Note

Pyrophosphate- and ATP-dependent
Phosphofructokinases in Pollen
of *Typha latifolia*Tooru FUNAGUMA, Yasuo HIBINO,
Satoshi FUKUMORI and Akira HARALaboratory of Biological Chemistry,
Faculty of Agriculture,
Meijo University,
Tenpaku-ku, Nagoya 468, Japan

Received April 7, 1987

PPi-PFK (EC 2.7.1.90) was first described with *Entamoeba histolytica* by Reeves *et al.*¹⁾ and then found in many leaf cells,^{2~5)} potato tubers,⁶⁾ mung beans,⁷⁾ germinating pea seeds,⁸⁾ castor bean seedlings,⁹⁾ mung hypocotyl,¹⁰⁾ and *Euglena gracilis*.¹¹⁾ The enzyme catalyzes the following reversible reaction: $\text{Fru-6-P} + \text{PPi} \rightleftharpoons \text{Fru-1,6-P}_2 + \text{Pi}$, and its reaction is strongly stimulated by Fru-2,6-P₂. Therefore, the enzyme could theoretically be involved in both glycolytic and gluconeogenic carbon flows. On the other hand, ATP-PFK (EC 2.7.1.11) catalyzes an essentially irreversible reaction: $\text{Fru-6-P} + \text{ATP} \rightarrow \text{Fru-1,6-P}_2 + \text{ADP}$. The enzyme from plants is not influenced by Fru-2,6-P₂, unlike the enzyme from mammalian sources.^{3~6,9,10)} Fru-2,6-P₂ is also a potent inhibitor for fructose 1,6-bisphosphatase.¹²⁾

Mature pollen from *Typha latifolia* germinates in about 30 min followed by a rapid pollen-tube elongation on an agar medium with or without sucrose. Carbohydrates such as sucrose and starch which are present in abundance in the pollen¹³⁾ are thought to be used as an energy source for synthesis of new cell wall polysaccharides. We have reported^{14~17)} on some enzymes related to the metabolism of carbohydrates, and recently detected a new PPi-PFK activity in mature pollen. This paper deals with the presence of PPi-PFK and ATP-PFK in mature and germinating pollens of *T. latifolia*, and the effects of Fru-2,6-P₂ on their activities.

Mature pollen grains of *T. latifolia* were collected in a paddy field at Nishin-cho, Aichi prefecture in June 1986, dried at room temperature overnight and stored at -20°C. The following materials were from the sources indicated in parenthesis: Fru-6-P and Fru-2,6-P₂ (Sigma), aldolase, triosephosphate isomerase and glycerol-3-phosphate dehydrogenase (Boehringer), NADH (Kohjin)

and ATP (Seikagaku Kogyo). Other reagents were of analytical grade.

Five hundred milligrams of each of the pollens was cultured on a 1.5% agar medium at 30°C and collected in 5 ml of buffer (10 mM HEPES-NaOH-5 mM β -mercaptoethanol-5 mM MgCl₂-1 mM EDTA · Na₂-10% (v/v) glycerol, pH 7.2) at the indicated times. After individually disrupting the suspension in a Teflon-glass homogenizer at about 2,000 rpm for 10 min, they were centrifuged at 20,000 × g for 20 min and the supernatants were dialyzed against the same buffer. Then each dialysate was used for assays of PPi- or ATP-PFK activity and protein. PPi- or ATP-PFK activity was assayed by measuring Fru-1,6-P₂ produced. The reaction mixture in 1.6 ml contained 50 mM Tris-HCl, pH 8.0, 5 mM Fru-6-P, 5 mM MgCl₂, 0.15 mM NADH, 2.5 mM PPi or 2 mM ATP, 2 units of aldolase, 20 units of triosephosphate isomerase, 4 units of glycerol-3-phosphate dehydrogenase, Fru-2,6-P₂ at the indicated concentrations, and the extract from pollen. The reaction was started at 37°C by the addition of PPi or ATP, and then the decrease in absorbance at 340 nm was measured. One unit of enzyme was defined as the amount of enzyme required to catalyze the formation of 1 μ mol of Fru-1,6-P₂ per min under the above conditions. Protein was measured by a modification¹⁸⁾ of Lowry's method with bovine serum albumin as standard.

Figure 1 shows the changes in PPi-PFK activity (A), ATP-PFK activity (B) and protein (C) during culture of

FIG. 1. Changes in PPi-PFK Activity, ATP-PFK Activity, and Protein during Culture of Pollen from *T. latifolia*.

