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IN VITRO FORMATION OF GLUCOSE-6-PHOSPHATE FROM GLYCERALDEHYDE-3-PHOSPHATE BY LIVER CYTOSOL FROM FED AND STARVED RATS. EFFECT OF DIVALENT CATIONS ON THE CONVERSION RATE

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SUMMARY

Liver cytosol preparations from fed rats are shown to form glucose-6-phosphate from glyceraldehyde-3-phosphate at a rate of 1.6 μmoles·min^{-1}·g liver wet weight^{-1} in presence of 0.4 mM Mg^{2+}. This rate is more than doubled by 30 μM EGTA and/or Mg^{2+}-concentrations ≥2 mM. 48 hours starvation increases the rate of glucose-6-phosphate formation at 0.4 mM Mg^{2+} to 3.0 μmoles·min^{-1}·g liver wet weight^{-1} and greatly diminishes the effect of EGTA and of higher Mg^{2+}-concentrations. Inhibition of glucose-6-phosphate formation by Ca^{2+} and Zn^{2+} is shown to be more pronounced with cytosol from fed than from 48 hours starved rats.

INTRODUCTION

Gluconeogenesis from dihydroxiacetone or glycerol in rat liver has been reported to be stimulated by starvation (1,2,3). This stimulation of glucose production from substrates entering gluconeogenesis at the triosephosphate level has mainly been attributed to inhibition of the glycolytic key enzymes phosphofructokinase (4) or pyruvate kinase (3). A mode of stimulation involving activation of fructose bisphosphatase was considered unlikely because, with one exception (5), little (6) or no difference has been found between fructose bisphosphatase activity from fed and starved rat liver (7) or rabbit liver (8). Pontremoli et al (9) even observed a decrease of fructose bisphosphatase activity during the first 48 hours of starvation. On the other hand, it has been shown by Horecker and collaborators (10) that Zn^{2+} is a potent inhibitor of fructose bisphosphatase, and it was postulated (10,11,12) that variations in the cytosolic Zn^{2+}-concentration might be important for the regulation of gluconeogenesis. Furthermore, Garfinkel et al (13), on the basis of computer simulation studies, have proposed a role of cytosolic Mg^{2+}-concentration in the regulation of fructose bisphosphatase and hence of gluconeogenesis.

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In the present study, the influence of divalent cations and starvation on the conversion of glyceraldehyde-3-P to glucose-6-P by rat liver cytosol was studied in an in vitro system.

MATERIALS AND METHODS

Male albino Wistar rats from the Swiss Vitamine Institute in Basel, weighing 180 to 230 g were either fed or starved for 48 hours prior to use.

For the preparation of cytosols, 2 grams of liver (wet weight) from rats killed by decapitation were immediately homogenized at 4°C with 10 ml of a buffer containing 0.14 M sucrose, 0.05 M potassium phosphate buffer pH 7.2 and 2 mM GSH. Final volume was 12 ml. The homogenate was centrifuged for 1 hour at 100,000 x g and 4°C. Protein concentrations in the resulting undiluted cytosols were 16.7 ± 1.6 mg (n=24) for fed and 19.2 ± 2.2 mg (n=21) for starved rats (mean ± S.D.)per ml supernatant.

Initial rates of glucose-6-P formation (see below) were found to be stable for at least 3 hours when the undiluted cytosols from starved rats were kept on ice. Cytosols from fed rats were diluted 1:2 with homogenization buffer directly after centrifugation in order to stabilize the rate of glucose-6-P formation which otherwise increased with time.

Initial rates of glucose-6-P formation from glyceraldehyde-3-P by rat liver cytosols were measured at 30°C using NADP and an at least 50-fold excess of glucose-6-P dehydrogenase as indicator system. The basic assay mixture (3 ml) contained 33 mM TRIS-HCl buffer pH 7.4, 0.66 mM GSH, 0.51 mM NADP, 3.5 units of glucose-6-P dehydrogenase and rat liver cytosol corresponding to 8.3 mg liver wet weight. Further additions are indicated in the legends. In a preincubation, endogenous glucose-6-P present in the cytosol was oxidized before the reaction was started by addition of glyceraldehyde-3-P. The reaction was followed by measuring the formation of NADPH at 340 nm. After a lag phase of approximately 1 minute, the rate became linear for at least 2 to 3 minutes and this linear phase was taken as initial rate of glucose-6-P formation. With cytosol from fed but not from starved rats, a slight NADPH-formation from 6-P-gluconolactone was observed, which was, however, not influenced by the effectors used in this study and was therefore neglected.

