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# Acceleration of Glycolysis in the Presence of the Non-phosphorylating and the Oxidized Phosphorylating Glyceraldehyde-3-Phosphate Dehydrogenases

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**Abstract**—Mild oxidation of glyceraldehyde-3-phosphate dehydrogenase in the presence of hydrogen peroxide leads to oxidation of some of the active site cysteine residues to sulfenic acid derivatives, resulting in the induction of acylphosphatase activity. The reduced active sites of the enzyme retain the ability to oxidize glyceraldehyde-3-phosphate yielding 1,3-diphosphoglycerate, while the oxidized active sites catalyze irreversible cleavage of 1,3-diphosphoglycerate. It was assumed that the oxidation of glyceraldehyde-3-phosphate dehydrogenase by different physiological oxidants must accelerate glycolysis due to uncoupling of the reactions of oxidation and phosphorylation. It was shown that the addition of hydrogen peroxide to the mixture of glycolytic enzymes or to the muscle extract increased production of lactate, decreasing the yield of ATP. A similar effect was observed in the presence of non-phosphorylating glyceraldehyde-3-phosphate dehydrogenase catalyzing irreversible oxidation of glyceraldehyde-3-phosphate into 3-phosphoglycerate. A role of glyceraldehyde-3-phosphate dehydrogenase in regulation of glycolysis is discussed.

*Key words*: glycolysis, glyceraldehyde-3-phosphate dehydrogenase, non-phosphorylating glyceraldehyde-3-phosphate dehydrogenase, acylphosphatase activity, hydrogen peroxide, sulfenic acid

According to Pasteur's effect, the transition from anaerobic fermentation to aerobic metabolism results in a change in the intensity of glycolysis, the glycolytic rate decreasing in the presence of oxygen. Pasteur's effect is considered to be due to the inhibition of phosphofructokinase by ATP produced during oxidative phosphorylation. If such a mechanism takes place, glycolysis must decelerate to the rate that is sufficient to provide mitochondria with NADH and pyruvate. However, if we refer to the reaction of the substrate phosphorylation catalyzed by glyceraldehyde-3-phosphate dehydrogenase (GAPDH, EC 1.2.1.12) and the subsequent 3-phosphoglycerate kinase reaction yielding ATP, it becomes obvious that in the lack of ADP glycolysis must completely cease rather than decelerate. Without constant consumption of 1,3-diphosphoglycerate by 3-phosphoglycerate kinase (that is impossible at a low concentration of ADP), the reversible reaction catalyzed by GAPDH stops and, as a result, the accumulation of NADH and 3-phosphoglycerate formation also cease. It can be assumed that some mechanisms must exist allowing glycolysis to proceed under aerobic conditions even at a low content of ADP, although with less efficiency than in the absence of oxygen.

It is known that the rate of glycolysis decreases at low concentrations of ADP and inorganic phosphate. However, the process is accelerated significantly with the addition of enzymes capable of hydrolyzing 1,3-diphosphoglycerate or ATP. Meyerhof demonstrated that the addition of ATPase to yeast extracts obtained by autolysis and containing low concentrations of ADP and inorganic phosphate raised the glycolytic rate (first published in 1948, republished in 1994) [1]. The acceleration of glycolysis was also observed with the addition of arsenate or muscle acylphosphatase, i.e., under conditions promoting hydrolysis of 1,3-diphosphoglycerate [2]. Many years later (in 1996), it was shown that the expression of the human muscle acylphosphatase in *Saccharomyces cerevisiae* increased the rate of ethanol production by these microorganisms [3].

Obviously, the intensity of glycolysis could be controlled by an enzyme possessing acylphosphatase activity in the presence of oxygen, but exhibiting no acylphos-

*Abbreviations*: GAPDH) glyceraldehyde-3-phosphate dehydrogenase phosphorylating (EC 1.2.1.12); GAPN) glyceraldehyde-3-phosphate dehydrogenase non-phosphorylating (EC 1.2.1.9); G-3-P) glyceraldehyde-3-phosphate.

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phatase activity under anaerobic conditions. The appearance of the acylphosphatase activity on incubation of one of the glycolytic enzymes, GAPDH, in the presence of a number of oxidants was demonstrated by Allison et al. [4-6]. This activity arose on oxidation of the enzyme by mild oxidants: *o*-iodosobenzoate, iodine monochloride, and trinitroglycerin. Under the conditions employed, the cysteine residues of the active site of the enzyme oxidized yielding sulfenic acid derivatives. However, such an oxidation resulted in the complete disappearance of the dehydrogenase activity of the enzyme, and the authors did not consider any possibility of the physiological significance of this process.

It was shown in our laboratory that incubation of GAPDH in the presence of a low concentration of hydrogen peroxide (comparable to the concentration of the enzyme) resulted in the oxidation of some of the Cys149 residues of the active site of the enzyme yielding sulfenic acid derivatives. The oxidation caused 25-30% decrease in the dehydrogenase activity, this being accompanied by the appearance of acylphosphatase activity [7, 8]. According to our assumption [8, 9] such a bifunctional enzyme exhibiting both the dehydrogenase and the acylphosphatase activities can be involved in regulation of the rate and efficiency of glycolysis, mediating effects of oxidants and reductants.

