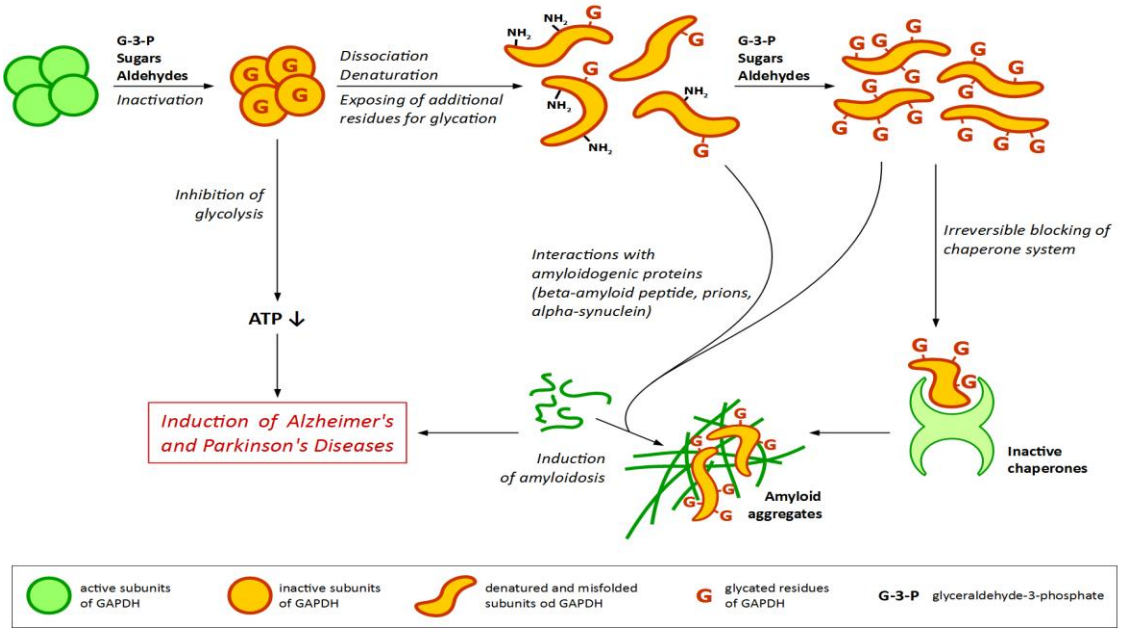

Figure 3. Scheme "Possible effects of glycation of GAPDH: inhibition of glycolysis and interaction with other proteins".

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