Regulation of Locust Fat-Body Phosphorylase

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1. Glycogen phosphorylase of locust fat-body was partially purified by differential centrifugation and dissociation from glycogen particles at two pH values. 2. Optimum activity was obtained at pH 6.6–6.7. 3. The calculated apparent K_m values for glycogen and glucose 1-phosphate were 0.08% and 10–13 mM respectively. 4. 5'-AMP activated in the range 5 μ M–1 mM. 5. Glucose 6-phosphate is a competitive inhibitor for the substrate glucose 1-phosphate ($K_i = 1.7$ mM). 5'-AMP abolishes this inhibition. Glucose weakly inhibits ($K_i = 25$ –30 mM), but trehalose does not inhibit even at 100 mM. 6. It is suggested that glucose 6-phosphate is a major regulator of glycogen phosphorylase activity in locust fat-body.

The insect fat-body is an organ analogous in many functional aspects to mammalian liver. It serves as a main storage organ for accumulation of energy reserves, and contains the various enzyme systems required for deposition and degradation of these reserves (Stevenson & Wyatt, 1964; Wiens & Gilbert, 1967a,b; Steele, 1969; Goldsworthy, 1970). Adult locust fat-body contains deposits of both glycogen and lipid. Glycogen is utilized for flight initiation, and fat-reserves for sustained periods of flight (Weis-Fogh, 1952). In the cockroach *Leucophaea maderae*, fat-body glycogen serves during the cycles of egg maturation in the adult female (Wiens & Gilbert 1967a).

The enzymic release of glucose 1-phosphate from glycogen has been shown to be directly monitored by the allosteric enzyme glycogen phosphorylase (α -1,4-glucan-orthophosphate glycosyltransferase; EC 2.4.1.1) in mammalian systems, which have been extensively studied (for reviews see Ryman & Whelan, 1971). Glycogen phosphorylase activity is affected in a complex manner by various small molecules and ions, and by the reversible transition of the enzyme molecule from active to inactive form, dependent on the activity of a specific kinase and phosphatase. Some aspects of this control might be expected to differ in insect fat-body wherein trehalose is synthesized from UDP-glucose and glucose 6-phosphate (Murphy & Wyatt, 1964, 1965). The present paper deals with the partial purification and properties of fat-body glycogen phosphorylase in the migratory locust (*Locusta migratoria*).

Experimental

Materials

Chemicals. Glucose 1-phosphate (dipotassium salt) was obtained from Fluka (Buchs, Switzerland) and recrystallized from ethanol. Glucose 6-phosphate

was obtained from Pierce Chemical Co. (Rockford, Ill., U.S.A.). Glycogen (oyster white) was a product of Nutritional Biochemicals Corp. (Cleveland, Ohio, U.S.A.) and was purified from contaminating nucleotides by dialysis (Lowry *et al.*, 1963). AMP was obtained from Calbiochem (Lucerne, Switzerland). All other chemicals were of the highest grade commercially obtainable.

Insects. Stock colonies of *Locusta migratoria* were reared in plastic screened cages in groups of 100 with a 12 h photoperiod and at 30°C. The diet was changed daily and was composed of fresh grass supplemented with flaked oats. Adults 1–2 weeks after eclosion were used for preparation of fat-body glycogen phosphorylase.

Methods

Enzymic activity. Glycogen phosphorylase was assayed as described by Sutherland & Wosilait (1956) by determining the release of P_i from glucose 1-phosphate. The reaction mixtures contained (unless otherwise noted): 10 μ g of enzyme protein (determined by the method of Lowry *et al.*, 1951), 0.5% glycogen, 30 mM-glucose 1-phosphate, 0.1 M-NaF and 0.05 M-Tris-maleate buffer, pH 6.6, in 1 ml. The reaction was started by introducing the enzyme solution and after a 30 min incubation at 30°C activity was terminated with 5% trichloroacetic acid and 0.1 M-sodium acetate as described by Lee (1966). P_i was determined by the method of Fiske & Subbarow (1925). Preliminary experiments indicated that activity was proportional to protein concentration up to 30 μ g/ml, and constant for incubation periods up to 60 min.