In the present work, we investigated the effect of hydrogen peroxide on glycolysis. Besides, we studied the effect of the microbial non-phosphorylating glyceraldehyde-3-phosphate dehydrogenase (GAPN, EC 1.2.1.9) on glycolysis, since this enzyme catalyses irreversible oxidation of glyceraldehyde-3-phosphate yielding 3-phosphoglycerate and can cause an effect similar to that caused by the oxidized GAPDH.

## MATERIALS AND METHODS

In this work we used the following chemicals: ADP, ATP, NADP, Hepes, glyceraldehyde-3-phosphate, and pyruvate kinase from Sigma (USA); NAD, hydrazine, and lactate dehydrogenase from ICN (USA); hydrogen peroxide from Merck (Germany); 2-mercaptoethanol from Ferak (Germany); bioluminescent reagent from Hughes Whitlock Ltd. (England).

**Isolation of enzymes.** Glyceraldehyde-3-phosphate dehydrogenase, 3-phosphoglycerate kinase, 3-phosphoglycerate mutase, and enolase were isolated from rabbit muscles according to Scopes [10].

Glyceraldehyde-3-phosphate dehydrogenase non-phosphorylating from *Streptococcus mutans* was isolated from the superproducing strain of *Escherichia coli* DH5α transformed with the psk*Bst*II plasmid bearing the gene of GAPN [11]. The plasmid was kindly provided by Prof. Branlant (University of Nancy, France). GAPDH from rabbit muscles and GAPN were stored at 4°C as a crystal suspension in half-saturated ammonium sulfate in 50 mM $KH_2PO_4$, pH 8.0, containing 5 mM 2-mercaptoethanol. Before experiments, GAPN was dissolved in 20 mM $KH_2PO_4$, pH 8.0, and desalted on a G-25 Sephadex column equilibrated with the same buffer.

**Protein assay.** Concentrations of homogeneous GAPDH and GAPN were determined spectrophotometrically at 280 nm using the absorption coefficient $A_{280}^{0.1\%} = 1$. Total protein concentration in muscle extract was determined using the biuret reaction.

**Assaying of the activity of GAPN.** The activity of GAPN was determined by measuring the accumulation of NADPH at 340 nm. The reaction mixture contained 1 mM NADP, 1 mM G-3-P, 50 mM Taps, and 5 mM 2-mercaptoethanol, pH 8.2. The specific activity of the enzyme was 55-60 U/mg.

**Assaying of the dehydrogenase and the acylphosphatase activities of GAPDH.** The dehydrogenase activity of GAPDH was determined by measuring the accumulation of NADH at 340 nm in a mixture of the following composition: 50 mM $KH_2PO_4$, 50 mM glycine, 1 mM NAD, 1 mM G-3-P, pH 9.0 (25°C).

The acylphosphatase activity of GAPDH was determined spectrophotometrically by measuring the accumulation of NADH in the system of the following coupled reactions:

$$\text{G-3-P} + \text{NAD}^+ \underset{\text{(E-SH)}}{\overset{\text{P}_i}{\longleftrightarrow}} \text{1,3-DPG} + \text{NADH} + \text{H}^+ \quad (1)$$

$$\text{1,3-DPG} \underset{\text{(E-SOH)}}{\longrightarrow} \text{3-PG} + \text{P}_i \quad (2)$$

$$\text{G-3-P} + \text{NAD}^+ \underset{\text{(E-SH + E-SOH)}}{\longrightarrow} \text{3-PG} + \text{NADH} + \text{H}^+ \ (1 + 2),$$

where E-SH is the reduced form of GAPDH, E-SOH is the oxidized form of the enzyme, G-3-P is glyceraldehyde-3-phosphate, 1,3-DPG is 1,3-diphosphoglycerate, and 3-PG is 3-phosphoglycerate. The reaction mixture contained 50 mM Hepes, 1 mM $KH_2PO_4$, pH 7.6, 5 mM EDTA, 0.5 mM $NAD^+$, 0.5 mM G-3-P, and 500 μg of the oxidized GAPDH. The preparation of the enzyme oxidized under mild conditions contained both the reduced and oxidized SH-groups, this allowing determination of the acylphosphatase activity without addition of the reduced form of GAPDH. In the absence of SOH-groups, no accumulation of NADH is observed in this system, since the concentration of inorganic phosphate is too low to shift the equilibrium towards the formation of 1,3-diphosphoglycerate.

**Determination of the content of the sulfenic acid derivatives among the products of oxidation of GAPDH.** The total content of the oxidized cysteine residues was

estimated by the decrease in the dehydrogenase activity of the enzyme. The content of the SOH groups was determined considering their ability to be reduced in the presence of sodium arsenite yielding SH-groups.

