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Oxidative inhibition of red blood cell ATPases by glyceraldehyde

M.L. Mira, F. Martinho, M.S. Azevedo and C.F. Manso

Centro de Metabolismo e Endocrinologia, Instituto de Química Fisiológica, Faculdade de Medicina, Lisboa (Portugal)

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Glyceraldehyde and other simple monosaccharides autoxidize under physiological conditions, forming dicarbonyl compounds and hydrogen peroxide via intermediate free radicals. These products may have deleterious effects on cell components. In this paper we study the effect of glyceraldehyde autoxidation on red-cell ATPase activities. The autoxidation of glyceraldehyde in imidazole-glycylglycine buffer, measured by oxygen consumption, depends on the buffer concentration and decreases in the presence of superoxide dismutase and catalase. The addition of DETAPAC inhibits the autoxidation almost completely. When human red-blood-cell membranes are incubated with glyceraldehyde, the red-blood-cell ATPase activities decrease significantly. The addition of DETAPAC, GSH and DTE (dithioerythritol) protects the enzyme from inactivation, but superoxide dismutase and catalase have no effect. Methylglyoxal (a dicarbonyl which is analogous to hydroxypyruvaldehyde derived from glyceraldehyde autoxidation) proved to have a powerful inhibitory action on ATPase activities. The addition of DTE completely protects the enzyme from inactivation, suggesting that the sulphydryl groups of the active site of the enzyme are the critical targets for dicarbonyl compounds.

Introduction

Simple monosaccharides, such as glyceraldehyde, autoxidize under physiological conditions, yielding α-ketoaldehydes, H_2O_2 and free radical intermediates ($O_2^{\cdot-}$, $HO^{\cdot}$ and hydroxyalkyl radical) [1].

The autoxidation is catalysed by hydrogen peroxide and trace metal ion contaminants and according to these observation it was proposed a mechanism for simple monosaccharide autoxidation by Thornalley et al. [1,2]. The rate of monosaccharide autoxidation is also influenced by pH and the buffer system used for pH control [1]. The products of monosaccharide autoxidation may induce damage of cell components [2–4], including protein modifications [4–7]. The free-radical products may attack proteins, modifying the activities of enzymes, producing crosslinks and fragmentation [5,6,8] and increasing the susceptibility to enzyme-catalyzed proteolysis [5]. Also α-dicarbonyls may play a role in the modification of proteins by reacting with amino-acid residues.

Besides the effects due to the products of monosaccharide autoxidation, the nonenzymatic glycosylation of proteins must be taken into account, too [9–11]. The glycosylation proceeds through the formation of a Schiff base between a carbonyl group and an α- or ϵ-amino

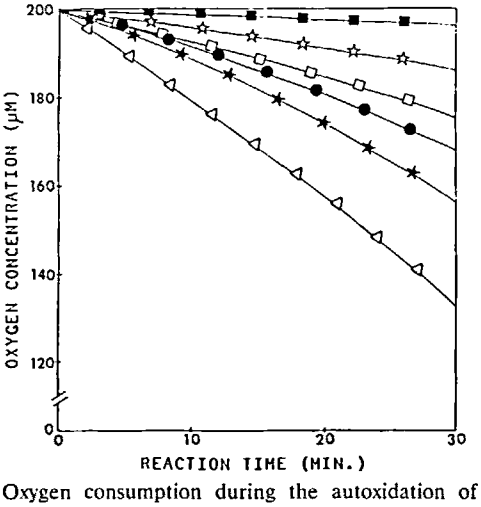

Fig. 1. Oxygen consumption during the autoxidation of 50 mM DL-glyceraldehyde in 25 mM (☆), 50 mM (★) and 100 mM (△), imidazole-glycylglycine buffers (pH 7.4). Effects of: +750 U/ml SOD (●), +700 U/ml catalase (CAT) (□), +5 μM DETAPAC (■) in 50 mM imidazole-glycylglycine buffer (pH 7.4).

Correspondence: M.L. Mira, Centro de Metabolismo e Endocrinologia, Instituto de Química Fisiológica, Faculdade de Medicina, 1600 Lisboa, Portugal.

group in a protein, together with the Amadori rearrangement to yield a relatively stable ketoamine.

Membrane ATPases may be one of the main targets of the effects described, and their inactivation has deleterious consequences, leading to osmotic lysis [5]. The occurrence of these processes in vivo may explain some of the diabetic complications which are attributed to hyperglycemia [12–14].

We report in this paper the effects of glyceraldehyde and of methylglyoxal on the ATPase activities of red-blood cell membranes. Methylglyoxal is a dicarbonyl which is analogous to hydroxypyruvaldehyde derived from glyceraldehyde autoxidation.

