Biochemistry (Moscow), Vol. 70, No. 11, 2005, pp. 1251-1255. Translated from Biokhimiya, Vol. 70, No. 11, 2005, pp. 1521-1526.
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Originally published in Biochemistry (Moscow) On-Line Papers in Press, as Manuscript BM04-395, May 1, 2005.

A New Role of Phosphoglucose Isomerase. Involvement of the Glycolytic Enzyme in Aldehyde Metabolism

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Received December 28, 2004
Revision received March 22, 2005

Abstract—Lipid peroxidation in biological membranes is accompanied by malonic dialdehyde (MDA) formation, but the problem of its further metabolism in cytoplasm remains unsolved. The experimental data obtained in this work showed that the liver fraction prepared by centrifugation at 10,000*g* contained phosphoglucose isomerase and enzymes of the glyoxalase system. In this fraction in the presence of GSH there is an aggregate of reactions taking place both in membranes (lipid peroxidation) and outside membranes (MDA conversion to methylglyoxal and further to neutral D-lactate). This means that MDA is slowly accumulated because it is a substrate of aldehyde isomerase (MDA ↔ methylglyoxal). Most probably, phosphoglucose isomerase serves as this enzyme. We concluded that D-lactate should be regarded as the end product of two different parametabolic reactions: lipid peroxidation or protein glycation.

Key words: lipid peroxidation, malonic dialdehyde, methylglyoxal, aldehyde isomerase, D-lactate

Lipid peroxidation (LPO) in biological membranes is almost universally associated with initiation of processes of degradation in a cell. The main mechanism of LPO initiation is interaction of primary (oxygen containing) radicals with polyunsaturated fatty acids [1]. In the intracellular membranes of eukaryotes, where the percentage of polyunsaturated fatty acids is substantial, the process of generation of lipid peroxides (LH → LOOH) proceeds at a high rate [1, 2]. Like protein glycation, this process can be attributed to parametabolic reactions [3]. Malonic dialdehyde (MDA) is a key product of lipid peroxidation, whereas the product of protein glycation is the ketoaldehyde methylglyoxal (MG). The two aldehydes are highly reactive compounds, and increase in their concentrations is associated with a number of pathological states [4, 5]. Therefore, in the presence of either of the two aldehydes in a cell, they should be converted into neutral compounds. This conversion takes place in mitochondrial matrix and cytoplasm.

There is an effective glyoxalase system in cells. This system catalyzes the conversion of MG into D-lactate, a neutral product [6]. This system includes two enzymes, glyoxalase I and glyoxalase II. There are mitochondrial and cytoplasmic glyoxalases, and the glyoxalase system is GSH-dependent [7]. In addition, there is a mechanism of MDA metabolism in liver mitochondria. This mechanism includes MDA oxidation by mitochondrial aldehyde dehydrogenase with further decarboxylation resulting in acetate formation and CO_2 evolution [8]. Although initiation of LPO in membranes of endoplasmic reticulum is necessarily accompanied by MDA formation in cytoplasm, the problem of its further metabolism remains unsolved.

The MDA molecule contains two aldehyde groups, whereas the MG molecule contains one aldehyde group and one keto group. According to [9], the problem of MDA metabolism would be solved if an isomerase capable of converting MDA into MG were found in cells (Scheme 1).

In this work, we studied production of MDA and D-lactate in the 10,000*g* liver fraction. The goal of this work was to elucidate the possible pathway of MDA conversion into MG (catalyzed by the isomerase of the glycolytic pathway) and further to D-lactate. It is well known that lipid peroxidation results in peroxide formation (LH → LOOH) and decay (LOOH → MDA). Lipid oxidation in the fraction of liver microsomes brings about an increase in the level of MDA. As far as the 10,000*g* liver fraction is concerned, accumulation of either MDA (if MDA is the end product) or another compound (if MDA, being the

Abbreviations: MDA) malonic dialdehyde; MG) methylglyoxal; LPO) lipid peroxidation.