GAPDH from rabbit muscles (7 μM) was incubated in the presence of different concentrations of hydrogen peroxide in 50 mM Hepes, pH 7.6, containing 0.5 mM NAD. After 30 min of incubation at 20°C, the samples were supplemented with 50 mM sodium arsenite and incubated for 1 h at the same temperature. The ratio of the SOH-groups in the samples of the oxidized GAPDH was calculated as $(A_{red} - A_{ox})/A \cdot 100\%$, where $A_{ox}$ is the dehydrogenase activity of GAPDH after the oxidation by hydrogen peroxide, $A_{red}$ is the dehydrogenase activity of GAPDH after the treatment with sodium arsenite, and $A$ is the original activity of the enzyme. The ratio of other products of oxidation was calculated as $(A - A_{red})/A \cdot 100\%$.

**Preparation of muscle extract.** Rat muscles were minced with scissors and placed in cooled buffer (20 mM $KH_2PO_4$, 0.8 mM $MgCl_2$, 3.4 mM KCl, 68.4 mM NaCl, pH 7.6) in the ratio of 1 : 2 w/v. After 15 min, debris was removed by centrifugation (12,000*g*, 10 min). To remove fine particles, the extract was centrifuged again at 30,000*g* for 10 min. To remove glycolytic substrates, the supernatant was passed through a G-25 Sephadex column equilibrated with 10 mM $KH_2PO_4$, 0.8 mM $MgCl_2$, pH 7.6.

**Assay for lactate content.** After different time intervals, aliquots were taken from the reaction mixture and $HClO_4$ was added to the final concentration of 1 M to stop glycolysis. Then the samples were neutralized with a saturated solution of $Na_2CO_3$. To determine the lactate content, 0.1 ml of the resulting solution was added to 0.9 ml of the assay mixture containing 50 mM glycine, 50 mM hydrazine, 1 mM NAD, and 10 units of lactate dehydrogenase, pH 9.0. The samples were incubated at 25°C for 1 h together with a control sample containing 1 ml of the assay mixture, and then concentration of NADH was measured in the samples with respect to the control.

**Assay for ATP content.** The content of ATP was determined by measuring the luminescence of samples in the presence of the luciferin–luciferase reagent. Aliquots (20 μl) were taken from the reaction mixture and added into 1 ml of a solution containing 2.5% trichloroacetic acid and 0.2 mM EDTA, pH 7.4. After 20-min incubation, 20 μl of the sample was diluted with 200 μl of 0.1 M Tris-acetate buffer containing 2 mM EDTA, pH 7.75. The luminescence of the samples was measured 30 sec after starting the reaction by the addition of the luciferin–luciferase reagent. The measurements were performed using an LKB 1256 luminometer (LKB, Sweden).

## RESULTS AND DISCUSSION

In our previous work we showed that incubation of purified GAPDH in the presence of low concentrations of hydrogen peroxide resulted in the appearance of the acylphosphatase activity due to oxidation of some of the Cys149 residues of the active site yielding sulfenic acid derivatives. The reduced Cys149 residues retained their ability to catalyze the dehydrogenase reaction [7].

Figure 1 demonstrates the dependence of the acylphosphatase activity on concentration of $H_2O_2$ (curve *3*). The maximal acylphosphatase activity is observed at the ratio of 1-2 moles of hydrogen peroxide per 1 mole GAPDH (calculated per tetramer), the subsequent increase in concentration of hydrogen peroxide resulting in a decrease in the acylphosphatase activity. The figure also shows the content of the sulfenic acid derivatives (curve *2*) among the products of oxidation of GAPDH after its incubation in the presence of different concentrations of $H_2O_2$. As seen from the figure, the maximal acylphosphatase activity corresponds to the maximal content of the SOH-groups. The content of the sulfenic acid derivatives decreases with increase in $H_2O_2$ concentration due to accumulation of the products of the higher extent of oxidation (sulfinic and sulfonic acids). Thus, the increase in $H_2O_2$ concentration above a certain level results in the inhibition of both the dehydrogenase and the acylphosphatase activities.

Based on the presented experiments and the hypothesis formulated previously concerning the possibility of uncoupling of oxidation and phosphorylation in glycoly-

