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INTERACTION OF A RAT-KIDNEY ENDOPLASMIC RETICULUM FRACTION WITH GLYCOLYTIC ENZYMES

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SUMMARY

The effects on glycolysis of a rat-kidney microsomal membrane preparation have been studied. The membrane preparation stimulates glycolytic activity more than 50%. The site of this stimulation is shown to be the 3-phosphoglycerate kinase reaction. Evidence is presented that the stimulation is due to ATPase activity of the membrane interacting with ATP associated with the kinase enzyme. The membrane preparation is shown to oxidize DPNH generated by glycolysis. The observed interaction between enzymes of glycolysis and the membrane is interpreted as a channeling of metabolic energy into the membrane for active transport and demonstrates one mechanism whereby active transport stimulates a metabolic pathway.

INTRODUCTION

A fundamental problem in cell biology is the manner in which the cell provides metabolic energy for transport activity at the membrane and how transport activity affects metabolic pathways in the cell[1]. In electron micrographs of kidney cells the cytoplasm appears to be in almost continuous contact with the endoplasmic reticulum or internal cytoplasmic membrane system and with the external cell membrane[2]. ATPase activity associated with coupled sodium and potassium transport has been found in a rat-kidney preparation consisting of membrane fragments of the endoplasmic reticulum system[3]. The present paper shows that in a reconstructed system prepared from rat kidney there is marked stimulation of a glycolytic enzyme system derived from the cell cytoplasm in the presence of the membrane fragments. Evidence is presented for an apparent channeling into the membrane of metabolic energy generated in glycolysis. The specific enzymic sites of interaction are defined.

METHODS

Preparation of the rat-kidney endoplasmic reticulum fraction and supernatant fraction employed have been described in the preceding paper[3]. These fractions were prepared in 0.25 M sucrose with no $^{2+}$M EDTA. Lactate analyses were carried out according to the procedure of BARKER AND SUMMERSON[4] and pyruvate analyses according to the procedure of FRIEDEMANN AND HAUGEN[5]. Incubations were carried out at 37°

in a Dubnoff metabolic incubator shaking at 80 cycles/min. Suitable aliquots of incubation mixtures were deproteinized with cold 5 % trichloroacetic acid and aliquots of the trichloroacetic acid supernatant after centrifugation were analyzed for end products of glycolysis.

Sodium salts of Fru-1,6-P_2, Glc-6-P, ADP and ATP; the calcium salt of 3-phosphoglyceric acid; *d,l*-glyceraldehyde 3-phosphate, DPN, DPNH and crystalline enzymes were obtained from the Sigma Chemical Company. 3-Phosphoglycerate was employed in these experiments as the potassium salt.

Activity of the 3-phosphoglycerate kinase was assayed according to the procedure of BUCHER[6,7]. Acylase activity was assayed by the method of GRISOLIA *et al.*[9].

Microoxygen-uptake measurements of DPNH ioxidation were carried out with a platinum electrode oxygraph manufactured by the Gilson Electronics Co., Middleton, Wisc. Myosin was prepared according to the procedure of SZENT-GYÖRGYI[8].

RESULTS

The effect of adding the endoplasmic reticulum fraction to the glycolyzing supernatant fraction is to increase by 50 % or more the rate of glycolysis throughout the 60-min experimental period. Fig. 1 shows that the increase in rate of glycolysis upon addition of the endoplasmic reticulum fraction is directly related to the amount of the endoplasmic reticulum fraction added. The rate of glycolysis increases as more of the endoplasmic reticulum fraction is added until it finally levels off at a maximal rate. Only trace levels of contamination by the glycolytic enzyme system (of the order of 1 or 2 % of the equivalent supernatant fraction) are found in the endoplasmic reticulum fraction. Activity of 3-phosphoglycerate kinase was tested separately with similar results.

