STUDY ON THE REGULATORY ROLE OF FRUCTOSE-1,6-DIPHOSPHATE IN THE FORMATION OF AMP IN RAT SKELETAL MUSCLE.

A MECHANISM FOR SYNCHRONIZATION OF GLYCOLYSIS AND THE PURINE NUCLEOTIDE CYCLE

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Summary: Fructose-1,6-diphosphate strongly inhibited adenylosuccinate synthetase purified from rat skeletal muscle. This compound was found to be a non-competitive inhibitor of all substrates of the enzyme. No other glycolytic intermediates affected adenylosuccinate synthetase activity. From these findings, it was proposed that this inhibition might play an important role in the oscillation of glycolysis in skeletal muscle.

INTRODUCTION

Ghosh and Chance (1) first observed a reciprocal oscillation between the concentrations of fructose-6-phosphate and fructose-1,6-diphosphate during glycolysis in yeast cells. Later Frenkel (2) reported a similar glycolytic oscillation in beef heart extracts depending on modifiers of the activity of phosphofructokinase [EC 2.7.1.11], a rate-limiting enzyme of glycolysis. Recently, Tornheim and Lowenstein (3) reported that glycolytic oscillation occurred on addition of glucose to particle-free extracts of rat skeletal muscle and suggested that the purine nucleotide cycle (IMP → adenylosuccinate → AMP → IMP) might be closely linked to this oscillation, because the concentration of AMP, an activator of phosphofructokinase, also fluctuated synchronously with oscillation of glycolytic intermediates. Therefore, we tested whether glycolytic intermediates played a critical role in regulation of the AMP concentration.

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This paper reports the inhibitory effect of fructose-1,6-diphosphate on adenylosuccinate synthetase (IMP: L-aspartate ligase (GDP), [EC 6.3.4.4]), a member of the purine nucleotide cycle, and discussion of its physiological significance.

MATERIALS AND METHODS

Male Wistar HLA strain rats were used throughout. Adenylosuccinate synthetase was assayed in reaction mixture containing in 250 µl, IMP (1 mM), GTP (0.5 mM), L-aspartate (4 mM), imidazole-HCl buffer, pH 6.8 (30 mM), $MgCl_2$ (8 mM) and enzyme solution. The reaction was initiated by addition of aspartate and terminated by addition of perchloric acid after incubation for 5 minutes at 37°. The adenylosuccinate formed in the supernatant was determined by the method of Lieberman (4). Adenylosuccinase (adenylosuccinate AMP-lyase, [EC 4.3.2.2]) and AMP deaminase (AMP aminohydrase, [EC 3.5.4.6]) were assayed by the methods of Carter and Cohen (5) and Smiley et al. (6), respectively. Protein was determined by the method of Lowry et al. (7) with bovine serum albumin as a standard.

Adenylosuccinate synthetase from rat skeletal muscle was purified by the method of Ogawa et al. (8). The purified preparation formed a single band on SDS-polyacrylamide gel electrophoresis. AMP deaminase was crystallized by a modification of the method of Smiley et al. (6). Adenylosuccinase was partially purified by phosphocellulose column chromatography.

IMP and GTP were purchased from Kyowa Hakko (Tokyo). All glycolytic intermediates used were products of Sigma (U.S.A.) except glucose-6-phosphate, fructose-6-phosphate, and fructose-1,6-diphosphate which were from Boehringer (Germany).

RESULTS AND DISCUSSION

The effects of glycolytic intermediates on adenylosuccinate synthetase are shown in Table 1. Fructose-1,6-diphosphate was found to be the most potent inhibitor of the intermediates tested. Triose phosphate seemed to be slightly inhibitory, but inorganic ortho-phosphate was as inhibitory at the same concentration (data not shown). This suggests that triose phosphates may not act as specific inhibitors of the enzyme.

The dose-response curve of the effect of fructose-1,6-diphosphate on enzyme activity is shown in Fig. 1. The minimal inhibitory concentration of fructose-1,6-diphosphate was 10 µM and 2 mM fructose-1,6-diphosphate caused almost complete inhibition.

The concentration of fructose-1,6-diphosphate in rat skeletal muscle at the crest of glycolytic oscillation has not been accurately determined

Table 1. Effects of glycolytic intermediates on adenylosuccinate synthetase activity

Addition	inhibition (%)
Glucose	5
Glucose-6-phosphate	2
Fructose-6-phosphate	8
Fructose-1,6-diphosphate	80
Dihydroxyacetone phosphate	3
Glyceraldehyde-3-phosphate	0
2,3-Diphosphoglycerate	18
3-Phosphoglycerate	17
2-Phosphoglycerate	16
Phosphoenolpyruvate	16
Pyruvate	0
Lactate	6

Enzyme activity was determined in the presence of 2 mM of each glycolytic intermediate. The results are expressed as percentage inhibitions of the activity without glycolytic intermediates. Each reaction system contained 16 munits of enzyme. One unit of enzyme activity was defined as the amount of enzyme catalyzing the formation of 1 μmole of adenylosuccinate per min per mg protein.

yet, but the concentration in resting muscle was reported to be 40 to 60 μM (9-10). Thus it is quite possible that fructose-1,6-diphosphate participates in regulation of adenylosuccinate synthetase activity *in vivo*. Neither fructose-1,6-diphosphate nor other glycolytic intermediates affected the adenylosuccinase and AMP deaminase activites of rat skeletal muscle.