Glyceraldehyde-3-P was freshly prepared from glyceraldehyde-3-P-diethylacetal for each experiment and the added concentrations were determined spectrophotometrically (14). Free Ca^{2+} was determined with arsenazo III (Sigma, grade I, A-8891) as described by Harris (15) directly in the assay, in which glucose-6-P formation had been measured. The Ca^{2+}-standard curve was determined in an identical assay mixture. Mg^{2+} was determined in the assay mixtures by titration with 1 mM EDTA at pH 10.0. Eriochromblack T was used as indicator.

For the calculation of results 1 ml of undiluted cytosol was considered equivalent to 0.167 g liver wet weight.

Table I. Conversion of glyceraldehyde-3-P to glucose-6-P with liver cytosol from fed or 48 h starved rats

Additions to basic medium	Rate of glucose-6-P formation	
	Fed	Starved
	$\mu moles \cdot min^{-1} \cdot g\ liver^{-1}$	
10 mM $MgSO_4$, glyceraldehyde-3-P 0.33-0.50 mM	4.6 ± 0.2 (5)	4.9 ± 0.2 (9)
0.4 mM $MgSO_4$, glyceraldehyde-3-P 0.20-0.30 mM	1.6 ± 0.2 (24)	3.0 ± 0.2* (12)

Initial rates of glucose-6-P formation were determined as described under Materials and Methods. Each result represents the mean ± S.E.M. of the number of animals given in brackets; *p-value (fed vs starved) <0.001.

RESULTS

Maximal rates for the formation of glucose-6-P by rat liver cytosols were observed in the presence of 10 mM Mg^{2+} and glyceraldehyde-3-P concentrations of more than 0.3 mM. Under these conditions, initial rates with liver cytosols from either fed or 48 hours starved rats were about equal (table I). At 0.4 mM Mg^{2+}, which approximately corresponds to the cytosolic concentrations of free Mg^{2+} (16), the rate of glucose-6-P formation was reduced and a highly significant increase of glucose-6-P formation due to starvation was observed (table I).

As observed in analogous incubation experiments, the concentrations of dihydroxyacetone-P and fructose-1,6-P_2 remained almost constant during the linear phase of glucose-6-P formation whereas the concentration of glyceraldehyde-3-P decreased. The concentration of dihydroxyacetone-P reached 30 to 40 % of the added glyceraldehyde-3-P concentration. Fructose-1,6-P_2 amounted to about 10 µM. Thus at initial glyceraldehyde-3-P concentrations ≤0.3 mM, dihydroxyacetone-P and fructose-1,6-P_2 were both within the range observed in rat liver (17,18).

As shown in fig. 1, the Mg^{2+}-dependence of glucose-6-P formation from glyceraldehyde-3-P was more pronounced with cytosols from fed than from 48 hours starved rats. As a consequence, gluconeogenic activity varied with the nutritional state at Mg^{2+}-concentrations below 1 mM. At Mg^{2+}-concentrations of 2 mM and higher and/or in presence of EGTA, cytosols from fed or starved rats

