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Maximum activities and properties of glucose 6-phosphatase in muscles from vertebrates and invertebrates

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1. The maximum catalytic activities of glucose 6-phosphatase were measured in a large number of muscles from vertebrates and invertebrates. The activities range from less than 0.1 to 8.0 μmol/min per g fresh wt. at 30°C: the highest activity, observed in the flight muscle of the wasp (*Vespa vulgaris*), is similar to that in rat liver. The hydrolytic activity was shown to be specific towards glucose 6-phosphate. 2. The pH optimum was 6.8 and the K_m was approx. 0.6 mM (flight muscle of a moth). 3. Almost all of the glucose 6-phosphatase activity from extracts of the flight muscle of a moth and the pectoral muscle of a pigeon were recovered in the cytosolic fraction (i.e. 150000 *g* supernatant). 4. During development of the locust (*Schistocerca gregaria*), the activity of the phosphatase in the flight muscle increased during the first 3 days after the final moult. 5. The activity of glucose 6-phosphatase from insect and avian muscle was separated from that of non-specific phosphatase on a Bio-Gel P-100 column. 6. For the activities from 63 muscles, there was a strong positive correlation between those of glucose 6-phosphatase and hexokinase, but no correlation between the activities of glucose 6-phosphatase and fructose bisphosphatase. It is suggested that the role of glucose 6-phosphatase in muscle is either to produce glucose from glucose 6-phosphate derived from glycogen or to provide the enzymic basis for a substrate ('futile') cycle between glucose and glucose 6-phosphate in muscle to improve the sensitivity of the mechanism that regulates the rate of glucose phosphorylation.

It is well established that a specific glucose 6-phosphatase (EC 3.1.3.9) exists in mammalian liver and kidney, where it plays an important role in the release of glucose from glucose 6-phosphate which has been produced from glycogen or gluconeogenic precursors. Although the ability to hydrolyse glucose 6-phosphate has been reported for extracts of muscle and other tissues (for review see Nordlie, 1971), the activities were very low. Furthermore, in many cases, the activities were measured by histochemical techniques, so that it was not possible to distinguish between a specific glucose 6-phosphatase and a non-specific phosphatase. However, the purification of glucose 6-phosphatase from rat brain (Anchors & Karnovsky, 1975) indicated the existence of a specific enzyme in nervous tissue, so that it seemed important to establish whether the enzyme also existed in muscle. The report of a simple, reproducible and precise radiochemical assay for glucose 6-phosphatase (Kitcher *et al.*, 1978) prompted a systematic investigation of the activities of this enzyme in muscle from animals across the animal kingdom. In addition, the maximum activities of hexokinase (EC 2.7.1.1) and, if they were not already available, the maximum activities of fructose bisphosphatase (EC 3.1.3.11) were measured. The results of this investigation, together with some of the properties of glucose 6-phosphatase from insect flight and vertebrate muscles, are presented and discussed in this paper.

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Materials and methods

Chemicals and enzymes

All chemicals and enzymes were obtained from Boehringer Corp. (London) Ltd., London W5 2TZ, U.K., except for the following: radiochemicals were obtained from The Radiochemical Centre, Amersham, Bucks. HP7 9LL, U.K.; Bio-Gel P-100 was obtained from Bio-Rad Laboratories, Richmond, CA, U.S.A.: Triton X-100, dithiothreitol, propan-2-ol and ethyl acetate were obtained from

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BDH Chemicals, Poole, Dorset BH12 4NN, U.K.; EDTA and all inorganic chemicals were obtained from Fisons Scientific Apparatus, Loughborough, Leics. LE11 0RG, U.K.

Source of animals

Animals were obtained from the sources given by Newsholme & Taylor (1969), Beis & Newsholme (1975) and Zammit & Newsholme (1976). Moths were bred in the Department or obtained from commercial sources (R. Adams, Las Palmas, and R. Baxter, London). Some of the more common moths were caught locally in a light-trap. Flies were a gift from Shell Biosciences Laboratory, Sittingbourne, Kent, U.K. Locusts were used 7–14 days after the final moult. Flies were used 7–14 days after emerging from pupae. All other insects were of undetermined age, but they were known to be capable of flight. Apart from rats, for which only male animals were used, muscle tissue was obtained from male and female animals indiscriminately.