Enzyme preparation. Fat-bodies, collected from groups of 20 adult locusts, were homogenized in 20 ml of ice-cold 0.05 M-Tris-maleate buffer, pH 7.5, containing 0.1 M-NaF, and the homogenate was

Table 1. *Steps in the partial purification of locust fat-body glycogen phosphorylase*

A unit is defined as the amount of enzymic activity that releases 1 μmol of P_i per min under optimum conditions of reaction. Assay conditions were as described in the Materials and Methods section.

	Total activity (units)	Specific activity (units/mg of protein)	Yield (%)	Degree of purification
Tissue homogenate	17.5	0.22	100	1
pH 7.5 precipitate (A)	12.3	0.44	70	2
pH 7.5 supernatant (A)	1.7	0.033	—	—
pH 6.0 precipitate (B)	—	—	—	—
pH 6.0 supernatant (B)	6.1	15.3	33	7

Fig. 1. *Effect of pH on locust fat-body glycogen phosphorylase activity*

Assay conditions were as described under 'Methods'. The reaction mixture contained 0.1 M-NaF in 0.05 M-Tris-maleate buffer, 0.5% glycogen and 30 mM-glucose 1-phosphate. Reaction time was 15 min at 30°C.

centrifuged in the cold (4°C) in a Sorvall RC-2B centrifuge at 37000g for 40 min. Most of the glycogen phosphorylase activity was found in the precipitate (A) (see Table 1). The supernatant was discarded and the precipitate dispersed in 0.05 M-Tris-maleate buffer, pH 6.0, containing 0.1 M-NaF. At this pH the glycogen phosphorylase dissociates from the precipitate. Activity was collected in the supernatant (B) after additional centrifuging at 37000g for 30 min. This supernatant represents a 70-fold purification of

the original tissue homogenate and served for the experiments reported herein.

Results

Effect of pH, temperature, substrate concentration and AMP on activity of glycogen phosphorylase

pH. This was studied, with saturating concentrations of glucose 1-phosphate and glycogen, in 0.05 M-Tris-maleate buffer over the effective pH range of this buffer. A sharp optimum of activity at pH 6.6–6.7 was noted (Fig. 1). The pH optimum of locust fat-body glycogen phosphorylase is similar to that of silkworm fat-body (Stevenson & Wyatt, 1964) and blowfly flight muscle (Childress & Sacktor, 1970) and of many other organisms (see, e.g., Chen & Segel, 1968; Shepherd & Segel, 1969).

Temperature. Enzyme activity was measured for an initial incubation period of 15 min at the optimum pH and was found to increase linearly up to 37°C (Fig. 2).

Substrate concentration. Glycogen phosphorylase utilizes glycogen as substrate in the direction of degradation, and glucose 1-phosphate in the direction of synthesis. At high concentrations of acceptor glycogen, the K_m of the locust enzyme for glucose 1-phosphate is independent of the glycogen concentration, and the K_m for glycogen is similarly independent of glucose 1-phosphate concentrations at high values of the latter. The K_m values were determined from Lineweaver-Burk plots to be 10–13 mM-glucose 1-phosphate and 0.08% glycogen (Figs. 3 and 4).

In separate experiments, preincubation of the fat-body glycogen phosphorylase with glycogen, for periods up to 20 min, was found to be unnecessary, in contrast with the activation of rabbit muscle phosphorylase after preincubation with glycogen (Wang *et al.*, 1965).

AMP. Activation by AMP increased steadily in the range 5 μM –1 mM-AMP, to 75% above that of control reaction mixtures. At 10 mM-AMP the degree

Fig. 2. *Effect of temperature on locust fat-body glycogen phosphorylase activity*
Conditions were as described under 'Methods' and in Fig. 1.

Fig. 3. *Effect of glucose 1-phosphate on locust fat-body glycogen phosphorylase activity*
○, 0.05% Glycogen; ●, 0.2% glycogen. All other conditions were as described under 'Methods' and in Fig. 1. $K_m = 10-13$ mM.

of activation decreased somewhat, to 62% above that of the controls (Fig. 5).

