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# Fructose-1,6-bisphosphatase in the fat body of the cockroach *Periplaneta americana* larvae

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Fructose-2,6-bisphosphate (Fru-2,6-$P_2$[1]) stimulates 6-phosphofructo-1-kinase (PFK-1) and inhibits fructose-1,6-bisphosphatase (FBPase-1). As the result, glycolysis is activated while gluconeogenesis is decreased [1]. Both enzymes are multimodulated, i.e. precisely controled by numerous effectors in response to hormonal and metabolic demands [2, 3].

Insect fat body is one of the tissues in which both glycolysis and gluconeogenesis were found to occur [4]. The level of Fru-2,6-$P_2$ in insect muscle is known to change during flight [5] and in fat body during starvation [6]; moreover, PFK-1 is stimulated by this metabolite [5, 6]. However, the studies on insect FBPase-1 are limited to only a few which were carried out on muscle, prior to the discovery of the role of Fru-2,6-$P_2$ [7, 8].

This communication presents some properties of FBPase-1 from larval fat body of *Periplaneta americana* and preliminary results which indicate that Fru-2,6-$P_2$ inhibits the activity of this enzyme.

The last instar larvae of the cockroach *Periplaneta americana* reared on standard diet, were used. The fat body from abdomen was dissected. For enzyme assays, the clear supernatant obtained after centrifugation of 10% homogenate at 100000 × *g* for 60 min was used.

FBPase-1 activity was measured spectrophotometrically by following the rate of $NADP^+$ reduction at 340 nm and 25°C in a coupled enzyme assay system according to Storey & Bailey [4].

The pH optimum for the *Periplaneta americana* fat body FBPase-1 was at 7.8 in Tris/HCl and at 8.0 in glycylglycine/NaOH buffer (Fig. 1). The $V_{max}$ of the enzyme was lower by 60% in glycylglycine/NaOH buffer than in Tris/HCl buffer. The pH optimum for this enzyme was similar to those for the FBPase-1 from the flight muscle of the bumble-bee [8] and of purified FBPase-1 from the mantle of sea mussel [9].

Like FBPase-1 from other sources, the enzyme from fat body has an absolute requirement for

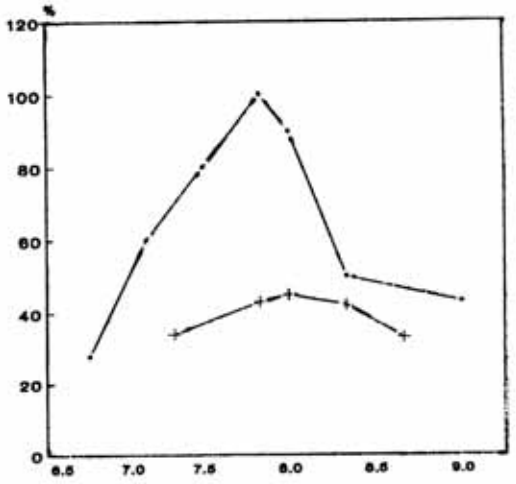


*Fig. 1. Effect of pH on activity of the fat body FBPase-1.*
The activity was determined in the presence of 0.100 mM Fru-1,6-$P_2$, 0.010 mM $MgCl_2$ and 50 mM: ■, Tris/HCl, or +, glycylglycine/NaOH buffers

[1]Abbreviations: Fru-2,6-$P_2$, fructose-2,6-bisphosphate; FBPase-1, fructose-1,6-bisphosphatase; PFK-1, 6-phosphofructo-1-kinase

divalent cations (Fig. 2). It seems that $Mg^{2+}$ better fulfils the enzyme's requirement for a divalent ion cofactor than does $Mn^{2+}$. The fat body FBPase-1 activity changed sigmoidally with $Mg^{2+}$ concentration, like those of the beef liver enzyme [10] and the rabbit muscle enzyme [11].

Monovalent cations were less effective in activation of the enzyme (Fig. 2). Maximum activity of the $K^+$ stimulated enzyme was 60% that with $Mg^{2+}$ as the cofactor. $Na^+$ activated the enzyme only to 30% of its $V_{max}$ with $Mg^{2+}$. Ammonium ions did not activate FBPase-1.

The enzyme activity responded hyperbolically to increasing concentrations of the substrate, Fru-1,6-$P_2$ (Fig. 3). The Michaelis constant determined from the double reciprocal plot (Fig. 3) was 0.0108 ± 0.0020 mM Fru-1,6-$P_2$. This value is very close to 0.010 determined for the purified FBPase-1 from the flight muscle of bumble-bee [8].

Figure 4 presents the influence of the effector Fru-2,6-$P_2$ on the activity of the fat body FBPase-1. It has been found that Fru-2,6-$P_2$ is a powerful inhibitor of this enzyme. The presence of 1 µM Fru-2,6-$P_2$ resulted in the loss of about 40% of the enzyme activity.