Preparation of homogenates

Muscle was removed from animals as soon as possible after death. It was cut into small pieces, if necessary, and homogenized in a Polytron set at position 10 for 10s at 0°C; 4 or 10vol. of extraction medium was used according to the expected activity of the enzyme. The extraction medium for glucose 6-phosphatase consisted of 100mM-triethanolamine, 5mM-EDTA, 5mM-$MgCl_2$, 30mM-mercaptoethanol and 100mM-KCl at pH7.3. Preliminary experiments established that this provided the highest activity of the phosphatase in muscles from selected animals from different phyla. The extraction buffer for hexokinase consisted of 150mM-triethanolamine, 15mM-$MgCl_2$, 1.6mM-EDTA, 3mM-KCl and 8mM-mercaptoethanol at pH7.5. The extraction medium for fructose bisphosphatase was as described by Crabtree *et al.* (1972).

Preparation of cell fractions

Cell fractions from both bumble-bee and pigeon muscle were prepared by the method of Schöttler (1971). The extraction medium used consisted of 20mM-Hepes [4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid], 300mM-mannitol and 1mM-EDTA, pH7.4, and 1mg of bovine serum albumin/ml of medium. Muscle was removed, cut into small pieces and gently homogenized with 10 or 6 vol. of extraction medium in a 50ml glass homogenizer with a Teflon pestle (the space between the pestle and homogenizer was such that, with 30ml of water in the homogenizer at 4°C, the pestle sank to the bottom under gravity in 20–25s). The homogenate was filtered through two layers of muslin and then centrifuged at 600*g* for 5min at 0°C. The 600*g* pellet was washed twice in extraction medium before resuspension in the medium, and the 600*g* supernatant was centrifuged at 5000*g* for 20min: the pellet was washed twice in extraction medium before resuspension in that medium. The 5000*g* supernatant was centrifuged at 150000*g* for 2h at 0°C; the pellet was washed twice in extraction medium before resuspension.

Assay of enzyme activities

Glucose 6-phosphate activity was assayed by the method of Kitcher *et al.* (1978). The assay medium contained 50mM-Pipes (1,4-piperazinediethanesulphonic acid), 10mM-glucose 6-phosphate and 0.02μCi of [U-^{14}C]glucose 6-phosphate adjusted to pH6.8 with KOH. A volume of assay medium (0.1ml) was incubated with 0.05ml of extract at 30°C for various periods of time up to 10min. The reaction was terminated by addition of 0.5ml of a saturated solution of $Ba(OH)_2$ followed by 0.5ml of 0.3M-$ZnSO_4$. A control was included in which the $Ba(OH)_2$ was added before the extract. After centrifugation in an Eppendorf Microfuge for 2min, 0.6ml of supernatant was mixed with 5.0ml of scintillation fluid [obtained by dissolving 2.0g of 2,5-diphenyloxazole and 0.5g of 1,4-bis-(5-phenyloxazol-2-yl)benzene in 500ml of toluene plus 250ml of Triton X-100 (Patterson & Greene, 1965)] and the radioactivity measured in a Beckman liquid-scintillation system (model LS230). Glycerol 3-phosphate phosphatase was assayed in a manner identical with that for glucose 6-phosphatase, except that glucose 6-phosphate was replaced by 10mM-glycerol 3-phosphate (containing 0.02μCi of [U-^{14}C]glycerol 3-phosphate).

Hexokinase was assayed as described by Zammit & Newsholme (1976). Fructose bisphosphatase was assayed as described by Crabtree *et al.* (1972). The activity of *p*-nitrophenyl phosphate was assayed as described by Bergmeyer *et al.* (1974). Pyruvate kinase was assayed as described by Zammit *et al.* (1978). Citrate synthase was assayed as described by Alp *et al.* (1976).

Assay of metabolites

Glycerol was assayed by the method of Garland & Randle (1962) and glucose was assayed by the method of Slein (1965).