Inhibition of glycogen phosphorylase

Inhibition by glucose. Glucose is a competitive inhibitor of glycogen phosphorylase for the substrate glucose 1-phosphate (Fig. 6) with a K_i of 25-30 mM calculated from the formula $K_p = K_m (1 + i/K_i)$. The

Fig. 4. *Effect of glycogen concentration on locust fat-body glycogen phosphorylase activity*
○, 8 mM-Glucose 1-phosphate; ●, 16 mM-glucose 1-phosphate. All other conditions were as described under 'Methods' and in Fig. 1. $K_m = 0.08\%$ (0.8 mg/ml) glycogen.

Fig. 5. *Effect of AMP concentration on locust fat-body glycogen phosphorylase activity*
Conditions were as described under 'Methods'. AMP concentration was in the range 1 μM-10 mM.

Fig. 6. Inhibition of locust fat-body glycogen phosphorylase activity by glucose

●, 25 mM-Glucose; ○, no glucose. All other conditions were as described under 'Methods' and in Fig. 1. $K_i = 25\text{--}30\text{ mM}$.

Fig. 7. Inhibition of locust fat-body glycogen phosphorylase activity by glucose 6-phosphate

●, 1.5 mM-Glucose 6-phosphate; ○, no glucose 6-phosphate. All other conditions were as described under 'Methods' and in Fig. 1. $K_i = 1.7\text{ mM}$.

fact that 1 mM-AMP totally abolishes this inhibition indicates that AMP is involved in the preferential binding of glucose 1-phosphate to the enzyme molecule, as suggested by Wang *et al.* (1965).

Inhibition by glucose 6-phosphate. Glucose 6-phosphate is a competitive inhibitor of glycogen phosphorylase for the substrate glucose 1-phosphate (Fig. 7) ($K_i = 1.7\text{ mM}$). In a manner similar to that obtained in the glucose inhibition of glycogen phosphorylase, 1 mM-AMP completely abolishes the inhibition by 2 mM-glucose 6-phosphate. This situation is the reverse of that encountered with bovine spleen glycogen phosphorylase, which is activated by AMP, and glucose 6-phosphate is a competitive suppressor of this activation (Kamogawa & Fukui, 1971).

Trehalose, at concentrations up to 0.1 M, did not inhibit locust fat-body glycogen phosphorylase. Trehalose does not inhibit moth fat-body glycogen phosphorylase (Wiens & Gilbert, 1967b).

Discussion

Locust fat-body glycogen phosphorylase exhibits several unusual characteristics, which relate to the functions of the insect fat-body in its surrounding milieu. First, although AMP does stimulate phosphorylase, no fractions were obtained, during any of numerous attempts, which absolutely required AMP and lacked activity in its absence; all active fractions were stimulated to the same extent. The apparent role of this nucleotide in control of glycogenolysis differs radically in various organisms: AMP plays a central role in mammalian systems via activation of

inactive phosphorylase *b*, but has no effect on protozoan or yeast glycogen phosphorylase (Blum, 1970; Sagardia *et al.*, 1971). In the mammalian systems in which AMP does act, its stimulatory effect is attenuated by glucose 6-phosphate, which serves to override AMP activation by competitive inhibition (see, e.g., Morgan & Parmeggiani, 1964; Kamogawa & Fukui, 1971). In locust fat-body glycogen phosphorylase the role of these effectors appears to be reversed: glucose 6-phosphate serves as an inhibitor exhibiting high affinity relative to glucose 1-phosphate as substrate, whereas AMP acts to suppress this inhibition totally, with the net result that activity is restored to its normal value.

The suggested role of glucose 6-phosphate as a primary regulator of glycogenolysis in locust fat-body is attractive in view of its known function in trehalose biosynthesis. This disaccharide is formed in locust fat-body (Candy & Kilby, 1959, 1960, 1961; Mikolaschek & Zebe, 1967) in a two-step reaction, with glucose 6-phosphate as one of the initial substrates (Murphy & Wyatt, 1964, 1965) and is secreted into the haemolymph, where it serves as the major pool of energy (see review by Wyatt, 1967). No appreciable amount of trehalose accumulates *in vivo* in fat-body, and this is in accord with its lack of effect *in vitro* on locust fat-body glycogen phosphorylase. Trehalose controls its own biosynthesis in insects by feedback inhibition (Murphy & Wyatt, 1965), and its utilization during energy expenditure would result in a lower concentration of intracellular glucose 6-phosphate in the fat-body, thereby re-activating

glycogen phosphorylase, which, with phosphoglucomutase, would ultimately restore the resting-state concentration of glucose 6-phosphate. The mobilization of glycogen from the fat-body would presumably be attenuated in an actively feeding insect by production of glucose 6-phosphate from exogenous dietary glucose. It is suggestive that in flight muscle of blowflies glycogen phosphorylase is not inhibited by glucose 6-phosphate (Childress & Sacktor, 1970), and that there too the site of trehalose synthesis is the fat-body (Clegg & Evans, 1961).

