Phosphofructokinase in Rabbit Dental Pulp is Less Sensitive to ATP Inhibition.

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Abstract

ATP-dependent profiles of phosphofructokinase (PFK) activity were determined in both crude and Sephadex G-25-filtered fractions from rabbit dental pulp. ATP had a dual effect on PFK as an activator and an inhibitor, according to its concentration. Gel-filtered PFK showed a similar profile to that of crude PFK, indicating a lack of low-molecular-weight effector(s) for PFK in rabbit dental pulp. For complete inhibition of the PFK in rabbit dental pulp, 5 mM ATP was required. This level of ATP is much higher than that required for other typical isozymes of PFK from liver, skeletal muscle or brain. It is postulated that differences in the properties of PFK isozymes are due not only to the subunit structure but also to the presence of other effectors.

Introduction

Phosphofructokinase (ATP: D-fructose 6-phosphate 1-transferase, EC 2.7.1.11, PFK) catalyzes the phosphorylation of fructose 6-phosphate (Fru-6-P) by ATP to form fructose 1, 6-bisphosphate (Fru-1, 6-P2) and is considered to be one of the key enzymes in the glycolytic pathway. The activity of this enzyme is controlled by the concentrations of a large series of effectors such as Fru-1, 6-P2, AMP, NH4+, Pi, citrate, H+ and fructose 2, 6-bisphosphate (Fru-2, 6-P2)11-21, and also of its two substrates, Fru-6-P and ATP. ATP is an allosteric effector for PFK, and acts not only as a substrate but also as an inhibitor. Fru-6-P is the most potent and physiologically important regulator of PFK, and reverses the ATP-induced inhibition of PFK.

Since glycolysis in dental pulp was first reported by IMMENKAMP[3] in 1934, carbohydrate metabolism has been studied by many investigators[4-9]. However, there have been few detailed studies concerning the regulation of glycolysis in dental pulp. The regulation of PFK has still not been elucidated. This communication describes the effects of ATP on the crude and the partially purified PFKs from rabbit dental pulp by Sephadex G-25 column chromatography.
Materials and Methods

The following chemicals and purified auxiliary enzymes were obtained from Boehringer Mannheim GmbH (W. Germany): reduced nicotinamide adenine dinucleotide (NADH), adenosine 5'-triphosphate (ATP), adenosine 5'-monophosphate (AMP), fructose 6-phosphate (Fru-6-P), glucose 6-phosphate (Glu-6-P), aldolase (EC 4. 1. 2. 13), glyceraldehyde 3-phosphate dehydrogenase (EC 1. 1. 1. 8, GDH), triosephosphate isomerase (EC 5. 3. 1. 1, TIM), phosphoglucone isomerase (EC 5. 3. 1. 9, PGI), glucose 6-phosphate dehydrogenase (EC 1. 1. 1. 49, Glu-6-PDH). Sephadex G-25 was purchased from Pharmacia Fine Chemicals Co., Ltd. (Sweden). Other chemicals were obtained from local companies and were of the highest grade. Fru-6-P was treated with 0.1 N HCl at 25°C for 30 min to eliminate any contaminating Fru-2, 6-P2, as suggested by KRUGER et al[10].

Rabbit heads were purchased from a local slaughterhouse and the dental pulps were removed from the molar teeth. Liver, skeletal muscle (femoral muscle) and brain were also removed from rabbits under pentobarbital (30 mg/kg) anesthesia. The tissues were rinsed with homogenization buffer (pH 7.3), containing 50 mM Tris/phosphate buffer, 0.2 mM EDTA (ethylenediaminetetraacetic acid) and 1.0 mM dithiothreitol. After being minced with scissors, the tissues were homogenized with one volume of the homogenization buffer at 400 rpm for 90 in a Potter-Elvehjem-type homogenizer with a Teflon pestle. The homogenates were passed through three layers of gauze, and the filtrates were centrifuged at 105,000 x g for 60 min. The supernatant fractions were used as a crude enzyme fraction. Part of the supernatant fraction was passed through a Sephadex G-25 column (1 x 20 cm) to remove low-molecular-weight effectors for PFK. PFK was eluted from the column with the homogenization buffer, and the gel-filtered enzyme was used as a partially purified PFK fraction.