Expression of results

All enzyme activities are expressed as μmol/min per g of fresh tissue at 30°C. Since, in the present work, no account has been taken of such factors as season, diet, size, age and, in some cases, sex of the animals, and since precise quantitative interpretations are not made from these activities, a conventional statistical presentation was not meaningful. Activities are presented as means with ranges and number of determinations on separate

animals or tissue pooled from separate animals given in parentheses in Table 1. Any use of the reported activities for precise quantitative analysis must be made with caution.

Results and discussion

Ranges of enzyme activities

The activities of hexokinase, glucose 6-phosphatase and fructose bisphosphates in the muscles from different animals are presented in Table 1. The mean hexokinase activities range from less than 0.1 to 172 μmol/min per g (abdominal muscle of shrimp and flight muscle of the wasp respectively); the mean glucose 6-phosphatase activities range from less than 0.1 to 8.0 μmol/min per g (some non-insect invertebrate muscles and flight muscle of the wasp respectively). The highest activity of glucose 6-phosphatase in the flight muscle of the wasp is similar to that in mammalian liver (see Scrutton & Utter, 1968, for the latter activities). In any individual insect flight muscle, the activity of glucose 6-phosphatase is, in general, 3–10% of the maximum activity of hexokinase. However, particularly in two families of moths (Bombycidae and Arctiidae) that were studied, the activities of glucose 6-phosphatase and hexokinase were similar (e.g. silkworms and tiger moths), and in one or two individual species the phosphatase activity was greater (e.g. buff ermine moth). In heart muscle from vertebrates, the activity of the phosphatase was usually much less than that of hexokinase. However, in the vertebrate skeletal muscle, except for the trout, the activity of the phosphatase was either similar to or higher than that of hexokinase. In some insects, the activities of glucose 6-phosphatase were measured in the fat-body; the activities are usually lower than those in the muscle (Table 1).

The activities of fructose bisphosphatase are included in Table 1 for comparison with those of glucose 6-phosphatase. There is no correlation between the activities of the two enzymes in the muscles investigated. For example, the flight muscles of the wasp and honey bee, in which fructose bisphosphatase activity is non-detectable, contain high activities of glucose 6-phosphatase; the activity of the bisphosphatase is very high in the *Bombus* species of bumble bee, whereas it is much lower in the cuckoo bumble bee (*Psithyrus* species), but the activities of the glucose 6-phosphatase are similar in the two bees (Table 1).

Specificity of muscle glucose 6-phosphatase

The radiochemical assay for glucose 6-phosphatase depends on the ability of a nascent precipitate at $BaSO_4$ and $Zn(OH)_2$ to bind the substrate, glucose 6-phosphate, but not the product, glucose. Hence, the activity of glucose 6-phosphatase is determined from the radioactivity appearing in the supernatant after removal of the precipitate. To show that glucose was the major product of the hydrolysis, the incubation was carried out with non-radioactive glucose 6-phosphate, under conditions identical with the radioactive assay, except that the reaction was stopped by addition of $HClO_4$ and glucose was measured enzymically (hexokinase and glucose 6-phosphate dehydrogenase). The radiochemical and spectrophotometric methods of assay gave almost identical activities for the enzyme from the flight muscles of the death's-head moth, bumble bee and garden tiger moth and from the pectoral muscle of the pigeon. It is assumed that, in other muscles not tested, the major product of hydrolysis is glucose.

Addition of up to 40 mM-glycerol 2-phosphate or -glycerol 3-phosphate to the assay medium for glucose 6-phosphatase (containing 10 mM-glucose 6-phosphate) caused no inhibition of the glucose 6-phosphatase, as measured by the radiochemical assay, in extracts of the flight muscles of the bumble bee, locust, death's-head hawk moth and garden tiger moth, and of sparrow pectoral muscle. However, the rate of glycerol release from 40 mM-glycerol 2-phosphate incubated with an extract of the flight muscle of bumble bee was 3.5, whereas the activity of glucose 6-phosphatase was 5.0 μmol/min per g. This suggests that a phosphatase capable of hydrolysing glycerol 2-phosphate exists in bumble-bee flight muscle, but, since glycerol 2-phosphate does not alter the rate of hydrolysis of glucose 6-phosphate, the two phosphatase activities must reside on separate enzymes. The addition of glucose 1,6-bisphosphate or fructose 1-phosphate (at 10 mM) to the assay medium had no effect on the activity of glucose 6-phosphatase from the garden tiger moth. The activity of bumble-bee extracts towards the usual non-specific phosphatase substrate, *p*-nitrophenyl phosphate, was 1.0 μmol/min per g, or about 20% of that of glucose 6-phosphatase.