The results presented above indicate that the *Periplaneta americana* fat body FBPase-1 resem-

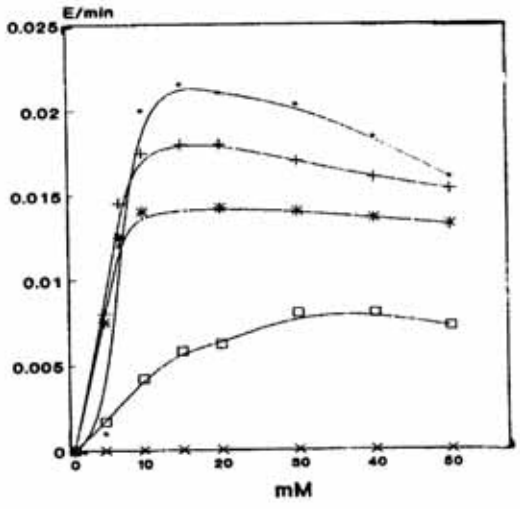


*Fig. 2. Effect of divalent and monovalent cations on activity of the fat body FBPase-1.*
Tris/HCl buffer was used in the presence of: ■, $Mg^{2+}$; +, $Mn^{2+}$; ✳, $K^+$; □, $Na^+$; x, $NH_4^+$

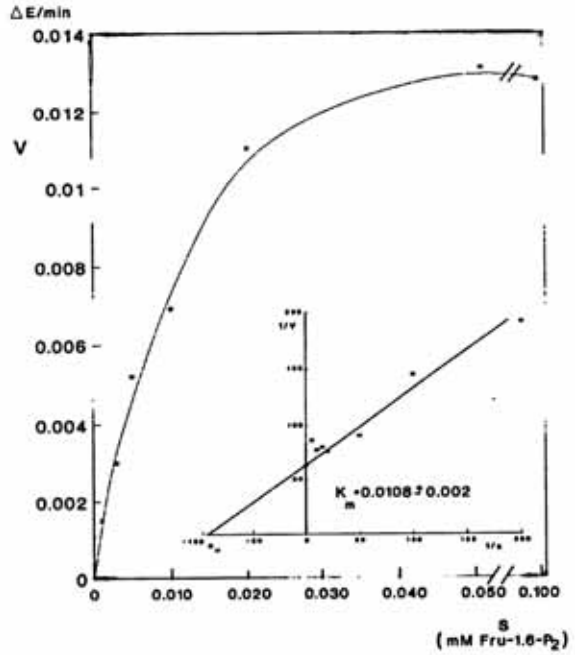


*Fig. 3. Effect of substrate concentration on activity of the fat body FBPase-1 at pH 7.8.*
Tris/HCl buffer was used. The $K_m$ value is the average of 7 determinations

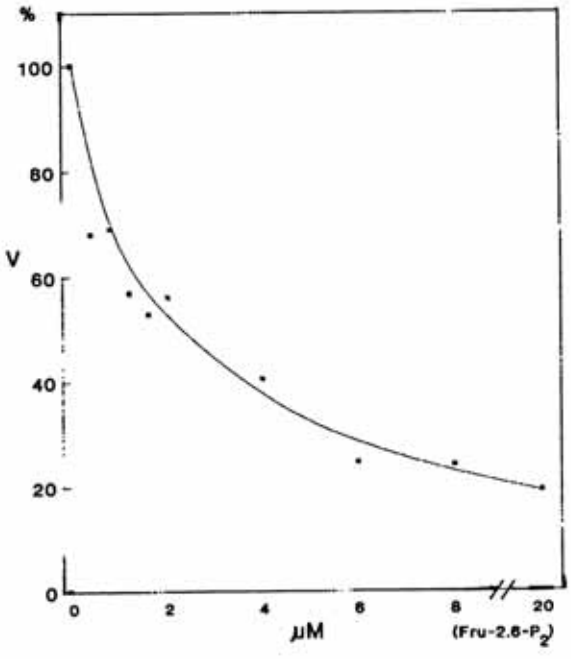


*Fig. 4. Inhibition of the fat body FBPase-1 by Fru-2,6-$P_2$.*
The activity was determined in the presence of: 0.100 mM Fru-1,6-$P_2$, 0.010 mM $MgCl_2$, 50 mM Tris/HCl buffer, pH 7.5. The activity in the absence of Fru-2,6-$P_2$ was 120 nmol/min per mg proteinand was taken as 100%

bles the enzyme from other sources in several respects, including pH optimum, activation by ion effectors and substrate affinity. It is the target enzyme for the inhibitory effect of Fru-2,6-$P_2$.

## REFERENCES


1. Hue, L. & Rider, M.H. (1987) *Biochem. J.* **245,** 313 - 324.
2. Pilkis, S.J. & Granner, D.K. (1992) *Annu. Rev. Physiol.* **54,** 885 - 909.
3. Tejwani, G.A. (1983) *Adv. Mol. Reg.* **54,** 121 - 194.
4. Storey, K.B. & Bailey, E. (1978) *Insect Biochem.* **8,** 121 - 125.
5. Storey, K.B. (1983) *FEBS Lett.* **161,** 256 - 268.
6. Kasprzyk, A. & Lesicki, A. (1988) *Proc. 14th Internat. Cong. Biochem. Prague* **1,** 75.
7. Newsholme, E.A., Crabtree, B., Higgins, S.J., Thornton, S.D. & Start, C. (1972) *Biochem. J.* **128,** 89 - 97.
8. Storey, K.B. (1978) *Biochim. Biophys. Acta* **523,** 443 - 453.
9. Barcia, R. & Ramos-Martinez, J.I. (1992) *Comp. Biochem. Physiol.* **101b,** 69 - 72.
10. Nimmo, H.G. & Tipton, K.F. (1975) *Biochem. J.* **145,** 323 - 334.
11. Marcus, C.J., Geller, A. M. & Byrne, W. L. (1979) *J. Biol. Chem.* **248,** 8567 - 8573.