Material and Methods

Material

DL-Glyceraldehyde, methylglyoxal, imidazole, glycylglycine, Tris-ATP, glutathione (GSH), dithioerythritol (DTE), diethylenetriaminepentacetic acid (DETAPAC), superoxide dismutase (bovine erythrocytes 3020 units/mg protein) and catalase (bovine liver 2000 units/mg protein) were obtained from Sigma. All other chemicals were of the highest purity available from Merck.

Oxygen consumption by glyceraldehyde

Oxygen consumption by autoxidizing DL-glyceraldehyde in imidazole-glycylglycine buffer (pH 7.4) at 37 °C was followed using a Clark-type oxygen electrode (Gilson 5/6 oxygraph).

The imidazole-glycylglycine buffer was used instead of the phosphate buffer used by Thornalley et al. in their studies of monosaccharides autoxidation [1,2] because we needed to measure P_i concentrations to determine the activities of ATPases. Data presented represent the mean of five experiments.

Preparation and incubation of erythrocyte membranes

The membranes were prepared as described by Reinila et al. [15]. The last washing was done with 25 mM imidazole-glycylglycine buffer (pH 7.4). A membrane suspension in the imidazole-glycylglycine buffer was prepared with a final protein concentration of 1.6 mg/ml as determined by the Christian-Warburg method. The membrane incubations lasted 4 h at 37 °C, in a shaking water bath, with the additions as indicated in Results. Then each membrane suspension was centrifuged at $3500 \times g$ for 20 min at 4 °C and the membranes were washed twice with the imidazole-glycylglycine buffer before the assay for ATPases.

Assay method for ATPases

The ATPase activities were measured by the enzymatic liberation of inorganic phosphate (P_i) from Tris-ATP, using the colorimetric essay of Reinila et al. [15] with imidazole-glycylglycine buffer instead of Tris-HCl buffer. The 'total' ATPase activity was measured in the presence of Na^+, K^+ and Mg^{2+} ions in the reaction mixture and Mg^{2+}-ATPase activity was determined only in the presence of the Mg^{2+} ions. The difference between the 'total' ATPase and Mg^{2+}-ATPase represents Na^+/K^+-ATPase.

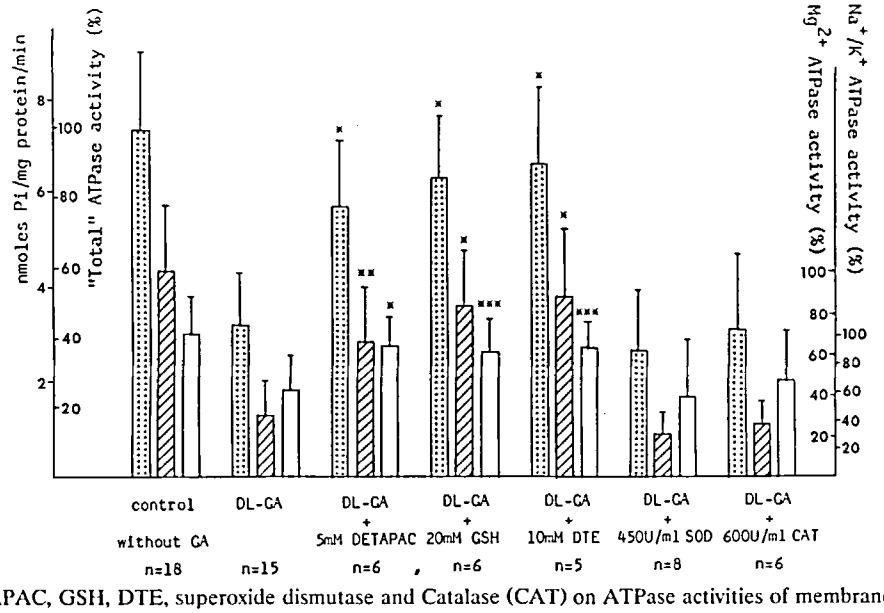

Fig. 2. Effects of DETAPAC, GSH, DTE, superoxide dismutase and Catalase (CAT) on ATPase activities of membranes incubated with 10 mM DL-glyceraldehyde (DL-GA). The incubations and the determination of ATPase activities were done as described in Material and Methods. 'Total' ATPase activity, ⊡; Mg^{2+}-ATPase activity, ▨; Na^+/K^+-ATPase activity, □. Columns are the means of 5–18 experiments in triplicate and vertical bars are S.D. The DL-GA values differ significantly from controls ($P < 0.001$). *: $P < 0.001$; **: $P < 0.01$; ***: $P < 0.02$ versus the DL-GA corresponding values.