Although high activities of glucose 6-phosphatase were detected in extracts of the flight muscles of honey bees and wasps, no detectable fructose bisphosphatase activity (Table 1) was found, in confirmation of previous work (see Crabtree *et al.*, 1972). In many other muscles, the activities of the two enzymes were very different, suggesting that the two activities are due to different enzymes.

Separation of glucose 6-phosphatase from non-specific phosphatases

Glucose 6-phosphatase has been separated from non-specific phosphatase activity (*p*-nitrophenyl phosphatase and glycerol 3-phosphate phosphatase) on a Bio-Gel P-100 column. A supernatant fraction obtained after centrifugation for 1 h at 50000 *g* of an extract of the flight muscle of the death's-head moth

Table 1. *Maximal activities of hexokinase, glucose 6-phosphatase and fructose bisphosphatase in tissues from vertebrates and invertebrates*

Enzyme assays are described in the Materials and methods section. Activities are presented as means with ranges and numbers of animals or pools of tissue from different animals given in parentheses. The asterisk indicates that fructose bisphosphatase activity was taken from Opie & Newsholme (1967), Crabtree *et al.* (1972) or Newsholme & Crabtree (1973, 1978): these latter activities were measured at 25°C.

		Enzyme activities (μmol/min per g fresh wt. of tissue)		
Animal	Tissue	Hexokinase	Glucose 6-phosphatase	Fructose bisphosphatase
Annelida				
Ragworm (*Nereis viriens*)	Dorsal longitudinal	3.3 (2.3–4.4) (3)	0.4 (0.3–0.5) (3)	0.4*
Mollusca				
Common limpet (*Patella vulgaris*)	Foot muscle	1.0 (0.9–1.1) (3)	<0.1 (3)	—
Common whelk (*Buccinum undatum*)	Radula retractor	3.8 (3.6–4.0) (3)	<0.1 (3)	0.6*
Crustacea				
Shrimp (*Crangon crangon*)	Abdominal flexor	<0.1 (3)	0.1 (3)	—
	Heart	3.4 (3.0–3.8) (3)	0.4 (0.3–0.6) (3)	—
Insecta				
Diptera				
Housefly (*Musca domestica*)	Flight muscle	29.5 (26.2–31.1) (3)	2.6 (2.4–2.7) (3)	—
Fleshfly (*Sarcophaga barbata*)	Flight muscle	28.8 (22.4–34.7) (3)	1.9 (1.4–2.5) (3)	1.3*
Blowfly (*Phormia terranova*)	Flight muscle	38.3 (33.3–41.7) (3)	2.8 (2.5–3.1)	0.8*
(*Calliphora erythrocephala*)	Flight muscle	42.1 (37.8–46.0) (3)	2.9 (2.7–3.2)	0.9*
Hymenoptera				
Honey-bee (*Apis mellifera*) (worker)	Flight muscle	64.0 (58.4–67.2) (3)	3.5 (2.6–4.0)	<0.05 (3)
Bumble-bee (*Bombus terrestris*) (male)	Flight muscle	96.7 (89.6–127) (3)	7.7 (7.4–8.0)	62*
(*Bombus pascuorum*) (male)	Flight muscle	145 (127–168) (3)	5.0 (4.8–5.3)	—
(*Bombus pascuorum*) (worker)	Flight muscle	153 (136–167) (3)	5.0 (4.3–5.8)	—
(*Bombus hortorum*) (male)	Flight muscle	116 (111–121) (3)	5.1 (4.5–5.9) (3)	—
(worker)	Flight muscle	117 (116–119) (3)	4.9 (4.8–4.9) (3)	22*
Wasp (*Vespa vulgaris*) (male)	Flight muscle	172 (155–180) (3)	8.0 (7.2–9.3) (3)	<0.05 (3)
Cuckoo bumble bee (*Psithyrus vestalis*) (male)	Flight muscle	67.8 (60.0–73.7) (3)	4.0 (3.8–4.3)	2.2*
Lepidoptera				
Cabbage white (*Pieris brassica*)	Flight muscle	14.4(13.3–16.3) (3)	1.4 (1.3–1.4)	—
(*Anglais urticae*)	Flight muscle	17.5 (16.6–17.7) (3)	1.8 (1.7–1.9)	—
	Fat-body	7.2 (5.8–8.8) (3)	1.4 (1.3–1.7)	—
Elephant hawk moth (*Deilephila elpenor*)	Flight muscle	27.9 (23.7–31.8) (3)	2.6 (2.0–3.3)	—
	Fat-body	9.8 (9.0–10.8) (3)	1.0 (0.9–1.2)	—
Death's-head hawk moth (*Acherontia atropos*)	Flight muscle	28.6 (28.3–29.3) (3)	4.6 (4.5–4.7)	0.2 (0.2) (2)
	Fat-body	5.8 (3.7–7.0) (3)	0.6 (0.5–0.7)	4.7 (4.0–5.4) (2)
Poplar hawk moth (*Laothae populi*)	Flight muscle	7.1 (5.4–8.1) (3)	1.5 (1.1–2.0)	0.2 (0.2) (2)
	Fat-body	1.0 (0.8–1.1) (3)	0.3 (0.2–0.4)	1.6 (1.4–1.8) (2)