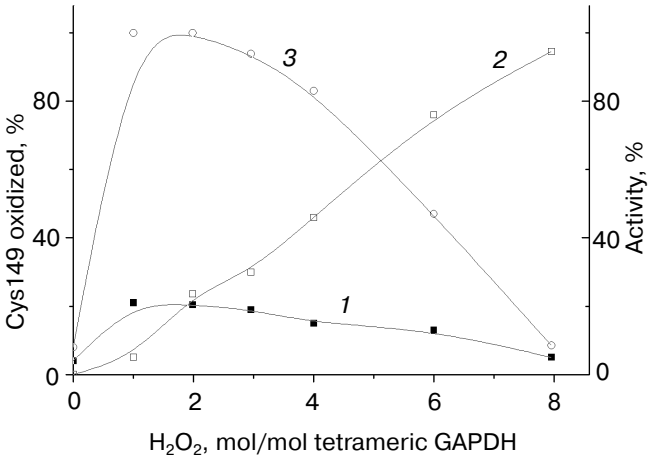


**Fig. 1.** Content of the sulfenic acid derivatives of Cys149 among the products of oxidation of GAPDH by hydrogen peroxide and its correlation to the acylphosphatase activity of the enzyme: sulfenic acid derivatives of Cys149 (left ordinate, curve *1*); other products of oxidation (curve *2*); acylphosphatase activity of GAPDH (right ordinate, curve *3*). The reaction mixture contained 50 mM Hepes, pH 7.6, 0.5 mM NAD, 7 μM rabbit muscle GAPDH, and $H_2O_2$ as indicated. The acylphosphatase activity and the content of the sulfenic acid derivatives were determined as described in "Materials and Methods".

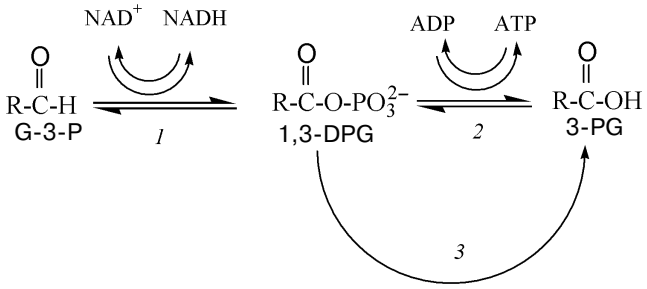


**Fig. 2.** Pathways of oxidation of glyceraldehyde-3-phosphate and the coupled reactions: glyceraldehyde-3-phosphate dehydrogenase reaction catalyzed by reduced GAPDH (*1*), phosphoglycerate kinase reaction (*2*), acylphosphatase reaction catalyzed by oxidized GAPDH (*3*), where G-3-P stands for glyceraldehyde-3-phosphate, 1,3-DPG is 1,3-diphosphoglycerate, and 3-PG is 3-phosphoglycerate.

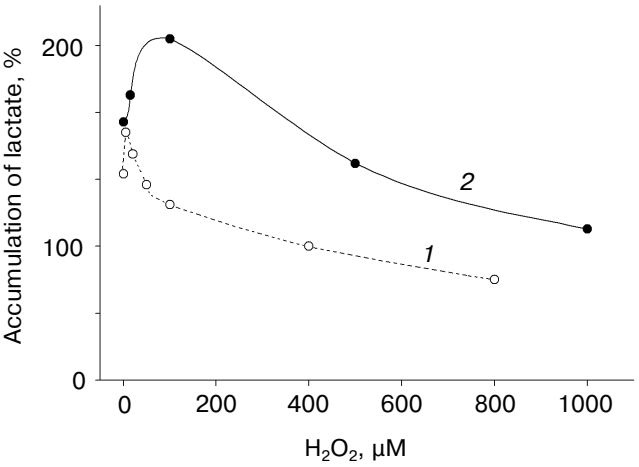


**Fig. 3.** Accumulation of lactate in the mixture of glycolytic enzymes (*1*) and in muscle extract (*2*) in dependence on hydrogen peroxide concentration: *1*) reaction mixture containing 2.3 μM GAPDH, 0.7 μM 3-phosphoglycerate kinase, 1 μM phosphoglycerate mutase, 0.3 μM enolase, 0.3 μM pyruvate kinase, 0.4 μM lactate dehydrogenase, 1 mM NAD, 10 mM $KH_2PO_4$, and 10 mM $MgSO_4$ in 50 mM Hepes, pH 7.6, was incubated in the presence of different concentrations of $H_2O_2$ or 2 mM 2-mercaptoethanol (as the control) for 30 min at 20°C. Then glycolysis was initiated by the addition of 1 mM ADP and 3 mM glyceraldehyde-3-phosphate, and the mixture was incubated then at 37°C for 1 h. The reaction was stopped by the addition of $HClO_4$, and the lactate content was determined as described in "Materials and Methods". The lactate content in the sample containing 2 mM 2-mercaptoethanol was taken as 100%. *2*) Muscle extract prepared as described in "Materials and Methods" was diluted with buffer (10 mM $KH_2PO_4$, 0.8 mM $MgSO_4$, pH 7.6), so that the total protein concentration constituted 1 mg/ml. The conditions for the experiment are given above in (*1*). The lactate content in the sample containing 2 mM 2-mercaptoethanol was taken as 100%.

sis [8], we assumed that the ADP-independent pathway of cleaving of 1,3-diphosphoglycerate catalyzed by the oxidized GAPDH (Fig. 2, reaction *3*) under certain conditions can accelerate glycolysis with a simultaneous decrease in the yield of ATP according to the scheme presented in Fig. 2.