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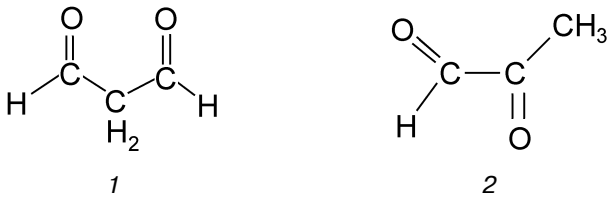


1 *2*

Structural formulae of aldehydes: *1*) malonic dialdehyde; *2*) methylglyoxal

Scheme 1

end product of the membrane process, is a substrate of another reaction taking place in cytoplasm) should be anticipated. If the 10,000*g* liver fraction contained enzymes of the glyoxalase system, in the presence of GSH this fraction would be characterized by a set of specific reactions taking part both inside and outside of membranes. In our opinion, the following succession of reactions in which MG is an intermediate product seems to be most probable:

$$\text{LOOH} \rightarrow \text{MDA}, \quad (1)$$

$$\text{MDA} \leftrightarrow \text{MG}, \quad (2)$$

$$\text{MG} \rightarrow \text{D-lactate}, \quad (3)$$

i.e. decay of lipid peroxides, aldehyde isomerization, and activation of the glyoxalase system in the presence of GSH.

Considering this, we measured in the studied system the accumulation of MDA, MG, and D-lactate under various conditions.

MATERIALS AND METHODS

Wistar rats were used; the 10,000*g* liver fraction was prepared by a conventional method using a Beckman J-21 centrifuge (Beckman, USA). The liver homogenate was centrifuged 10 min at 1000*g* (twice), and the supernatant was centrifuged 10 min at 10,000*g*. The supernatant (fraction containing membranes of endoplasmic reticulum and cytosol) was isolated many times, and each time the resulting preparation was tested for glucose-6-phosphate dehydrogenase activity. Experimental samples were incubated at 37°C under continuous shaking in flasks using glucose-6-phosphate and glutathione as additions. LPO was initiated by the addition of 100 μM $FeSO_4$ and 0.5 mM ascorbate into the incubation medium. Aliquots from the incubation medium were used for the determination of aldehydes (MDA, methylglyoxal) and D-lactate at certain time intervals (5-10 min).

Two methods were used to measure the level of MDA produced in Fe-ascorbate-dependent LPO: measurement of optical absorption of MDA complex with thiobarbituric acid (λ = 535 nm, $\varepsilon = 1.56 \cdot 10^5$ $M^{-1} \cdot cm^{-1}$) [10], and HPLC on a C_{18} column with a fluorescence detector (λ_{ex} = 520 nm, λ_{fl} = 560 nm). Methylglyoxal was measured using the method based on derivatization of aldehyde with 4-hydroxy-2,5,6-triaminopyrimidine (Fluka, USA) during 45 min at 60°C [11]. Fluorescence (λ_{ex} = 352 nm, λ_{fl} = 450 nm) was used for MG determination.

In addition, glyoxalase I activity was also measured; it is responsible for the reaction MG → S-D-lactoylglutathione. The formation of this intermediate was registered to measure initial rate of the reaction (λ = 240 nm, $\varepsilon = 3370$ $M^{-1} \cdot cm^{-1}$) [12].

The level of D-lactate was measured using a method based on NAD^+ reduction, which accompanies conversion of D-lactate into pyruvate and NADH detection [13]. D-Lactate dehydrogenase (Sigma, USA) was used for this purpose.