Yellow-underwing moth (*Noctua pronuba*)	Flight muscle	21.4 (19–25) (3)	1.8 (1.7–1.9) (3)	<0.05*	
Tobacco horn worm (*Manduca sexta*)	Flight muscle	25.6 (24.8–27.2) (3)	2.0 (1.8–2.4) (3)		—
Buff-tip moth (*Phalera bucephala*)	Flight muscle	9.0 (8.0–9.9) (3)	1.6 (1.4–1.7) (3)	<0.05*	
Garden tiger (*Arctia caja*)	Flight muscle	3.2 (2.8–3.5) (3)	1.8 (1.4–2.0) (3)		—
Silkworm moth (*Bombyx mori*)	Flight muscle	2.2 (2.0–2.5) (3)	1.6 (1.1–3.1)		—
	Fat-body	3.4 (2.1–4.5) (3)	0.3 (0.2–0.4)		—
Heart and dart moth (*Agrotis exclamationis*)	Flight muscle	45.8 (34.8–53.1) (3)	2.2 (1.9–2.4)		—
Buff-ermine moth (*Spilosoma lutea*)	Flight muscle	0.7 (0.6–0.9) (3)	1.0 (0.8–1.1)		—
Orthoptera					
Locust (*Schistocerca gregaria*)	Flight muscle	10.3 (8.5–12.1) (3)	1.5 (1.6–2.3)	0.9*	
(*Locusta migratoria*)	Flight muscle	13.8 (11.7–17.3) (3)	2.2 (2.1–2.2)	0.4*	
Coleoptera					
Rosechafer (*Pachnoda ephippiata*)	Flight muscle	4.9 (4.3–5.3) (3)	2.2 (1.7–2.6) (3)		—
Trichoptera	Fat-body	8.4 (6.1–10.1) (3)	1.0 (0.6–1.6) (3)		—
Caddis fly (*Phryganea sp.*)	Flight muscle	12.6 (11.9–13.4) (3)	2.7 (2.2–3.6)		—
Pisces					
Rainbow trout (*Salmo gairdneri*)	Red	0.4 (0.3–0.6)	<0.1 (3)	0.2*	
	White	0.1 (0.1) (3)	<0.1 (3)	0.7*	
Amphibia					
Frog (*Rana temporaria*)	Sartorius	0.7 (0.6–0.9) (3)	1.5 (1.2–1.8) (3)	0.6*	
	Gastrocnemius	0.4 (0.3–0.4) (3)	1.5 (1.1–2.1) (3)	0.6*	
Toad (*Xenopus laevis*)	Sartorius	0.5 (0.4–0.6) (3)	0.8 (0.7–0.9) (3)		—
	Gastrocnemius	0.4 (0.3–0.5) (3)	0.8 (0.7–1.0) (3)	0.6	
Aves					
Domestic fowl (*Gallus domesticus*)	Heart	3.9 (3.2–4.3) (3)	0.2 (0.2) (3)	<0.05*	
	Pectoral muscle	0.6 (0.5–0.7) (3)	0.3 (0.2–0.3) (3)	2.5*	
Pigeon (*Columba livia*)	Heart	5.5 (4.7–6.2) (3)	1.6 (1.2–2.3) (3)		—
	Pectoral muscle	2.3 (2.1–2.4) (3)	1.8 (1.5–2.0) (3)	0.5*	
Sparrow (*Passer domesticus*)	Heart	3.4 (2.9–3.9) (3)	1.2 (0.9–1.4) (3)		—
	Pectoral muscle	1.6 (1.1–2.1) (3)	2.1 (1.9–2.3) (3)		—
Mammalia					
Laboratory mouse	Heart	10.4 (10.0–11.1) (3)	0.5 (0.5–0.6) (3)	<0.05*	
Laboratory rat	Heart	6.4 (6.0–6.7) (3)	0.3 (0.2–0.3) (3)	<0.05*	
	Red portion of quadriceps	1.5 (1.3–1.8) (3)	1.8 (1.5–2.0) (3)		—
	White portion of quadriceps	0.5 (0.4–0.6) (3)	1.5 (1.3–1.6) (3)	0.3*	
	Epitrochlearis	0.6 (0.4–0.8) (3)	0.7 (0.6–0.8) (3)		—
Laboratory rabbit	Heart	6.8 (6.4–7.1) (3)	0.5 (0.4–0.6) (3)	<0.05*	
	Semitendinosus	2.2 (2.0–2.4) (3)	3.4 (3.2–3.9) (3)	<0.05*	
	Adductor longus	0.4 (0.2–0.5) (3)	1.1 (1.0–1.2) (3)	0.9*	