To test this assumption, the mixture of glycolytic enzymes and muscle extract were incubated in the presence of hydrogen peroxide under the conditions providing the partial oxidation of the cysteine residues of GAPDH and the appearance of the acylphosphatase activity. According to literature data, no detectable effects of 5 mM hydrogen peroxide were observed on catalytic parameters of any glycolytic enzymes except for hexokinase (25% decrease in activity) and GAPDH (99% inhibition) [12]. The maximal concentration of $H_2O_2$ used in our experiments did not exceed 100 μM. Consequently, only GAPDH could be affected by the oxidant under these conditions.

To study the effect of the oxidized GAPDH on glycolysis, we used two systems: 1) a mixture of several isolated glycolytic enzymes (GAPDH, 3-phosphoglycerate kinase, 3-phosphoglycerate mutase, enolase, pyruvate kinase, and lactate dehydrogenase), and 2) cell-free extract of rat muscles liberated from low-molecular-weight components using G-25 Sephadex gel filtration (see "Materials and Methods"). Figure 3 shows the dependence of the accumulation of lactate on $H_2O_2$ concentration during the incubation of the enzyme mixture (curve *1*) or the muscle extract (curve *2*) in the presence of glyceraldehyde-3-phosphate and ADP.

As seen from Fig. 3, the lactate content is significantly higher after incubation of the enzyme mixture in the presence of $H_2O_2$ compared to that after incubation in the presence of the reductant (2-mercaptoethanol). In the case of the enzyme mixture, the addition of 5-10 μM $H_2O_2$ resulted in the maximal (1.7-1.8-fold) increase in the accumulation of lactate (Fig. 3, curve *1*). In the muscle extract, maximal accumulation of lactate exceeding 2.5-fold the control values was observed in the presence of 100 μM $H_2O_2$ (Fig. 3, curve *2*). The differences in the $H_2O_2$ concentrations required for oxidation of GAPDH in the enzyme mixture and in the muscle extract are likely due to the fact that there are many proteins in the muscle extract capable of interacting with $H_2O_2$ and decreasing its concentration.

It should be noted that the addition of 2-mercaptoethanol resulted in a significant decrease in the accumulation of lactate in both systems, this indicating that the acylphosphatase reaction can take place due to spontaneous oxidation of GAPDH in the presence of oxygen. This observation is in agreement with previous data according to which the purified enzyme exhibited the acylphosphatase activity in the absence of low-molecular-weight thiols [7].

Figure 4 shows the dependence of the accumulation of lactate on ADP concentration in the samples contain-

ing $H_2O_2$ or 2 mM 2-mercaptoethanol. As seen from the data, the maximal stimulation of glycolysis by hydrogen peroxide (more than 3-fold increase in the lactate accumulation) is observed at low concentrations of ADP (below 0.5 mM). Under these conditions, the ADP-dependent 3-phosphoglycerate kinase reaction is likely to become a rate-limiting step of glycolysis. Oxidation of a part of Cys149 of GAPDH by $H_2O_2$ induces the acylphosphatase activity of the enzyme, resulting in its ability to hydrolyze irreversibly 1,3-diphosphoglycerate. The appearance of the ADP-independent pathway of the hydrolysis of 1,3-diphosphoglycerate accelerates glycolysis, resulting in an increased accumulation of lactate.

The increase in the accumulation of lactate in the presence of hydrogen peroxide was demonstrated using other glycolytic substrates—glucose, glucose-6-phosphate, fructose-6-phosphate, and fructose-1,6-diphosphate. In the presence of 100 μM $H_2O_2$, the content of lactate accumulated in the muscle extract in the presence of the substrates indicated above increased by 25-50% compared to the samples containing 2-mercaptoethanol (data not shown). These data indicate that the effect of hydrogen peroxide on glycolysis is similar in the case of using different glycolytic substrates (from glucose to glyceraldehyde-3-phosphate). Evidently, if $H_2O_2$ affects other glycolytic enzymes, this does not affect glycolysis significantly compared to the effect caused by the oxidation of GAPDH.

Thus, we demonstrated that the glycolytic rate increases (at least at a low content of ADP) due to the oxidation of a part of the active sites of GAPDH by hydrogen peroxide. The efficiency of glycolysis as the process yielding ATP can be evaluated as the amount of ATP produced per 1 mole of the substrate. Table 1 presents the data on the content of lactate and ATP in the muscle extract after the incubation in the presence of G-3-P and ADP. As seen from the table, the content of lactate and ATP is significantly higher in the presence of $H_2O_2$ compared to that in the presence of 2-mercaptoethanol. However, the efficiency of glycolysis (the ratio ATP/lactate) is significantly higher in the presence of 2-mercaptoethanol. In the presence of the oxidant, 1.04 moles of ATP is produced per 1 mole of lactate, this corresponding to complete uncoupling of the oxidation and phosphorylation in the reactions catalyzed by GAPDH and 3-phosphoglycerate kinase (Fig. 2, reaction *3*). This observation suggests that in the latter case ATP is formed mainly in the pyruvate kinase reaction. Considering that 1 mole of glucose yields 2 moles of glyceraldehyde-3-phosphate, consuming 2 moles of ATP (hexokinase and phosphofructokinase reactions), the total yield of ATP per 1 mole of glucose is zero. Consequently, oxidation of GAPDH by hydrogen peroxide results in acceleration of glycolysis, decreasing the yield of ATP. These data indicated that uncoupling of the oxidation and phosphorylation in glycolysis is due to the mild oxidation of GAPDH.