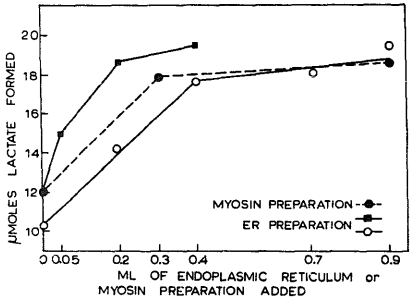

Fig. 1. The effect of varied amounts of added endoplasmic reticulum fraction or a myosin preparation on the rate of glycolysis (Fru-1,6-P_2 to lactate) carried out by the kidney supernatant fraction. Incubation flasks contained 300 μmoles KCl, 9 μmoles $MgCl_2$, 150 μmoles potassium phosphate (pH 7), 3 μmoles ADP, 670 μmoles nicotinamide, 0.033 μmole DPN and 30 μmoles Fru-1,6-P_2. The incubation volume was 3 ml. Each flask contained 0.5 ml of the kidney supernatant fraction. The amount of the endoplasmic reticulum fraction added is indicated in the graph. An amount of 0.25 M sucrose was added to each incubation flask so that the endoplasmic reticulum fraction plus added sucrose totalled 0.9 ml. Where a myosin preparation is added an amount of 0.6 M KCl was added to all flasks so that myosin plus KCl equalled 0.9 ml. Each incubation was for 60 min.

The section of the glycolytic pathway affected by the endoplasmic reticulum fraction is shown by the data presented in Table I to be between Fru-1,6-P_2 and 3-phosphoglycerate. Stimulation of glycolysis is found with either Glc-6-*P* or Fru-1,6-P_2 as substrate, but does not occur with 3-phosphoglycerate as substrate. With Glc-6-*P* or Fru-1,6-P_2 as substrate the formation of lactate is increased 50% or

TABLE I

THE EFFECT OF THE ENDOPLASMIC RETICULUM FRACTION ON GLYCOLYSIS

Each incubation contained 300 μmoles KCl, 9 μmoles $MgCl_2$, 150 μmoles potassium phosphate (pH 7), 3 μmoles ADP, 3 μmoles ATP, 670 μmoles nicotinamide, 0.033 μmole DPN and 30 μmoles substrate. The incubation volume was 3 ml. Each flask contained 0.5 ml of the kidney supernatant fraction. Each flask contained either 0.9 ml of the endoplasmic reticulum fraction or 0.9 ml of 0.25 M sucrose. Experiments were paired with and without the endoplasmic reticulum fraction (ER) for each kidney preparation. The incubations were for 60 min. The results are reported ± S.E. of the mean.

Reaction	*Number of preparations*	*μmoles lactate or pyruvate formed*	
		Without ER	*ER added*
Glc-6-*P* to lactate	1	12.5	18.6
	1	16.6	25.4
Fru-1,6-P_2 to lactate	12	13.7 ± 1.06	23.2 ± 1.34
Fru-1,6-P_2 to lactate (arsenate $2.8 \cdot 10^{-3}$ M)	5	16.9 ± 0.44	17.3 ± 0.57
3-Phosphoglycerate to pyruvate	4	14.2 ± 0.69	14.2 ± 0.45

more. It should be noted that with these substrates the pyruvate formed is about 20% of the total pyruvate and lactate formed as end products of glycolysis. This equilibrium is not greatly affected by the addition of the endoplasmic reticulum fraction. Therefore, lactate can serve as an overall index of glycolytic activity. When 3-phosphoglycerate was used as substrate in our preparation only pyruvate was formed since no DPNH is generated. The conversion of 3-phosphoglycerate to pyruvate is not stimulated by adding the endoplasmic reticulum fraction.

Lower levels of the following glycolytic cofactors did not appear to alter stimulation of glycolytic activity by the endoplasmic reticulum fraction. Stimulation was observed with phosphate levels as low as 3 mM. Stimulation was also observed with DPN levels from 10^{-7}–10^{-4} M. Stimulation was found at all magnesium levels between 1 and $12 \cdot 10^{-3}$ M. The following two reactions are part of the glycolytic pathway that is stimulated.

Glyceraldehyde 3-phosphate + DPN + P_i

$$\xrightarrow[\text{dehydrogenase}]{\text{glyceraldehyde 3-phosphate}} \text{1,3-diphosphoglycerate} + \text{DPNH}$$

1,3-Diphosphoglycerate + ADP

$$\xrightarrow[\text{kinase}]{\text{3-phosphoglycerate}} \text{3-phosphoglycerate} + \text{ATP}$$

If adequate levels of arsenate are present the glyceraldehyde 3-phosphate in the presence of its dehydrogenase forms the unstable 1-arseno-3-phosphoglycerate

which spontaneously decomposes to 3-phosphoglycerate[10,11]. This eliminates the 3-phosphoglycerate kinase reaction from the glycolytic sequence. When arsenate is added to a glycolyzing incubation the rate of glycolysis is enhanced[12]. Table I shows that when arsenate is present no stimulation occurs upon addition of the endoplasmic reticulum fraction. This observation points to the 3-phosphoglycerate kinase enzyme as the site of interaction with the endoplasmic reticulum fraction that affects the rate of glycolysis.