or an extract of pectoral muscle of a sparrow was prepared as described in the Materials and methods section. A volume (5 ml) was placed on a column of Bio-Gel P-100 (100–200 wet mesh) 98 cm in length and 2.9 cm in diameter. The column was equilibrated in a buffer containing 150 mM-triethanolamine, 5 mM-EDTA, 5 mM-$MgCl_2$, 30 mM-mercaptoethanol and 200 mM-NaCl at pH 7.3, which was also used for elution of protein. The glucose 6-phosphatase activity from the insect flight muscle was clearly separated from glycerol 3-phosphatase and *p*-nitrophenyl phosphatase (Fig. 1), but the activities were less clearly separated for the pectoral muscle of the sparrow (Fig. 2). In both cases, the maximum activities of the glucose 6-phosphatase were markedly greater than those of the non-specific phosphatases both before and after separation. From the position of standards (bovine serum albumin and chymotrypsinogen A), the molecular weight of the glucose 6-phosphatase in the avian muscle was estimated to be about 58000 and somewhat lower than this in the insect flight muscle.

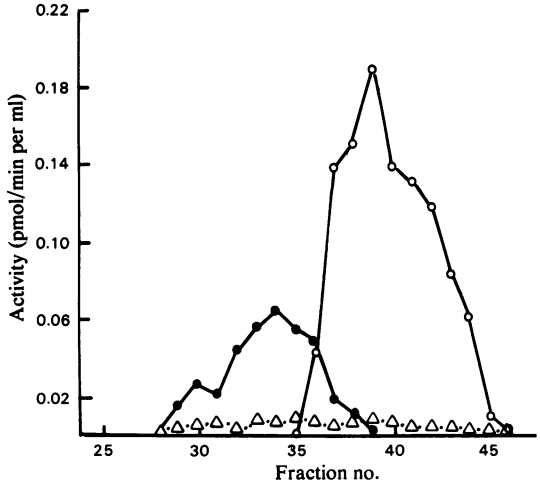

Fig. 1. *Separation of glucose 6-phosphatase from non-specific phosphatase activities in extract of insect flight muscle*

Flight muscle of the death's-head moth was extracted as described in the Materials and methods section, and 5 ml of a 50000 *g* supernatant was placed on a Bio-Gel P-100 column and eluted as described in the text. Fractions (5 ml) were collected and assays for glucose 6-phosphatase (O), glycerol 3-phosphate phosphatase (●) and *p*-nitrophenyl phosphatase (△) were carried out as described in the Materials and methods section.