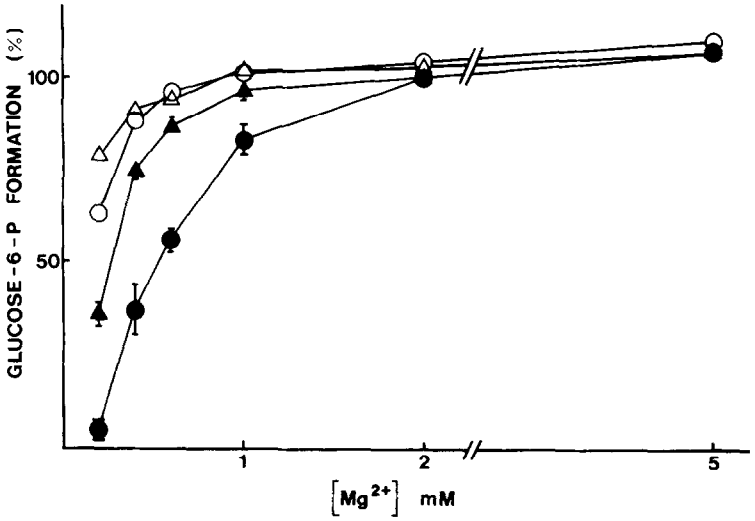

Figure 1. Influence of Mg^{2+} and EGTA on the conversion of glyceraldehyde-3-P to glucose-6-P with liver cytosol from fed and 48 h starved rats. Initial rates of glucose-6-P formation were measured in absence (●,▲) or presence (○,△) of 30 µM EGTA as described under Materials and Methods. Liver cytosols from either fed (●,○) or 48 h starved rats (▲,△) were used. The basic medium was supplemented with $MgSO_4$ and 0.22 - 0.31 mM glyceraldehyde-3-P. Mg^{2+}-concentrations present in the individual assays were determined as described under Materials and Methods. Results are expressed in % of the activity at 2 mM Mg^{2+} in absence of EGTA. Each point represents the mean from 3 individual livers ± S.E.M.

did not differ with respect to glucose-6-P formation. EGTA increased glucose-6-P formation at Mg^{2+}-concentrations below 2 mM. This stimulation was three times larger with cytosols from fed rats as compared to 48 hours starved rats (fig. 1). The EGTA-concentration required for half-maximal stimulation was about 4 µM with cytosol from fed and <1 µM with cytosol from 48 hours starved rats.

In fig. 2, glucose-6-P formation is shown in relation to the ratio of Mg^{2+}/free Ca^{2+}. Since these ratios varied from experiment to experiment, individual data from 3 fed and 3 starved livers are given. The Mg^{2+}-concentrations in these experiments were between 0.3 and 0.5 mM, the free Ca^{2+}-concentrations between 2 and 35 µM. Under these conditions, glucose-6-P formation with cytosol from fed rats was very sensitive to changes in the Mg^{2+}/free Ca^{2+} ratio, 30 % inhibition being reached at ratios between 31 and 38. Starvation diminished the regulatory effect of the

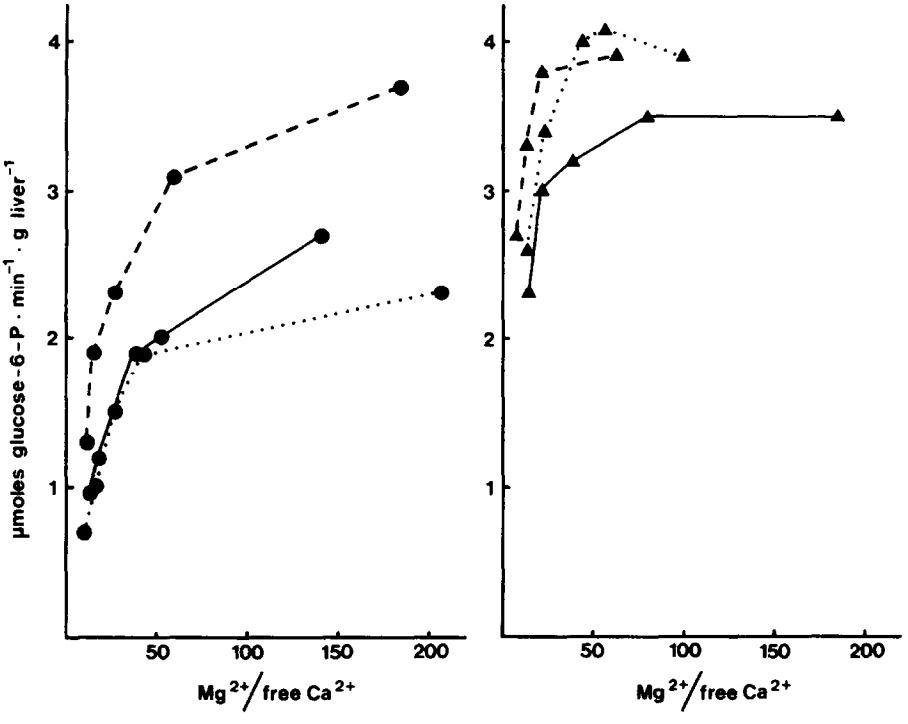

Figure 2. Influence of changing Mg^{2+}/free Ca^{2+} ratios on glucose-6-P formation from glyceraldehyde-3-P with liver cytosols from 3 fed (●) and 3 48 h starved (▲) rats. Initial rates of glucose-6-P formation were measured as described under Materials and Methods. The basic medium was supplemented with 0.4 mM $MgSO_4$, 10 µM EGTA, 0 - 30 µM $CaCl_2$ and 0.22 - 0.27 mM glyceraldehyde-3-P. Mg^{2+} and free Ca^{2+} were determined directly in the individual assays as described under Materials and Methods.