ATPase activity of the endoplasmic reticulum fraction has been previously described[3]. It was postulated that the ATPase activity of the endoplasmic reticulum fraction is responsible for the stimulation of glycolysis and that other active ATPase preparations should also stimulate glycolysis. Several myosin preparations from rabbit skeletal muscle with high ATPase activity were added to the kidney glycolyzing system. Stimulus of glycolysis ranging from 50–90% is obtained with these preparations. One of these experiments is plotted in Fig. 1.

ATPase activity could serve to maintain maximal levels of glycolysis by regenerating adequate substrate levels of ADP from ATP as the ADP is depleted by 3-phosphoglycerate kinase activity. High substrate levels of ADP would be expected to substitute for such an effect. The experiment plotted in Fig. 2 shows that a stimulation of glycolysis by the endoplasmic reticulum fraction takes place at almost all substrate levels of ADP. The only exception is at the very lowest ADP level studied. This may be due to the ADPase activity of the endoplasmic reticulum fraction destroying the limited ADP available. The soluble glycolyzing fraction with or without the endoplasmic reticulum fraction added will carry out glycolysis if ATP substitutes for ADP. An experiment with varying levels of ATP in the absence of ADP resulted in glycolytic activities almost identical to those seen with ADP in Fig. 2. If the ATPase

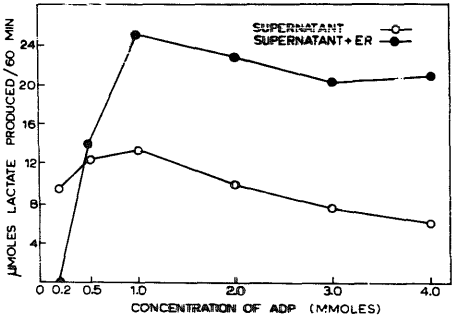

Fig. 2. The effect of varying levels of ADP on glycolysis (Fru-1,6-P_2 to lactate) carried out by the kidney supernatant fraction in the presence and absence of the endoplasmic reticulum fraction. Substrate, cofactor, and enzyme additions are described in the legend of Fig. 1. ADP levels are those indicated on the abscissa of the graph. Each incubation was for 60 min.

activity of the endoplasmic reticulum fraction is the cause of the increased glycolysis, this ATPase is producing its effect by acting on ATP formed at the 3-phosphoglycerate kinase enzyme site rather than merely regenerating ADP in the incubation medium.

Fig. 3 is a study of the effect of the endoplasmic reticulum fraction on crystalline yeast 3-phosphoglycerate kinase enzyme activity. The fraction has two effects on the 3-phosphoglycerate kinase enzyme reaction: The rate of the reaction is more than doubled and the reaction proceeds irreversibly to completion rather than to the equilibrium point reached with 3-phosphoglycerate kinase in the absence of the endoplasmic reticulum fraction. The reaction with 3-phosphoglycerate kinase is assayed by measuring at 340 mμ the absorbancy change resulting from DPNH formed by the dehydrogenase as glyceraldehyde 3-phosphate is converted by two enzymic reactions to 3-phosphoglycerate. The endoplasmic reticulum fraction contains an active DPNH oxidase which readily reoxidizes the DPNH formed in the above reactions to DPN. Deoxycorticosterone inhibits this DPNH oxidase and was added to the cuvettes in order to permit the measurement of the absorbancy change that results from the kinase reaction. The reaction of the glyceraldehyde 3-phosphate in the presence of arsenate is also shown for comparison. This reaction proceeds irreversibly and rapidly to completion. The initial absorbancy change that occurs on adding the endoplasmic reticulum fraction is due to turbidity of the particulate fraction.