Concentrations of the resulting products (MG and D-lactate) were calculated by the method of additions. The principle of this method is presented in Fig. 1. Point *1* shows the fluorescence level of the sample after NAD reduction in the reaction NAD^+ + D-lactate → NADH + pyruvate; point *2* shows the fluorescence level of the sample after the first addition (1 nmol NADH), and point *3* shows the fluorescence level obtained after the second addition (1 nmol NADH). The straight line passing points *2* and *3* intersects the axis of the abscissa. It traverses point *1* or below and the appropriate value on the abscissa axis denotes the NADH concentration, and then the desired value of D-lactate concentration in the used liver fraction. In the first case (MG) the prepared derivate of MG with 4-hydroxy-2,5,6-triaminopyrimidine (Sigma) was used as an addition aliquot, and in the second case (D-lactate) NADH was used.

Protein was assayed by the microbiuret method.

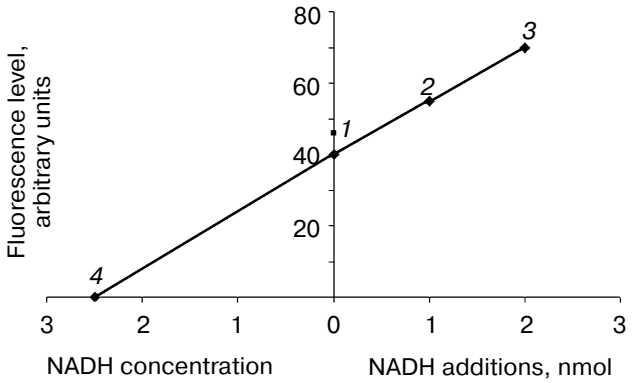


Fig. 1. Method of addition for determination of NADH and other fluorescence compounds. Point *1*, the fluorescence level of the sample after NAD^+ reduction; point *2*, the fluorescence level of the sample after the first addition (1 nmol NADH); point *3*, the fluorescence level of the sample after the second addition (1 nmol NADH); point *4*, NADH concentration in the sample.

RESULTS AND DISCUSSION

The changes in the MDA level in the 10,000*g* liver fraction (activity of glucose-6-phosphate dehydrogenase in the fraction in this case was 10 nmol/min per mg protein) are shown in Fig. 2. It follows from Fig. 2 that the initial level of MDA was 2 nmol/mg protein, and there was a delay in the increase in this level after the moment of initiation of lipid peroxidation (MDA level declined during the first 10 min of incubation); there was an increase in the level of MAD after 10 min of incubation, whereas after 20 min the MDA concentration was maintained at a constant level. A substantially different situation was observed in the presence of glucose-6-phosphate: there was a continuous increase in the concentration of MDA, and within 30 min it increased 2.5-fold relative to the initial level.

In our opinion, both MDA and MG can serve as substrates of phosphoglucose isomerase (enzyme catalyzing isomerization glucose-6-phosphate ↔ fructose-6-phosphate and MDA ↔ MG). Then, the rate of MDA conversion to MG should be decreased in the presence of glucose-6-phosphate in the reaction medium. Thus, this explains why kinetic curves *1* and *2* in Fig. 2 deviate from one another. Indeed, curve *2* shows true kinetics of MDA formation, whereas curve *1* represents a steady-state level of MDA concentration.

Samples of 10,000*g* fraction were incubated with or without GSH. These samples were used for assaying D-lactate (reactions (1)-(3)) and MG (reactions (1) and (2)). Curves of methylglyoxal accumulation in the 10,000*g* fraction in the presence and in the absence of GSH are shown in Fig. 3. It follows from Fig. 3 that MG was not accumulated in the presence of GSH, because it can be converted into D-lactate. Activity of glyoxalase I in this case was 100 nmol/min per mg protein.