Statistical methods

All the results are expressed as mean ± S.D.. The Student's *t*-test was used to evaluate the difference of the means between groups, accepting $P < 0.05$ as significant.

Results

Autoxidation of glyceraldehyde

With 50 mM glyceraldehyde in aqueous imidazole-glycylglycine buffer (pH 7.4) at 37 °C there is a great consumption of oxygen, which depends on the buffer concentration (Fig. 1).

The rate of oxygen consumption decreases slightly in the presence of superoxide dismutase and even more in the presence of catalase (Fig. 1).

The addition of 1 mM DETAPAC to glyceraldehyde inhibits almost completely the rate of oxygen consumption (Fig. 1).

Effects of glyceraldehyde on erythrocyte membranes ATPase activities

Fig. 2 shows that glyceraldehyde inhibits the 'total' ATPase and Na^+/K^+-ATPase in about 50% and the Mg^{2+}-ATPase in more than 50%. DETAPAC protects the ATPase activities (Fig. 2). GSH and DTE protect a little more the ATPase activities but superoxide dismutase and catalase seem to have no effect (Fig. 2). Albumin and heat inactivated superoxide dismutase, added as controls for non-specific protein effects, showed the same effect as active superoxide dismutase (data not shown).

Effect of methylglyoxal on erythrocyte membrane ATPase activities

Methylglyoxal is an analogue of hydroxypyruvaldehyde, which is formed from glyceraldehyde autoxidation. Its effects were studied to test the role of dicarbonyl compounds, generated from autoxidation of glyceraldehyde, on protein modification. We observed an inactivation of ATPase activities, which depend on methylglyoxal concentration (Fig. 3). We have tried to clarify the targets for the dicarbonyl compounds on the ATPase molecule. The enzyme possesses sulphydryl groups, which are essential for the activity, and an arginine residue in the nucleotide binding centre. So we have studied the effects of adding DTE (a protector of sulphydryl groups) or arginine to our assay system. We have observed that DTE protects the ATPase activities completely, but arginine seems to have no such effect (Fig. 4).

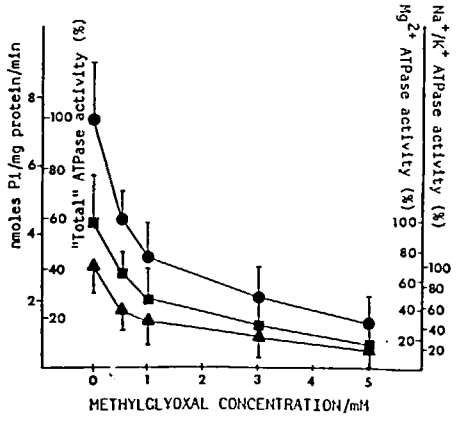

Fig. 3. Effects of methylglyoxal concentration (MG) on ATPase activities. The incubations and the determination of ATPase activities were done as described in Material and Methods. Data represent the means of 18 control experiments and five test experiments in triplicate ± S.D. 'Total' ATPase activity, •; Mg^{2+}-ATPase activity, ■; Na^+/K^+-ATPase activity, ▲.

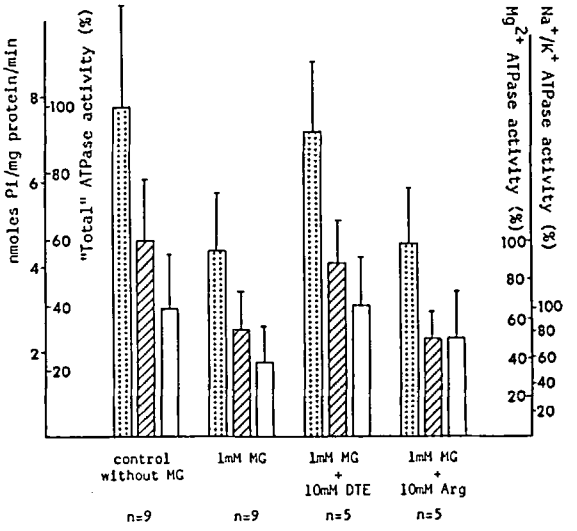

Fig. 4. Effects of DTE and of arginine (Arg) on ATPase activities of membranes incubated with 1 mM methylglyoxal (MG). DTE and arginine were added just before methylglyoxal. The incubations and the determination of ATPase activities were done as described in Material and Methods. 'Total' ATPase activity, ▨; Mg^{2+}-ATPase activity, ▨; Na^+/K^+-ATPase activity, □. Columns are the means of 5–9 experiments in triplicate and vertical bars are S.D. The methylglyoxal values differ significantly from controls ($P < 0.01$ for 'total' ATPase and Mg^{2+}-ATPase; $P < 0.05$ for Na^+/K^+-ATPase). DTE provides significant protection ($P < 0.01$ for 'total' ATPase; $P < 0.02$ for Mg^{2+}-ATPase; $P < 0.01$ for Na^+/K^+-ATPase).