PFK activities were assayed at pH 7.3 and pH 8.0, and these were designated as v and Vmax, respectively. The reaction mixture for the determination of maximum PFK activity (Vmax) under optimal assay conditions contained the following components in a final volume of 1.0 ml: 50 mM HEPES (N-2-hydroxyethylpiperazine-n'-2-ethanesulfonic acid), 100 mM KCl, 6.5 mM MgCl2, 1.0 mM NH4Cl, 5.0 mM KH2PO4, 0.3 mM NADH, 0.1 mM AMP, 0.5 U aldolase, 0.5 U GDH, 5.0 U TIM, 1.0 U PGI, 1.0 mM Fru-6-P, 3.0 mM Glu-6-P, 1.5 mM ATP and an appropriate amount of the PFK fraction. For determinaion of the regulatory properties of PFK, the assay was conducted at pH 7.3 (v) in a reaction mixture containing essentially the same components as those used for Vmax determination, except that 50 mM HEPES buffer (pH 7.3), 0.1 mM Fru-6-P, 0.3 mM Glu-6-P and various concentrations of ATP were used. The concentrations of ATP and Fru-6-P were measured enzymatically[11-12]. Both types of reaction were initiated by the addition of ATP, and reduction of NADH was monitored at 340 nm. PFK activities were expressed as v/Vmax.

Results and Discussion

Figure 1 summarizes the effects of ATP on both the crude and partially
puriﬁed PFKs from rabbit dental pulp. Both preparations were remarkably activated in the presence of a low concentration of ATP, but they were slightly suppressed with a further increase of ATP. When PFK activity was expressed as $v/V_{\text{max}}$, the ATP-dependent proﬁle of the partially puriﬁed PFK was quite similar to that of the crude PFK.

Recently, Fru-2, 6-P$_2$ was reported to be a powerful effector for the relief of PFK inhibition by ATP, and was also reported to be present in various tissues$^{[2]}$. In other tissues of rabbit, removal of low-molecular-weight substances, especially Fru-2, 6-P$_2$, made PFKs more sensitive to ATP-dependent inhibition (data not shown). However, gel-ﬁltration of the crude PFK from rabbit dental pulp did not increase its sensitivity to ATP-dependent inhibition.

![Fig. 1 Effect of gel-filtration on crude dental pulp PFK activity. Activities of the crude (○) and partially purified (■) PFKs were determined in medium containing 0.1 mM Fru-6-P and various concentrations of ATP.](image)

The activity of the partially puriﬁed PFK from rabbit dental pulp was determined at pH 7.3 in the presence of various concentrations of ATP to determine the nature of the PFK sensitivity to ATP-dependent inhibition. As shown in Fig. 2, partially puriﬁed PFK was suppressed only in the presence of a relatively high concentration of ATP, and 5.0 mM ATP was required for complete inhibition.

PFK is a tetramer, and the presence of three kinds of PFK isozymes designated A, B and C, has been reported in rabbit$^{[13]}$. The A and B isozymes are present as homotetramers and have been isolated in essentially homogeneous form from skeletal muscle and liver, respectively$^{[14-15]}$. The C isozyme has also been isolated
from brain[16], but brain PFK is a heterotetramer, and consists of a hybrid mixture of the A, B and C types of subunit[17]. In general, subunit composition is responsible for the difference in the properties of these isozymes. Therefore, to compare the properties of dental pulp PFK with those of other typical PFK isozymes, the sensitivity to ATP inhibition was determined at pH 7.3 using the partially purified PFKs from liver, skeletal muscle and brain (Fig. 3).

As shown in Fig. 3, the activities of both the liver and skeletal muscle enzymes were completely inhibited by 1.0 mM ATP. Complete inhibition of activity of the brain enzyme was achieved at 3.0 mM ATP. When the sensitivity of PFK to ATP-dependent inhibition was judged according to the minimum ATP concentra-
tion required to obtain complete inhibition of PFK, dental pulp PFK was the least sensitive, and the order of sensitivity was dental pulp < brain < liver = skeletal muscle. However, it has been reported that the order of sensitivity of typical PFK isozymes to ATP-dependent inhibition is A (muscle type) < C (brain type) < B (liver type)\[16\]. These results suggest that the separate kinetic properties of each isozyme do not appear to completely predict the kinetic properties of hybridized mixtures of these isozymes. Therefore, not only the subunit structure but also other factors are responsible for the control of sensitivity to ATP-dependent inhibition. Recently, a factor of Mr=13,800 has been purified from rat liver and shown to relieve liver PFK from ATP-dependent inhibition\[18,19\]. Phosphorylation is another candidate for the regulation of PFK, and data on this line have been obtained using Ascaris suum muscle\[20\].

In human dental pulp, the concentration of ATP calculated from the data of Bell and Larmas\[21\] is 1.0-1.5 mM. If the concentration of ATP in rabbit dental pulp is the same as that in human dental pulp, then ATP-dependent suppression of PFK cannot occur in rabbit dental pulp. Precise analysis of the regulation of PFK in rabbit dental pulp is currently in progress in this laboratory.

References

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