Properties of glucose 6-phosphatase of insect flight muscle

The presence of a high activity of the enzyme in insect flight muscle permitted an analysis of the properties of the enzyme to be carried out with extracts of muscle.

pH. The effect of pH was investigated for the enzyme from the flight muscles of a butterfly, a moth and a bumble bee, and from pigeon pectoral muscle. In all three cases the enzyme showed a broad pH optimum in the range tested, pH 6.2–7.8, with an optimum at pH 6.8 (Table 2).

K_m. For the enzyme from the bumble-bee (*Bombus pascuorum*) flight muscle, a K_m value of 0.6 mM was obtained from a double-reciprocal plot. The plot was linear over the concentration range 0.5–2.5 mM, indicating an absence of co-operative effects. A similar value has been obtained for the enzyme from flight muscle of the death's-head moth. A problem with the sampling assays, as used in the measurement of glucose 6-phosphatase activity, is the time taken during the assay to allow a sufficient amount of radioactive product to be formed for satisfactory detection (see Crabtree *et al.*, 1979). During this time, the substrate concentration may have been decreased to values that cause a decrease in the enzyme activity; this is a particular problem at the low substrate concentrations. Hence the value of 0.6 mM is probably an upper estimate of the real

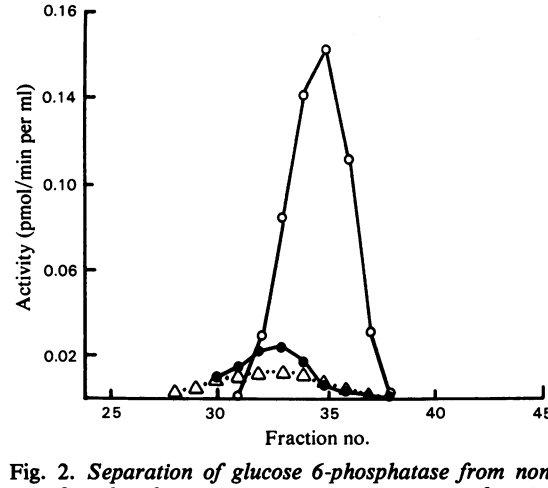

Fig. 2. *Separation of glucose 6-phosphatase from non-specific phosphatase activities in extracts of avian pectoral muscle*

The pectoral muscle of the sparrow was extracted and treated as described for insect flight muscle in Fig. 1. O. Glucose 6-phosphatase; ●, glycerol 3-phosphate phosphatase; △, *p*-nitrophenyl phosphatase.

Table 2. *Effect of pH on the activity of glucose 6-phosphatase from the flight muscles of the bumble bee (Bombus pascuorum), the moth (Noctua pronuba), the butterfly (Pieris brassica) and the pectoral muscle of the pigeon*

The enzyme activity was measured by using the buffer system and the assay method as described in the Materials and methods section.

	Glucose 6-phosphatase activity (μmol/min per g fresh wt.)			
pH	Flight muscle of *Bombus pascuorum*	Flight muscle of *Noctua pronuba*	Flight muscle of *Pieris brassica*	Pectoral muscle of the pigeon
6.2	6.0	1.4	0.8	1.0
6.5	6.1	1.7	1.1	1.2
6.8	6.5	1.9	1.5	1.7
7.0	6.4	1.4	1.3	1.1
7.2	6.0	1.4	—	—
7.5	5.5	1.1	1.2	1.0
7.8	5.3	0.8	0.8	0.8

Table 3. *Intracellular distribution of glucose 6-phosphatase, pyruvate kinase and citrate synthase in flight muscle of bumble bee (Bombus pascuorum) and pectoral muscle of the pigeon*

The enzyme activities are presented as μmol/min per g fresh muscle at 30°C. The supernatants and sediments were prepared as described in the Materials and methods section. It is assumed that the 600 *g* sediment represents the cell debris, the 5000 *g* sediment represents the mitochondrial fraction, the 150000 *g* sediment represents the sarcoplasmic reticulum and the 150000 *g* supernatant represents the cytosol.