Discussion

Thornalley at al [1,2] demonstrated that autoxidation of glyceraldehyde in phosphate buffer generates an α-ketoaldehyde and intermediates which are free-radicals. These compounds may exert several noxious effects such as, for example, protein modification. In order to study the effects of glyceraldehyde on red-blood-cell ATPases, it was necessary to study the autoxidation of glyceraldehyde in another buffer first. Imidazole glycylglycine buffer was chosen among some other buffers because the rate of glyceraldehyde autoxidation was maximal in it. In this buffer at pH 7.4 the

O_2 consumption increases with buffer concentration, and decreases in the presence of superoxide dismutase. This suggests the formation of $O_2^{\cdot}$ during the autoxidation. Catalase is also efficient, demonstrating the involvement of H_2O_2. The importance of metal ions is demonstrated in the presence of DETAPAC, which induces a significant decrease in autoxidation. We also tested the effect of GSH and DTE on glyceraldehyde oxidation, since these compounds can bind metals. However, we have found no inhibition of glyceraldehyde oxidation by these compouds in our incubation medium, 25 mM imidazole glycylglycine buffer (pH 7.4) (data not shown).

In the incubation studies of red-blood-cell membranes with glyceraldehyde a marked decrease in Na^+/K^+-ATPase and Mg^{2+}-ATPase is detected. Addition of superoxide dismutase or of catalase does not change the results, indicating that active forms of oxygen are probably not involved in this inhibition. As an alternative we can speculate that superoxide dismutase and catalase have a limited accessibility to the sites of $O_2^{\cdot}$ generation in the hydrophobic membranes.

The α-ketoaldehyde (a dicarbonyl), which results from the autoxidation of glyceraldehyde, may be then the responsible for the inhibition of ATPases. Our results with methylglyoxal seem to support this hypothesis. Wolff and Dean [6] also suggested that α-ketoaldehydes derived from glucose autoxidation appear to contribute to protein modification. The inhibition of ATPase activities by aldehydes has already been demonstrated; however, no mechanism for the inhibition was presented [16].

Na^+/K^+-ATPase has been reported to contain as many as 34 thiol groups, of which at least three are essential for activity and also to contain an arginine residue in the nucleotide binding centre [17,18].

When we incubate membranes with glyceraldehyde we find that DTE or GSH have a protector effect on the ATPase activities. This make us assume that the sulphydryl groups can be the critical targets for dicarbonyl compounds. Furthermore it is known that methylglyoxal reacts with thiol compounds [19,20]. In agreement with our assumption in the experiments with methylglyoxal and DTE, a complete protection on the ATPase activities is shown. The effect of DTE must probably be due to direct reaction with methylglyoxal whereby the reaction of methylglyoxal with the sulphydryl groups of the ATPase is competitively inhibited.

It has also been shown that the activities of Ca^{2+}-ATPase of sarcoplasmic reticulum [21] and of erythrocytes [22] and the activity of Na^+/K^+-ATPase of kidney [23,24] depend on sulphydryl groups of the enzymes.

We excluded the reaction of the dicarbonyls with the guanidinium groups of arginine, through the formation of Schiff bases, because these groups are very basic ($pK_a > 12$) having a positive charge which is stabilized by resonance. Only uncharged amino groups on the proteins can participate in the formation of Schiff bases [14,25]. Our experiments with methylglyoxal and arginine confirm our idea. The addition of arginine does not change the effect of methylglyoxal on the ATPase activities.

DETAPAC has a protective effect, which is in agreement with the decrease which is observed in glyceraldehyde autoxidation in the imidazole-glycylglycine buffer. DETAPAC (when it chelates the trace metal ion contaminants) must reduce hydroxypyruvaldehyde formation.

Our results tend to suport the hypothesis that the progressive inactivation of membrane ATPases, which is caused by dicarbonyl compounds, is probably due to the reaction with -SH groups. This fact may contribute to the cytotoxic effect of glucose in some diabetic complications by disturbing the ionic homeostasis of the cell. The work of Greene and Latimer [26] suports our idea. They demonstrated that neuronal Na^+/K^+-ATPase of diabetic patients is inhibited by elevated blood glucose concentration, but its activity is restored by the inhibitors of aldose redutase. These inhibitors block frutose formation [27] and are hydroxyl radical scavengers [28]. We are reminded that frutose, by nonenzymatic reaction, may give trioses, like glyceraldehyde and dihidroxyacetone [28], that can autoxidize into dicarbonyls [1], which are possibly the inhibitory agents.

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