Both PPi-PFK activity (A) and ATP-PFK activity (B) were assayed in the presence (○) and absence (●) of 1 μ M Fru-2,6-P₂. Protein (C) was expressed as the amount extracted from 500 mg of the pollen.

Abbreviations: PPi-PFK, inorganic pyrophosphate-dependent phosphofructokinase; Fru-6-P, fructose 6-phosphate; Fru-1,6-P₂, fructose 1,6-bisphosphate; Fru-2,6-P₂, fructose 2,6-bisphosphate; ATP-PFK, ATP-dependent phosphofructokinase; Pi, inorganic phosphate.

the pollen on an agar medium. As seen in (A), PPi-PFK activity was detected and also strongly enhanced by $1\ \mu\text{M}$ Fru-2,6-P₂. Wu *et al.*⁸⁾ reported that the sensitivity of PPi-PFK to activation by Fru-2,6-P₂ changes during germination of pea seeds, and that the enzyme exists in two convertible forms controlled by Fru-2,6-P₂. In contrast, no definite change of either activity in the presence or absence of Fru-2,6-P₂ was observed during the germination followed by pollen tube elongation (A). A very similar pattern of change in the activity was also obtained from an experiment of the culture on an agar medium with 2% sucrose. These results suggest that the regulation of the enzyme observed in germinating pea seeds is not present in *Typha* pollen. The protein level was constant during the culture, as seen in (C). ATP-PFK activity was also detected in the pollen, but it changed during the culture, differing from that of PPi-PFK, as seen in (B). The approximately 50% increase in activity observed at 2 hr of the culture may be due to activation or synthesis of the ATP-PFK. The enzyme was negligibly sensitive to Fru-2,6-P₂, like other plant enzymes.^{3-6,9,10)}

These results indicate the presence of PPi-PFK activated by Fru-2,6-P₂ and ATP-PFK insensitive to the effector in mature and germinating pollens of *T. latifolia*, and their participation in glycolysis and energy metabolism.

REFERENCES

- 1) R. E. Reeves, D. J. South, H. J. Blytt and L. G. Warren, *J. Biol. Chem.*, **249**, 7737 (1974).
- 2) N. W. Carral and C. C. Black, *Biochem. Biophys. Res. Commun.*, **86**, 20 (1979).
- 3) M. Smith, G. Meiskes, H-D. Soling and H. W. Heldt, *FEBS Lett.*, **145**, 217 (1982).
- 4) C. Cseke, N. F. Weeden, B. B. Buchanan and K. Uyeda, *Proc. Natl. Acad. Sci. U.S.A.*, **79**, 4322 (1982).
- 5) N. W. Carnal and C. C. Black, *Plant Physiol.*, **71**, 150 (1983).
- 6) E. Van Schaftigen, B. Lederer, R. Bartrons and H-G. Hers, *Eur. J. Biochem.*, **129**, 191 (1982).
- 7) D. C. Sabulase and R. L. Anderson, *Biochem. Biophys. Res. Commun.*, **103**, 848 (1981).
- 8) M-X. Wu, D. A. Smyth and C. C. Black, *Plant Physiol.*, **73**, 188 (1983).
- 9) N. J. Kruger, E. Kombrink and H. Beevers, *FEBS Lett.*, **153**, 409 (1983).
- 10) D. C. Sabulase and R. L. Anderson, *Biochem. Biophys. Res. Commun.*, **102**, 848 (1981).
- 11) K. Miyatake, T. Enomoto and S. Kitaoka, *Agric. Biol. Chem.*, **50**, 2417 (1986).
- 12) H-G. Hers, L. Hue and E. Van Schaftigen, *Trends Biochem. Sci.*, **7**, 329 (1982).
- 13) T. Watanabe, Y. Motomura and K. Aso, *Nippon Nōgeikagaku Kaishi*, **34**, 704 (1960).
- 14) T. Hondo, A. Hara and T. Funaguma, *Plant & Cell Physiol.*, **24**, 61 (1983).
- 15) T. Hondo, A. Hara and T. Funaguma, *Plant & Cell Physiol.*, **24**, 1535 (1983).
- 16) A. Hara, K. Kawamoto and T. Funaguma, *Plant & Cell Physiol.*, **21**, 1475 (1980).
- 17) A. Hara, S. Ebina and T. Funaguma, *Agric. Biol. Chem.*, **49**, 3539 (1985).
- 18) E. F. Hartree, *Anal. Biochem.*, **48**, 422 (1972).