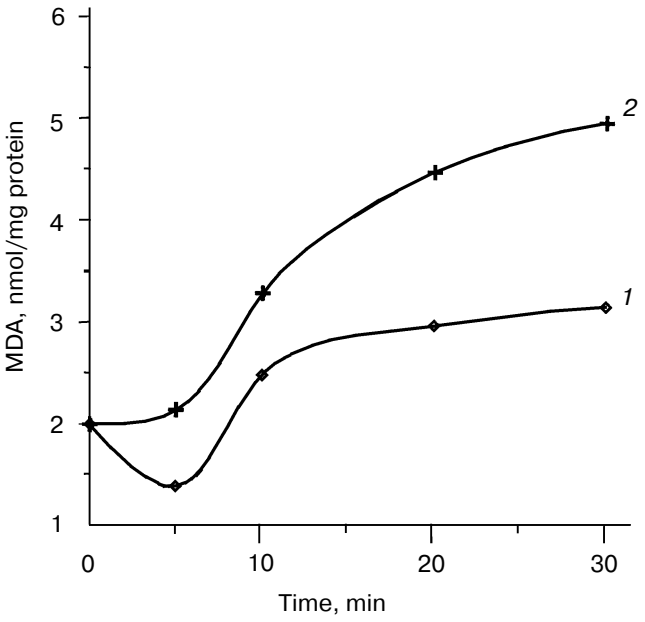


Fig. 2. Change in MDA level after initiation of LPO in the 10,000*g* liver fraction. Curves: *1*) without glucose-6-P; *2*) with glucose-6-P (1 mM). Incubation medium: 0.15 M KCl, 50 mM Tris-HCl (pH 7.7), 5 mM $MgCl_2$, 0.2 mM $Na_2P_2O_7$, 10 μM $FeSO_4$, 0.5 mM ascorbate, 1 mM GSH. Protein concentration, 1 mg/ml.

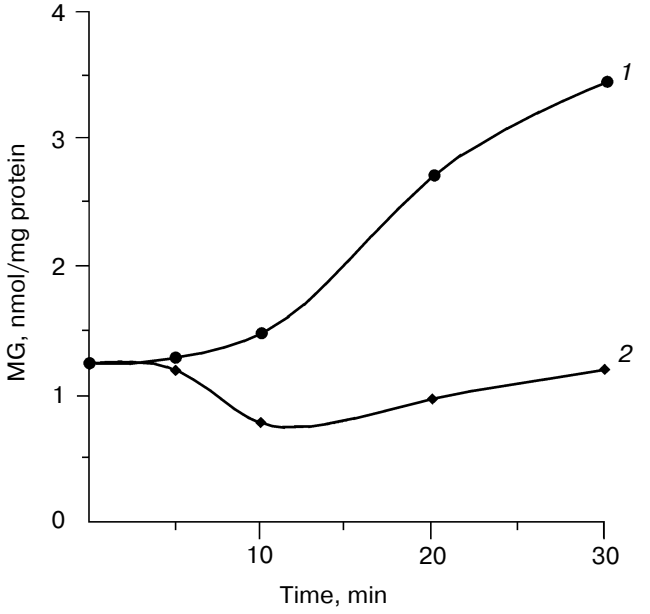


Fig. 3. Effect of glutathione on the accumulation of MG in the 10,000*g* liver fraction after initiation of LPO. Curves: *1*) without GSH; *2*) with GSH (1 mM). Conditions are the same as in Fig. 2 legend.

Finally, curves of accumulation of D-lactate in fraction 10,000*g* in the presence and in the absence of glucose-6-phosphate are shown in Fig. 4. It is evident that D-lactate is not directly produced from MDA. Perhaps MG can be an intermediate compound. It follows from Fig. 4 that in the experiment with glucose-6-phosphate the level of D-lactate was invariable (curve *2*), and this fact can be regarded as evidence of blockage of MDA conversion into MG and further into D-lactate. The level of D-lactate in the case of incubation of the same sample in the absence of glucose-6-phosphate was increased 2.5-fold (curve *1*), which was quantitatively consistent with the MDA concentration increase shown in Fig. 2 (curve *2*).