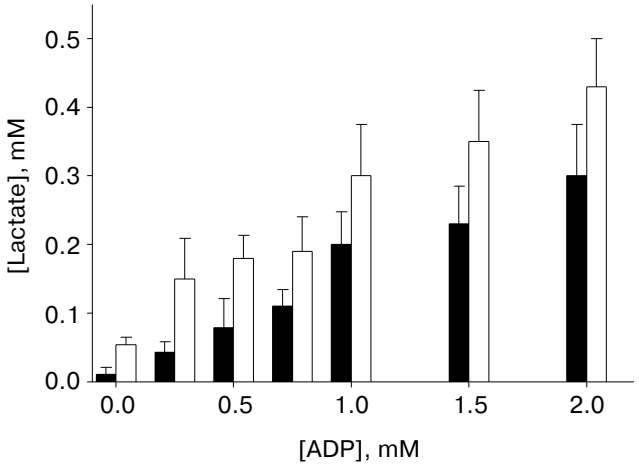


**Fig. 4.** Effect of $H_2O_2$ on the accumulation of lactate in the mixture of glycolytic enzymes in the presence of different concentrations of ADP. The experiments were performed as described Fig. 3 (*1*), but in the presence of different ADP concentrations. The samples contained 10 μM $H_2O_2$ (open rectangles) or 2 mM 2-mercaptoethanol (filled rectangles). The data of four independent experiments are presented as means ± SD.

In the next phase of the study, we investigated the effect of the non-phosphorylating glyceraldehyde-3-

**Table 1.** Accumulation of ATP and lactate during the incubation of muscle extract in the presence of 3 mM glyceraldehyde-3-phosphate and 1 mM ADP

| Incubation | Lactate, mM | ATP, mM | ATP/lactate | ATP/lactate (theoretical value) |
|---|---|---|---|---|
| In the presence of 1 mM 2-mercaptoethanol | 0.186 ± 0.022 | 0.31 ± 0.032 | 1.61 ± 0.14 | 2 (complete coupling) |
| In the presence of 100 μM $H_2O_2$ | 0.62 ± 0.038 | 0.65 ± 0.01 | 1.04 ± 0.08 | 1 (complete uncoupling) |

Note: The conditions of the experiment are given in the legend to Fig. 3. Concentrations of lactate and ATP were determined as described in "Materials and Methods".

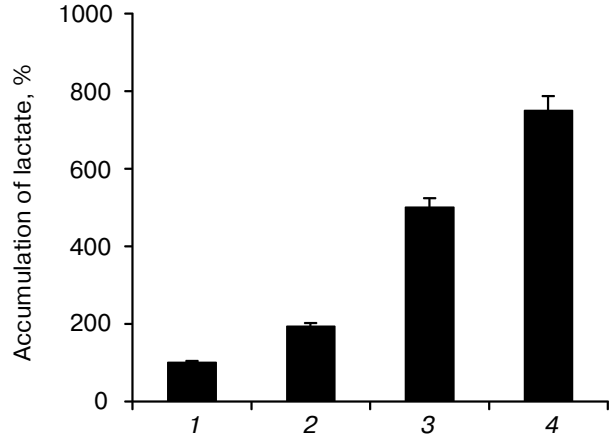


**Fig. 5.** Effect of GAPN on the accumulation of lactate in the muscle extract. The reaction mixture contained the muscle extract (protein concentration, 1 mg/ml), 10 mM $KH_2PO_4$, 0.8 mM $MgSO_4$, pH 7.6, 1 mM NAD, 1 mM ADP, 2 mM NADP, and 3 mM G-3-P. The mixture was incubated at 37°C for 45 min without additions (*2*), in the presence of 2 mM 2-mercaptoethanol without GAPN (*1*), or in the presence of 1 or 3.5 μM GAPN, respectively (*3*, *4*). Then NADH was added into the mixture to provide the complete conversion of pyruvate into lactate, and after 15 min of incubation the samples were assayed for lactate as described in "Materials and Methods".

phosphate dehydrogenase on the rate of glycolysis. According to our model, this enzyme must accelerate glycolysis due to the ADP-independent conversion of G-3-P to 3-phosphoglycerate (without formation of 1,3-diphosphoglycerate). The data presented in Fig. 5 show that the addition of GAPN to the muscle extract increased 5-7-fold the accumulation of lactate (depending on the amount of GAPN added into the sample). It should be noted that all experiments with GAPN were performed in the presence of NADP and NADH, because NADP is the cofactor of GAPN, and the addition of NADH was necessary for the complete conversion of pyruvate into lactate, since NADPH formed in the reaction catalyzed by GAPN cannot be used by lactate dehydrogenase.