As shown in Fig. 2, the double reciprocal plots of the initial rates with fructose-1,6-diphosphate and aspartate, IMP, and GTP indicate that fructose-1,6-diphosphate is a non-competitive inhibitor with all these substrates.

It is well known that nucleotides, such as AMP and ADP, and glycolytic

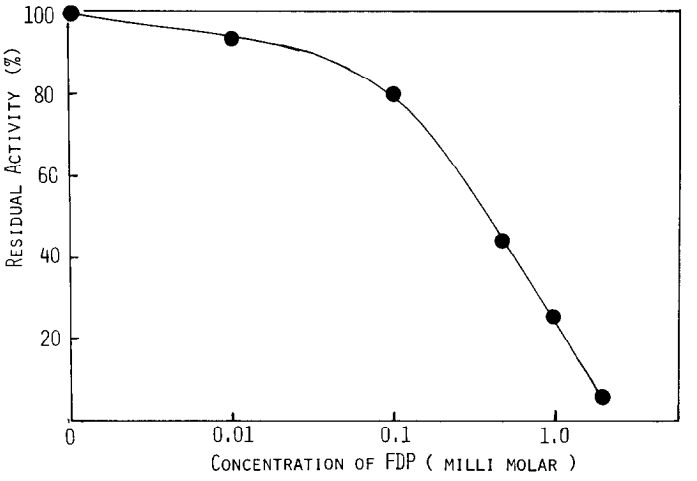

Fig. 1. Effects of various concentrations of fructose-1,6-diphosphate (EDP) on adenylosuccinate synthetase activity. The amount of enzyme used was the same as that given in the legend to Table 1.

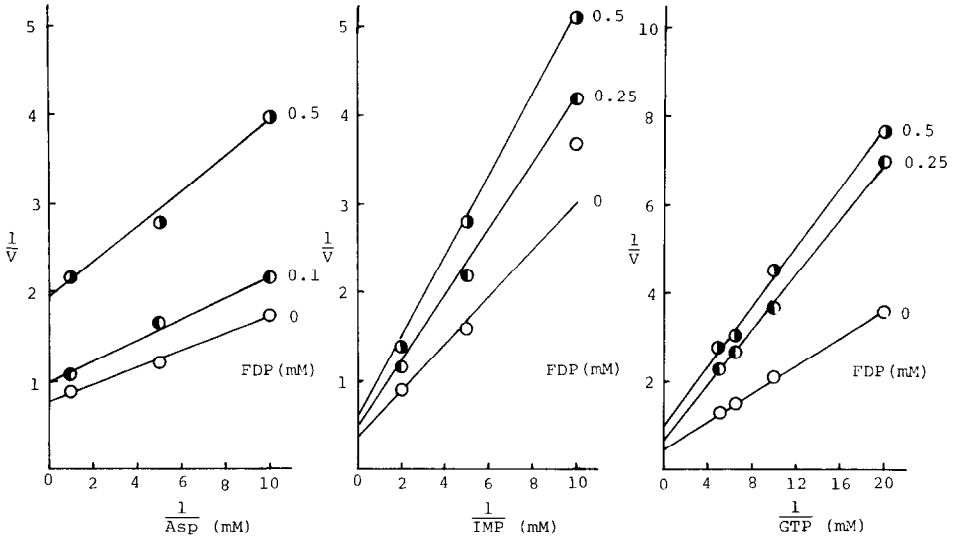

Fig. 2. Double reciprocal plots of the initial velocities of adenylosuccinate synthetase with various substrate concentrations in the presence of a fixed concentration of FDP.

intermediates, such as fructose-1,6-diphosphate and fructose-6-phosphate, activate phosphofructokinase, whereas ATP is inhibitory. According to Frenkel (2) and Tornheim and Lowenstein (3), the levels of AMP and ADP were highest when that of ATP was lowest during the glycolytic oscillation.

Tornheim and Lowenstein (11) also observed that the activating effect of fructose-1,6-diphosphate on phosphofructokinase depended strongly on the presence of AMP. These findings explain the sudden increase in phosphofructokinase activity. However, this mechanism cannot explain the oscillatory fluctuation of phosphofructokinase activity, because when once the enzyme activity has increased it does not return to the previous level while the concentration of AMP remains high.

Our findings give a clue to this problem, since they show that the accumulation of fructose-1,6-diphosphate inhibits adenylosuccinate synthetase, reducing AMP formation. ATP might also participate in this mechanism as an allosteric effector of AMP deaminase. When fructose-1,6-diphosphate is degraded by fructose diphosphate aldolase [EC 4.1.2.13], the inhibition of adenylosuccinate synthetase activity is relieved. Accordingly the inhibitory effect of fructose-1,6-diphosphate on adenylosuccinate synthetase seems to be critical for oscillation of glycolysis.

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