It follows from the results shown in Figs. 2-4 that in the 10,000*g* liver fraction accumulation of MDA is either observed (Fig. 2, curve *2*) or not observed (Fig. 2, curve *1*) within 30 min depending on incubation conditions. In the latter case, there is accumulation of MG (Fig. 3, curve *1*), which is converted into D-lactate in the presence of GSH in the incubation medium (Fig. 4, curve *1*). This means that in the 10,000*g* liver fraction there is an aldehyde isomerase. Most probably, this enzyme is phosphoglucose isomerase, because in the presence of glucose-6-phos-

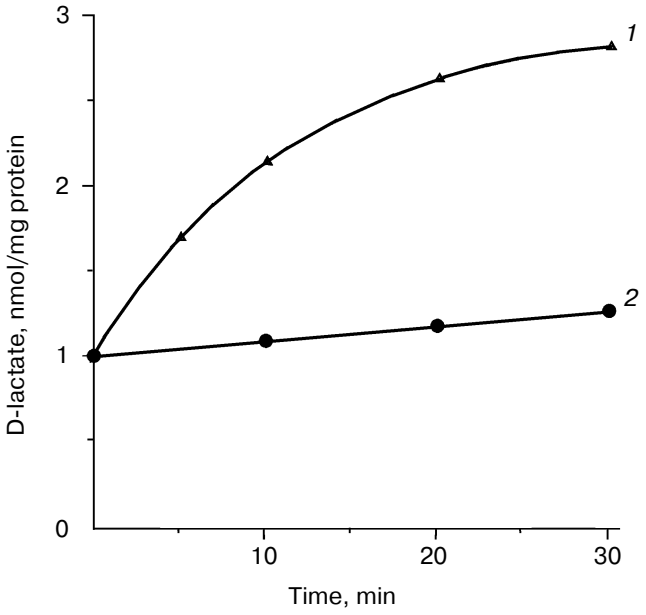
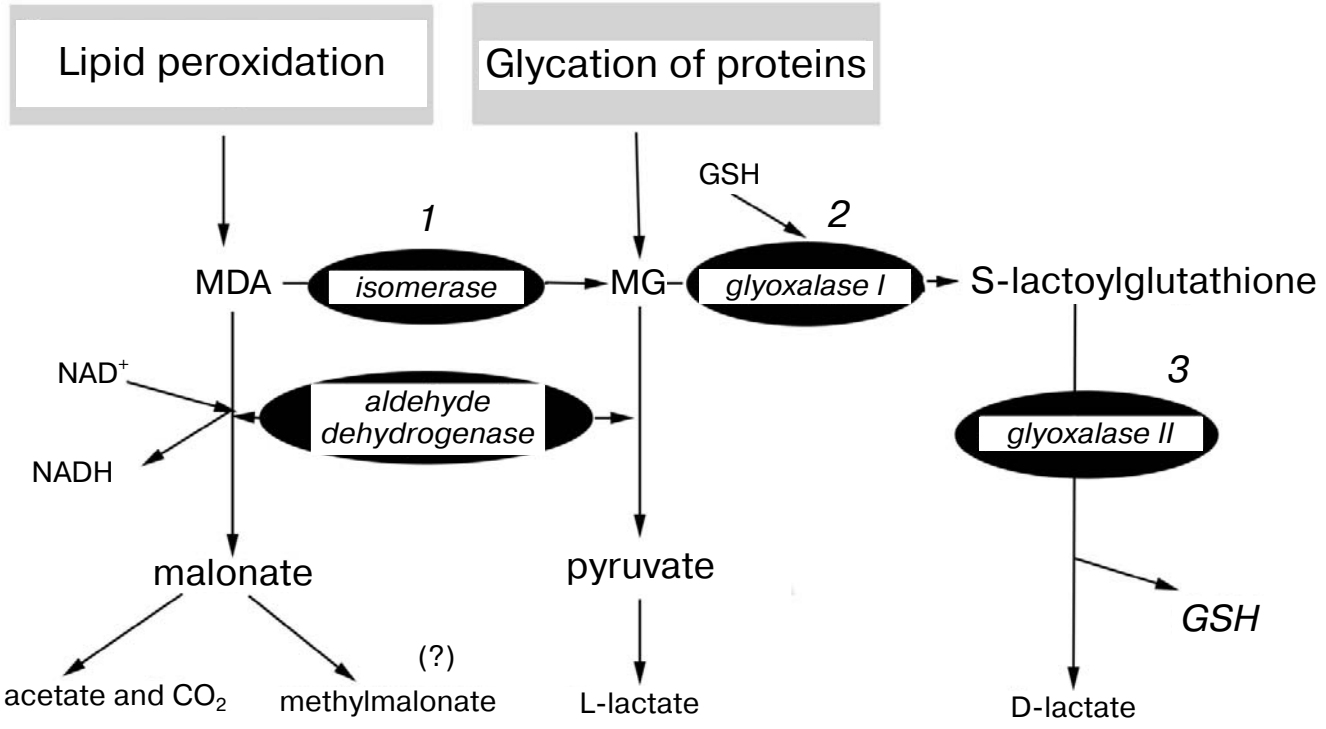


