The Phosphofructokinase of *Arthrobacter crystallopoietes*

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Arthrobacter crystallopoietes is a bacterium that undergoes sphere-rod morphogenesis when grown on a 0.5% glucose-minimal salts medium containing 0.2% of succinate (Ensign & Wolfe, 1964). Krulwich & Ensign (1969) have suggested that glucose is a poor substrate for growth of *A. crystallopoietes* and that enzymes such as PFK* involved in the metabolism of glucose may serve primarily a biosynthetic role rather than play a key role in the regulation of energy metabolism. Under normal environmental conditions it appears that succinate, amino acids and fatty acids provide the main sources of energy for the morphogenesis of *Arthrobacter* (Krulwich & Ensign, 1969).

In contrast, PFK enzymes isolated from a number of other plant, animal and bacterial sources have been found to be highly regulated enzymes closely associated with the control of cellular energy metabolism (Uyeda & Racker, 1965; Walker & Baily, 1969). An example of such regulation is demonstrated by the response of PFK to adenylate or energy-charge control (Atkinson, 1968; Atkinson & Walton, 1967) and its response to such allosteric effectors as citrate, phosphoenolpyruvate and NH_4^+ (Kelly & Turner, 1968; Underwood & Newsholme, 1967; Uyeda & Racker, 1965).

PFK was isolated from *A. crystallopoietes* by 10 min. sonication (Blackstone model SS-2C) in 50 mM-tris-HCl buffer, pH 8.0 at 4°. The disrupted cells were then centrifuged at 20000g for 30 min. at 4°. All the following procedures were carried out at 4°. The supernatant was treated with streptomycin sulphate (0.4 g./ml.) to remove nucleic acids and centrifuged at 20000g for 20 min. The pellet was discarded and the supernatant was brought to 50% saturation by addition of $(\text{NH}_4)_2\text{SO}_4$. The precipitated enzyme was removed by centrifugation at 20000g for 20 min. and resuspended in 50 mM-tris-HCl buffer, pH 8.0. The enzyme, purified five-fold, was then dialysed for 12 hr. against 50 mM-tris-HCl buffer, pH 7.5.

All assays were performed at 25° with a Gilford model 2000 recording spectrophotometer by the method of Bauman & Wright (1968). To detect production of fructose 1,6-diphosphate, excess of aldolase, triose phosphate isomerase, α -glycerophosphate dehydrogenase and NADH were added

*Abbreviation: PFK, phosphofructokinase.

to the reaction mixture. PFK activity was proportional to the rate of oxidation of NADH measured spectrophotometrically at 340 nm.

All chemicals employed were obtained through Sigma Chemical Co. (St Louis, Mo., U.S.A.) or Calbiochem (Los Angeles, Calif., U.S.A.), and all coupling enzymes were dialysed for 12 hr. against 50 mM-tris-HCl buffer, pH 7.5, to remove $(\text{NH}_4)_2\text{SO}_4$.

The PFK of *A. crystallopoietes* was found to follow simple Michaelis-Menten kinetics with respect to the substrate, fructose 6-phosphate, and demonstrated no sigmoidal kinetics, a characteristic often associated with regulatory enzymes. The enzyme appeared to lack any kind of adenylate or energy-charge control. Enzyme activity was not inhibited by excess of ATP (10 mM) or affected by ADP, AMP or 3',5'-(cyclic)-AMP in concentrations up to 10 mM. In addition, enzyme activity was neither inhibited by citrate (10 mM) or phosphoenolpyruvate (10 mM), nor activated by NH_4^+ (10 mM). Ferdinandus & Clark (1969) did, however, find the PFK of *A. crystallopoietes* to be selectively inhibited

Fig. 1. Hill plot of PFK with respect to substrate fructose 6-phosphate. A slope or interaction coefficient $n=1.0$ was obtained. The reaction mixture contained (final concentrations) 50 mM-tris-HCl buffer, pH 8.0, 5 mM- MgCl_2 , 1 mM-dithiothreitol, 0.2 mM-NADH, 1 mM-ATP, excess of aldolase, triose phosphate isomerase and α -glycerophosphate dehydrogenase, fructose 6-phosphate at the indicated concentrations and enzyme.

by free fatty acids. From a Hill plot (Atkinson, Hathaway & Smith, 1965) of PFK activity (Fig. 1) an interaction coefficient $n=1.0$ was obtained, suggesting the lack of interaction between substrate-binding sites or lack of homotropic co-operativity (Blangy, Buc & Monod, 1968).

The PFK of *A. crystallopoietes* is different from PFK isolated from other microbial sources in that the enzyme follows simple Michaelis-Menten kinetics and appears to lack an extensive type of regulation. Since glucose is not the main source of energy during the morphogenesis of *A. crystallopoietes*, perhaps the PFK of *A. crystallopoietes* has very little to do with the regulation of its own energy metabolism. This could explain the lack of response of PFK to adenylate or energy-charge control, and the lack of response to other effectors such as citrate, phosphoenolpyruvate and NH_4^+ . If energy regulation occurs at sites other than PFK during the morphogenesis, then one might expect a relatively simple type of PFK rather than a highly regulated PFK. This idea is further supported by the fact that Bauman & Wright (1968) have isolated a PFK from *Dictyostelium discoideum* that was found to be weakly regulated and followed simple Michaelis-Menten kinetics. A common characteristic of both *A. crystallopoietes* and *D. discoideum* is

that during morphogenesis each organism utilized fats or proteins as an internal energy source rather than exogenous glucose. Thus a highly regulated PFK to help control energy metabolism during morphogenesis may not be needed.

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- Atkinson, D. E. (1968). *Biochemistry*, **7**, 4030.
Atkinson, D. E., Hathaway, J. A. & Smith, E. C. (1965). *J. biol. Chem.* **240**, 2682.
Atkinson, D. E. & Walton, G. M. (1967). *J. biol. Chem.* **242**, 3239.
Bauman, P. & Wright, B. E. (1968). *Biochemistry*, **7**, 3653.
Blangy, D., Buc, H. & Monod, J. (1968). *J. molec. Biol.*, **31**, 13.
Ensign, J. C. & Wolfe, R. S. (1964). *J. Bact.* **87**, 924.
Ferdinandus, J. & Clark, J. B. (1969). *J. Bact.* **98** (in the Press).
Kelly, G. J. & Turner, J. P. (1968). *Biochem. biophys. Res. Commun.* **30**, 195.
Krulwich, T. A. & Ensign, J. C. (1969). *J. Bact.* **97**, 526.
Underwood, A. H. & Newsholme, E. A. (1967). *Biochem. J.* **104**, 296.
Uyeda, K. & Racker, E. (1965). *J. biol. Chem.* **240**, 4682.
Walker, P. R. & Baily, E. (1969). *Biochem. J.* **111**, 365.