Mg^{2+}/free Ca^{2+} ratio, the ratio for 30 % inhibition being reduced to about 14. However, the activating effect of EGTA cannot be attributed to complexation of Ca^{2+} alone. As shown in table II, readdition of an equimolar quantity of Ca^{2+} to a system containing 10 µM EGTA did not completely suppress the activating effect of the chelator although the concentration of free Ca^{2+} was higher than in the control. This indicates that beside Ca^{2+} at least one other inhibiting cation is complexed by EGTA. This ion may possibly be Zn^{2+}, which in parallel to Ca^{2+}, had a stronger effect on glucose-6-P formation with cytosol from fed than from 48 hours starved rats (table II).

DISCUSSION

The cytosolic system used in the present study forms glucose-6-P from glyceraldehyde-3-P at a rate which is at least 10 times

Table II. Influence of free Ca^{2+} and Zn^{2+} on the conversion of glyceraldehyde-3-P to glucose-6-P

Additions	Glucose-6-P formation		Free Ca^{2+}
	$\mu moles \cdot min^{-1} \cdot g\ liver^{-1}$	%	μM
Fed			
Control	1.0	44	8
+ EGTA	2.3	100	2
+ EGTA + $CaCl_2$	1.5	65	13
+ EGTA + $CaCl_2$ + $ZnSO_4$	0.8	35	13
48 h STARVED			
Control	3.0	86	8
+ EGTA	3.5	100	3
+ EGTA + $CaCl_2$	3.2	91	13
+ EGTA + $CaCl_2$ + $ZnSO_4$	2.4	69	13

Initial rates of glucose-6-P formation were determined as described under Materials and Methods. The basic medium was supplemented with 0.4 mM $MgSO_4$ and 0.25 - 0.27 mM glyceraldehyde-3-P. Other additions: EGTA, 10 µM; $CaCl_2$, 10 µM; $ZnSO_4$, 0.5 µM. Free Ca^{2+} was measured as described under Materials and Methods.

higher than the gluconeogenic rate from the same substrate observed by McDermott and Veneziale (19) in their in vitro system. At low Mg^{2+}-concentrations, the observed rates of glucose-6-P formation in our system are about equal to the gluconeogenic rate from trioses in perfused rat liver (1) and rat hepatocytes (3).

The doubling of glucose-6-P formation in response to starvation, cannot be attributed to inhibition of glycolytic key enzymes (3,4) because, in absence of the necessary cofactors, these enzymes are inoperative in the present system. In experiments with fructose-1,6-P_2 as substrate (not shown), in principle the same dependence of the conversion rate on EGTA, the Mg^{2+}-concentration and the nutritional state was observed as with glyceraldehyde-3-P. The present study was carried out with glyceraldehyde-3-P as substrate because with fructose-1,6-P_2 at non-saturating physiological concentrations, reaction rates were not linear due to the rapid change of substrate concentrations by aldolase and fructose bisphosphatase. Since glucose-6-P isomerase activity in rat liver (20) by far exceeds our ob-

served rates of glucose-6-P formation, the regulatory enzyme in our system is likely to be fructose bisphosphatase.

In earlier studies (7,9) the activation of fructose bisphosphatase by starvation has not been observed because the enzymatic activity was measured at Mg^{2+}-concentrations of 2 mM and higher. At these concentrations, also in our system no difference of glucose-6-P formation between cytosol from fed and starved rats was found.

With liver cytosol from fed rats, the rate of glucose-6-P formation is inhibited by divalent cations which compete with Mg^{2+} and are complexed by EGTA. This sensitivity towards the ratio of Mg^{2+}/inhibitory cation may provide a short term regulatory mechanism controlling the conversion of trioses to glucose by changing the concentration of either free Mg^{2+} or an inhibiting cation, possibly Ca^{2+} or Zn^{2+}.

The loss of sensitivity towards inhibiting cations upon starvation leads to a stable enhancement of the gluconeogenic rate in cytosol from 48 hours starved rats. It could be postulated that phosphorylation of fructose bisphosphatase as reported by Riou et al (21) might induce a different response of the enzyme towards divalent cations. The finding that the activities of phosphorylated and dephosphorylated fructose bisphosphatase differ only in the absence of EDTA (21) would be in agreement with the present observation that EGTA abolishes the difference between cytosol from fed and starved rats with respect to glucose-6-P formation.

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