Thus, we demonstrated that the alternative ADP-independent pathways that allow bypassing the phosphoglycerate kinase reaction accelerated glycolysis. If the suggested scheme is true (Fig. 2), in the excess of ATP the oxidized GAPDH together with 3-phosphoglycerate kinase must catalyze hydrolysis of ATP according to the scheme presented in Fig. 6. In such a system, 3-PG formed due to coupling of the dehydrogenase and acylphosphatase reactions (reactions *1* and *2*) is phosphorylated in the presence of ATP by 3-phosphoglycerate kinase (reaction *3*) yielding 1,3-diphosphoglycerate that is returned into the cycle. The ADP formed can be used in the pyruvate kinase reaction (*4*). The hydrolysis of ATP in this futile cycle allows glycolysis to proceed in the absence of ADP. This assumption is supported by the data presented in Table 2. Accumulation of lactate was observed in the presence of ATP (and no ADP) in the samples containing hydrogen peroxide that induced the acylphosphatase activity of GAPDH. No lactate accumulated in the samples containing 2-mercaptoethanol, i.e., in the presence of the completely reduced GAPDH. In the presence of the GAPN glycolysis can proceed in the presence of ATP (without addition of ADP) due to the ADP-independent conversion of G-3-P into 3-phosphoglycerate (Fig. 6, reaction *5*). Then 3-phosphoglycerate in the presence of ATP is phosphorylated by 3-phosphoglycerate kinase to yield 1,3-diphosphoglycerate and ADP. As seen from the data presented in Table 2, the addition of GAPN together with the corresponding cofactors into the

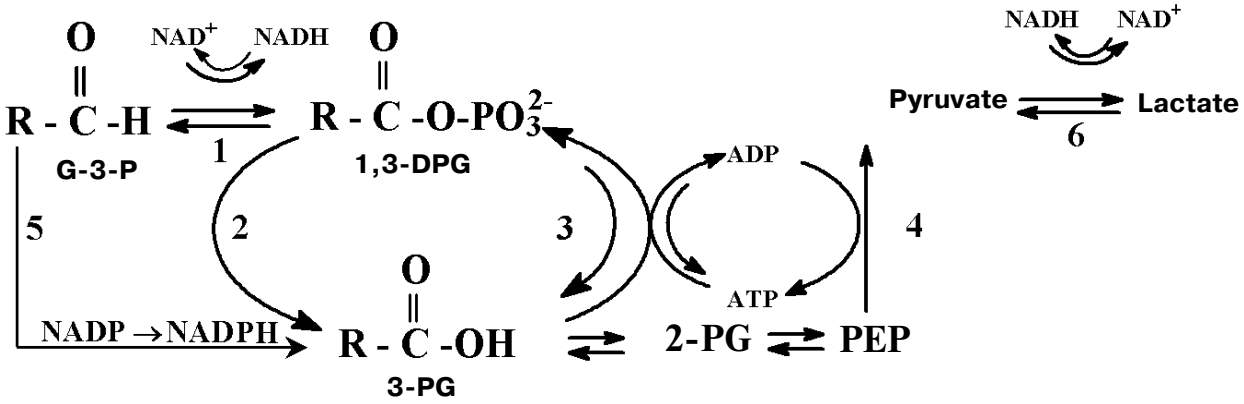


**Fig. 6.** Scheme of hydrolysis of ATP in the mixture of glycolytic enzymes in the presence of oxidized GAPDH (reactions *1*, *2*, and *3*) and in the presence of GAPN (reactions *5*, *3*, and *1*): *1*) GAPDH reduced; *2*) GAPDH oxidized; *3*) 3-phosphoglycerate kinase; *4*) pyruvate kinase; *5*) GAPN; *6*) lactate dehydrogenase. 2-PG stands for 2-phosphoglycerate; PEP, phosphoenol pyruvate.

**Table 2.** Effect of hydrogen peroxide and GAPN on the accumulation of lactate in the mixture of enzymes and muscle extract in the presence of 3 mM G-3-P and 1 mM ATP

| Incubation | Lactate, mM | | |
|---|---|---|---|
| | in the presence of 2-mercaptoethanol | in the presence of $H_2O_2$ | in the presence of GAPN |
| Mixture of enzymes | 0.024 ± 0.006 | 0.27 ± 0.008 | — |
| Muscle extract | 0.015 ± 0.005 | 0.18 ± 0.006 | 0.28 ± 0.01 |

Note: The conditions of the experiment are given in the legends to Figs. 3 and 5 (for GAPN), but 1 mM ATP was added instead of ADP. The concentration of $H_2O_2$ was 10 μM for the enzyme mixture and 100 μM for muscle extract.

muscle extract resulted in the accumulation of lactate. Consequently, the addition of GAPN to the mixture of glycolytic enzymes results in hydrolysis of ATP according to the scheme (Fig. 6), although this pathway is more complicated than the reaction catalyzed by the oxidized GAPDH because it requires the addition of the special enzyme and its cofactor.