Fig. 4. Effect of glucose-6-P on the accumulation of D-lactate in the 10,000*g* liver fraction after initiation of LPO. Curves: *1*) without glucose-6-P; *2*) with glucose-6-P (1 mM). Conditions are the same as in Fig. 2 legend.

phate, the main substrate of phosphoglucose isomerase, aldehyde isomerization (MDA ↔ MG) is blocked.

It is presently beyond doubt that LPO processes in the absence of pathology are controlled at several levels. It should be noted that LPO initiation is mediated by active oxygen species including superoxide, the content of the species being controlled by superoxide dismutase. The second level of protection is provided by lipid antioxidants (tocopherol, etc.) and lipid-radical cycles [14]. Finally, the third level of protection is provided by glutathione peroxidase. This enzyme catalyzes conversion of lipid peroxides into hydroxy acids. If a fraction of the peroxides decays giving rise to MDA, the following process of MDA conversion to MG and further to D-lactate is activated. This means that D-lactate should be regarded as the end product of two different parametabolic reactions—LPO and glycation of proteins (Scheme 2).

Although there is a control mechanism of aldehyde (MDA and MG) formation in cells, unfavorable conditions in an organism can activate parametabolic reactions and/or bacterial translocation; in the latter case, the content of bacterial cells (*E. coli*, etc.), including MG and D-lactate, escapes to the inner medium of the organism. In this case, there should be an increase in the ratio of two lactate forms (D-lactate/L-lactate) in blood plasma. In our opinion, the increase in the ratio may directly indicate activation of pathological processes in the organism.



Parametabolic reactions: LPO and glycation of proteins

Scheme 2

Nevertheless, conversion of aldehydes into D-lactate, a neutral product, is more favorable than reactions catalyzed by aldehyde dehydrogenase (MDA → malonate → methylmalonate), provided that both aldehydes themselves and their products exert negative effects. For example, MDA initiates formation of polymer structures (lipofuscin granules); MG is a nonspecific protein inhibitor; and methylmalonate inhibits creatine kinases [15].

It should be noted in conclusion that phosphoglucose isomerase is an enzyme of glycolysis catalyzing conversion of a single substrate to a single product (glucose-6-phosphate ↔ fructose-6-phosphate); the cofactor of the enzyme is $MgCl_2$ [16]. However, it is safe to suggest from the results of this work that glucose-6-phosphate is not the only substrate of phosphoglucose isomerase; MDA, the product of LPO, can serve as another substrate. It should be noted in this context that aldose reductase, another enzyme involved in glucose metabolism, not only catalyzes glucose conversion into sorbitol, but this enzyme can although use MG as a substrate.

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