It is proposed that the 3-phosphoglycerate kinase reaction is irreversible in the presence of the endoplasmic reticulum fraction because ATP formed by the 3-phosphoglycerate kinase enzyme is hydrolyzed by the fraction. In the complete glycolytic enzyme system stimulation of glycolysis occurs even at high ATP levels suggesting that hydrolysis of the ATP occurs at the kinase enzyme site. An alternate hypothesis would be an acylase cleavage by the endoplasmic reticulum fraction of the 1,3-diphosphoglycerate to yield 3-phosphoglycerate and phosphate. Such a reaction would permit glyceraldehyde 3-phosphate to be converted to 3-phosphoglycerate in the

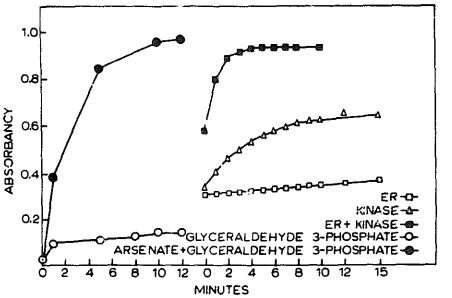

Fig. 3. Activity of crystalline 3-phosphoglycerate kinase in the presence and absence of the endoplasmic reticulum fraction. The initial additions to all 3 cuvettes were 150 μmoles potassium phosphate (pH 6.9), 1.2 μmoles $MgCl_2$, 1.2 μmoles ADP, 1.3 μmoles DPN, 1.5 μmoles *d,l*-glyceraldehyde 3-phosphate, 6 μmoles glutathione and 0.15 mg crystalline glyceraldehyde-3-phosphate dehydrogenase. 12 min was allowed for equilibration of the dehydrogenase reaction. Then 1 mg deoxycorticosterone in 0.02 ml ethanol was added to each cuvette and 0.1 μg of the crystalline yeast 3-phosphoglycerate kinase and/or 0.1 ml of the endoplasmic reticulum was added as indicated on the graph. Final incubation volume was 3 ml. The arsenate incubation contained no ADP, no $MgCl_2$, only 50 μmoles potassium phosphate (pH 6.9) and had in addition 225 μmoles sodium arsenate. The endoplasmic reticulum fraction employed in this experiment was freed of traces of kinase activity by repeated washings of the preparation.

presence of the dehydrogenase and endoplasmic reticulum fraction and in the absence of ADP and the kinase. The reaction, however, does not go unless ADP and the kinase are also present. When acylase activity was determined with acetyl phosphate as substrate, no acylase activity was found in the endoplasmic reticulum fraction.

The K_m for ADP in the crystalline yeast 3-phosphoglycerate kinase enzyme has been reported[7] as $2 \cdot 10^{-4}$ M. This was the approximate value found for our crystalline yeast preparation. The apparent K_m for ADP was also determined when the endoplasmic reticulum fraction was added to the kinase enzyme. Values in 3 determinations ranged from 2 to $4 \cdot 10^{-4}$ M which is essentially no change. ADPase activity present in the endoplasmic reticulum fraction may adversely affect the accuracy of these measurements. This absence of a significant change in the K_m is in contrast to studies of the interaction of creatine kinase and myosin ATPase[13] where lowering of the K_m for adenine nucleotides from $3 \cdot 10^{-4}$ M to 10^{-6} M was observed when both enzymes were present.

The endoplasmic reticulum fraction of kidney oxidizes DPNH. This can be measured both by absorbancy changes at 340 mμ indicating the disappearance of DPNH and by the uptake of oxygen measured with a platinum electrode oxygraph. Fig. 4 shows that there is an almost stoichiometric oxygen uptake when DPNH is added to the endoplasmic reticulum fraction.

A second interaction between the glycolytic enzyme system and the endoplasmic reticulum fraction is shown in Fig. 4. In glycolysis DPNH is generated by the oxidation of glyceraldehyde 3-phosphate. If only glycolytic enzymes are present most of this DPNH is reoxidized when pyruvic acid becomes lactic acid. In homogenates or reconstructed cell systems the mechanism of reoxidation of DPNH generated in the course of glycolysis has not been completely elucidated[14]. Although the rat-kidney