Thus, the experiments described above illustrate a new role of GAPDH in the regulation of glycolysis. The effect of oxidation and other types of modifications on the activity of GAPDH are often discussed in the literature. It is considered that any decrease in the activity of GAPDH must inhibit glycolysis and activate the hexose monophosphate shunt [13, 14]. However, the physiological significance of such an inhibition is limited because of a very high concentration of GAPDH in cells (about 10% of the total cytoplasmic proteins). For example, 50% inhibition of the intracellular GAPDH is observed in the presence of 100-1000 μM $H_2O_2$ [12, 15]. It should be noted that even 50% decrease in the activity of GAPDH could not affect glycolysis significantly, because the concentration and the activity of this enzyme are very high. Moreover, high concentrations of $H_2O_2$ required for inhibition of GAPDH can also disturb different cell functions due to oxidative damage of proteins and nucleic acids. Consequently, under these conditions it is difficult to estimate what effects are connected with the oxidation of GAPDH. We assume that the mild oxidation of GAPDH by low concentrations of hydrogen peroxide can play a regulatory role in glycolysis even in the cases when the decrease in the dehydrogenase activity of the enzyme is very small.

In the present work we demonstrated that the mild oxidation of GAPDH accelerate glycolysis in the section G-3-P–lactate due to uncoupling of the reactions catalyzed by GAPDH and 3-phosphoglycerate kinase. Such a pathway would be of no use in anaerobic conditions because of zero yield of ATP. However, under aerobic conditions, glycolysis is not the only possible source of energy. The possibility of cell growth under conditions of uncoupling of the oxidation and phosphorylation in glycolysis was demonstrated using the gene engineering approach. A mutant strain of *E. coli* lacking GAPDH was supplemented with the gene encoding a plant enzyme, NADP-dependent non-phosphorylating glyceraldehyde-3-phosphate dehydrogenase, capable of oxidizing G-3-P yielding 3-phosphoglycerate. Thus, the strain of *E. coli* was constructed that was able to perform glycolysis without the substrate phosphorylation, i.e., yielding no ATP. Such a strain appeared to be capable of growing in aerobic conditions [16]. This approach demonstrated that in the case of aerobic metabolism, glycolysis is required not only for ATP synthesis, but also for production of the substrates for mitochondria. Consequently, under certain conditions, glycolysis yielding no ATP can be useful.

It should be noted that reduced glutathione is always present in the cell under normal conditions, and its concentration is maintained at a high level by NADPH-dependent glutathione reductase. In the presence of glutathione or other reduced thiols the acylphosphatase activity of GAPDH is instantly blocked due to the interaction of the sulfenic acid with the SH-group of the thiol. The reaction is likely to yield a mixed disulfide, so the dehydrogenase activity is not restored:

$$\text{E-SOH} + \text{G-SH} \rightarrow \text{ES-SG} + H_2O.$$

The mechanism of S-thiolation of GAPDH observed in the presence of hydrogen peroxide in monocytes [13] and in endothelial cells [14] is likely based on the reaction presented above. However, some pathological states lead to damage of the mechanisms providing the reduction of glutathione. For example, the deficiency in glucose-6-phosphate dehydrogenase results in the incapability of glutathione reductase to recycle GS-SG because of the lack of NADPH. The glucose-6-phosphate dehydrogenase-deficient cells exhibit an enhanced sensitivity to oxidative stress. Thus, it cannot be excluded that uncoupling of glycolysis caused by the oxidation of GAPDH can be considered as a pathological state connected with the deficiency in the systems involved in the maintenance of the cell redox status.

Recently, *in vivo* experiments demonstrated that NO-producing cells, exhibiting an increased rate of glycolysis, had a decreased activity of GAPDH, and a low rate of ATP formation. The effect of the NO-donors on GAPDH *in vitro* appeared to be similar to that of hydrogen peroxide: these compounds inhibited the dehydrogenase activity of the enzyme, inducing acylphosphatase activity [17]. Based on these data, our assumption on the role of GAPDH in uncoupling of glycolysis [8], and the

experiments on the induction of the acylphosphatase activity of purified GAPDH [7], it was assumed that NO can uncouple glycolysis, inducing the acylphosphatase activity of GAPDH.

It is noteworthy that reactive oxygen species cause uncoupling of the oxidation and phosphorylation in mitochondria due to opening of the mitochondrial pores. This process is a defensive system that prevents the formation of reactive oxygen species [18]. Evidently, uncoupling of oxidation and phosphorylation in mitochondria must be supplemented with the analogous process in glycolysis that was revealed in the present work. Thus, the uncoupling of the oxidation and phosphorylation in glycolysis and mitochondria by peroxides triggers simultaneously the defensive system that protects cells from these compounds.

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