Finally, a further dissimilarity between mammalian liver systems and the locust fat-body is in the meagre inhibition by glucose encountered in the latter. In perfused rat liver, hyperglycaemia rapidly inactivates phosphorylase and is regarded as an important moderator of liver glycogen metabolism *in vivo* (Glinsmann *et al.*, 1970). Free glucose in locust fat-body is non-existent for all practical purposes, and its slight effect *in vivo* is assumed to lack physiological significance.

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References

- Blum, J. J. (1970) *Arch. Biochem. Biophys.* **137**, 65–74
 Candy, D. J. & Kilby, B. A. (1959) *Nature (London)* **183**, 1594–1595
 Candy, D. J. & Kilby, B. A. (1960) *Biochem. J.* **74**, 19P
 Candy, D. J. & Kilby, B. A. (1961) *Biochem. J.* **78**, 531–536
 Chen, G. S. & Segel, I. H. (1968) *Arch. Biochem. Biophys.* **127**, 175–186
 Childress, C. C. & Sacktor, B. (1970) *J. Biol. Chem.* **245**, 2927–2936
 Clegg, J. S. & Evans, D. R. (1961) *J. Exp. Biol.* **38**, 771–792
 Fiske, C. H. & Subbarow, Y. (1925) *J. Biol. Chem.* **66**, 375–400
 Glinsmann, W., Pauk, G. & Hern, E. (1970) *Biochem. Biophys. Res. Commun.* **39**, 774–782
 Goldsworthy, G. J. (1970) *Gen. Comp. Endocrinol.* **14**, 78–85
 Kamogawa, A. & Fukui, T. (1971) *Biochim. Biophys. Acta* **242**, 55–68
 Lee, Y. P. (1966) *Methods Enzymol.* **8**, 550–554
 Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
 Lowry, O. H., Schulz, D. W. & Passonneau, J. V. (1963) *J. Biol. Chem.* **239**, 1947–1953
 Mikolaschek, G. & Zebe, E. (1967) *J. Insect Physiol.* **13**, 1483–1488
 Morgan, H. E. & Parmeggiani, A. (1964) *J. Biol. Chem.* **239**, 2440–2445
 Murphy, T. A. & Wyatt, G. R. (1964) *Nature (London)* **202**, 1112–1113
 Murphy, T. A. & Wyatt, G. R. (1965) *J. Biol. Chem.* **240**, 1500–1508
 Ryman, B. E. & Whelan, W. J. (1971) *Adv. Enzymol. Relat. Areas Mol. Biol.* **34**, 285–443
 Sagardia, F., Gotay, I. & Rodriguez, M. (1971) *Biochem. Biophys. Res. Commun.* **42**, 829–835
 Shepherd, D. & Segel, I. H. (1969) *Arch. Biochem. Biophys.* **131**, 609–620
 Steele, J. E. (1969) *Comp. Biochem. Physiol.* **29**, 755–763
 Stevenson, E. & Wyatt, G. R. (1964) *Arch. Biochem. Biophys.* **108**, 420–429
 Sutherland, E. W. & Wosilait, W. D. (1956) *J. Biol. Chem.* **218**, 459–468
 Wang, J. H., Shonka, M. L. & Graves, D. J. (1965) *Biochemistry* **4**, 2296–2301
 Weis-Fogh, T. (1952) *Phil. Trans. Roy. Soc. London Ser. B* **237**, 1–36
 Wiens, A. W. & Gilbert, L. I. (1967a) *J. Insect Physiol.* **13**, 779–794
 Wiens, A. W. & Gilbert, L. I. (1967b) *Comp. Biochem. Physiol.* **21**, 145–159
 Wyatt, G. R. (1967) *Advan. Insect Physiol.* **4**, 287–360