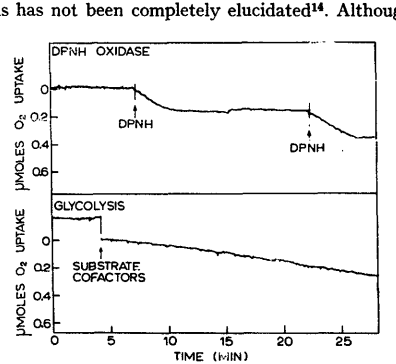

Fig. 4. Oxygen uptake of the endoplasmic reticulum fraction in the presence of DPNH or a glycolytic system as recorded from the platinum electrode. Reactions were measured at room temperature. The incubation mixture for DPNH oxidation contained 150 μmoles Tris buffer (pH 7) 200 μmoles KCl, 100 μmoles potassium phosphate buffer (pH 7) and 0.2 ml of the endoplasmic reticulum fraction. The incubation volume was 2.4 ml. 0.375 μmole DPNH was added as indicated on the chart. Mixtures for glycolysis contained 240 μmoles KCl, 220 μmoles potassium phosphate buffer (pH 7), 0.2 ml of the kidney supernatant fraction and 0.2 ml of the endoplasmic reticulum fraction. Cofactors and substrates indicated on the chart were 2.4 μmoles ADP, 2.4 μmoles $MgCl_2$ and 15 μmoles Fru-1,6-P_2. The final incubation volume was 2.4 ml.

glycolyzing system does use DPNH to form lactate, it is seen in Fig 4 that the glycolyzing system in the presence of the endoplasmic reticulum fraction has an oxygen uptake. No oxygen uptake is observed if the membrane fraction is absent. The following observations indicate that the source of this oxygen uptake is the DPNH generated by the glyceraldehyde-3-phosphate dehydrogenase reaction. The oxygen uptake in glycolysis when the endoplasmic reticulum fraction is present occurs with Fru-1,6-P_2 as substrate but does not occur if 3-phosphoglycerate is the substrate. The endoplasmic reticulum DPNH oxidase is inhibited more than 50% by 10^{-5} M deoxycorticosterone and is almost completely inhibited by 10^{-4} M deoxycorticosterone. (The DPNH oxidase found in the microsomal fraction of rat kidney is inhibited by many steroids[15].) Glycolytic oxygen uptake is almost completely inhibited by 10^{-4} M deoxycorticosterone.

It appears that the continuous production of DPNH in glycolysis results in a small but continuous DPNH oxidase reaction in the endoplasmic reticulum which can be visualized as a steady flow of electrons from DPNH to oxygen within the membrane.

DISCUSSION

The properties of ATPase activity found in this rat-kidney membrane preparation have been described in the preceding paper[3]. The data strongly indicated that this ATPase system is a component of the system for active transport of sodium and potassium across kidney membranes.

The present paper shows that this kidney membrane preparation stimulates glycolytic activity of the soluble cytoplasmic enzyme fraction by interacting with 3-phosphoglycerate kinase. The following experimental observations support the concept that this interaction stimulating glycolysis is probably due to ATPase activity of the membrane interacting specifically with ATP generated by the kinase. A myosin preparation with ATPase activity stimulates glycolysis to about the same extent. Acylase activity which, if present, could stimulate glycolysis by eliminating the kinase step is not found in the membrane. Varied levels of DPN, Mg^{2+} or P_i did not affect the stimulation. The stimulating action of the ATPase could be attributed to regeneration of ADP in the medium. However, high levels of ADP or ATP in the medium did not affect the stimulation. This means that if ATP hydrolysis stimulates glycolysis it must occur at the kinase site. The kinase reaction studied with a crystalline 3-phosphoglycerate kinase preparation was found to be accelerated by the membrane preparation with a shift of equilibrium toward 3-phosphoglycerate. This is readily explained if ATP generated by the kinase is hydrolyzed by the membrane.

The significance of this interaction is its apparent direct coupling of a metabolic reaction which generates ATP in the cytoplasm with a membrane ATPase that participates in active transport of sodium and potassium. It can be assumed that any metabolically generated ATP might have access to the membrane. However, these experiments do demonstrate a specific point of interaction that affects the overall rate of glycolysis. This direct demonstration adds to the accumulated evidence that glycolytic energy is used for active-transport processes.

The oxidation of glyceraldehyde 3-phosphate results in a second interaction with the membrane fraction. A part of the DPNH generated is oxidized by the membrane

fraction. This DPNH oxidation generates a steady flow of electrons from DPNH to oxygen in the membrane. This electron flow represents a measurable energy input into the membrane and is readily inhibited by 10^{-4} M nonpol arsteroid hormones. It is tempting to postulate that this interaction of DPNH generated by glycolysis and the DPNH oxidase of the membrane is a component of ion-transport systems in the membrane.

It should be noted that in this system nearly all of the DPNH formed in glycolysis is used to convert pyruvate to lactate. In the intact cell relatively little lactate is formed. It is possible that much more glycolytically generated DPNH is available to the membrane in the intact cell.

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