	Enzyme activities (μmol/min per g fresh wt.)											
	Glucose 6-phosphatase				Pyruvate kinase				Citrate synthase			
	Bumble bee		Pigeon		Bumble bee		Pigeon		Bumble bee		Pigeon	
	Total activity	% of that in crude extract	Total activity	% of that in crude extract	Total activity	% of that in crude extract	Total activity	% of that in crude extract	Total activity	% of that in crude extract	Total activity	% of that in crude extract
Crude extract	2.7	—	2.2	—	259.5	—	424.5	—	68.2	—	39.3	—
600 *g* sediment	0.2	6.6	<0.01	0	0.6	0.2	0.5	0.1	33.1	48.1	11.2	28
5000 *g* sediment	0.1	3.0	<0.01	0	0.4	0.1	0.2	<0.1	29.8	43.3	13.2	33
5000 *g* supernatant	2.1	78.5	2.1	95	250.7	96.6	429.1	101	7.4	10.7	12.7	32
150000 *g* sediment	0.1	1.6	<0.01	0	19.1	7.4	14.1	3.3	0.8	1.2	1.5	4
150000 *g* supernatant	1.9	72.0	2.2	100	211.6	81.6	419.7	99	4.9	7.1	9.9	25

K_m. Nonetheless, this value is similar to that reported for glucose 6-phosphatase from the liver (0.2–1.4 mM, depending on the pH; see Nordlie, 1971).

The K_m values of glucose 6-phosphatase are similar to the concentration of glucose 6-phosphate measured in the freeze-clamped flight muscle of the bumble bee (about 0.2 mM; Newsholme *et al.*, 1972) and the death's-head moth, about 0.4 mM.

Subcellular distribution of glucose 6-phosphatase

Subcellular fractions were prepared from bumble-bee flight muscle and pigeon pectoral muscle as described in the Materials and methods section. Pyruvate kinase and citrate synthase were used as markers of the cytosolic and mitochondrial compartments respectively. Almost all the glucose 6-phosphatase activity was recovered in the 150000 *g* supernatant, with less than 1% in the 150000 *g* sediment (Table 3). This suggests that the phosphatase is present in the cytosolic compartment or, if it is attached to the sarcoplasmic reticulum, that it is all released on homogenization. The distribution of glucose 6-phosphatase in the insect flight muscle and pigeon pectoral muscle is similar to that found for hexokinase in these two muscles (Vaughan *et al.*, 1973), but it is very different from that of the enzyme from liver and kidney (see Nordlie, 1971).

Inhibitors and activators

Although glucose 6-phosphatase from the mammalian liver is inhibited by citrate (Nordlie, 1971), this compound had no effect on the enzyme activity from the flight muscle of the moth. There was no

effect of the following compounds on glucose 6-phosphatase activity from the flight muscle of the garden tiger moth: ATP, ADP, AMP, GTP, GDP, GMP, CTP, CDP, CMP, UTP, UDP, UMP, ITP, IDP, IMP (0.1–2mM) or 3′:5′-cyclic AMP and 3′:5′-cyclic GMP (0.1mM) when tested at 10mM-glucose 6-phosphate. Similarly, there was no effect of P_i (up to 20mM).

Stability

The maximum activity of glucose 6-phosphatase was not modified by freezing the muscle (either insect or vertebrate) in liquid nitrogen before extraction, nor was it affected by freezing the muscle extract and storing at −20°C for several days. Furthermore, the activity was maintained in the extract at 0°C for up to 12h.

Activity of glucose 6-phosphatase during development of the locust

The question arose whether the glucose 6-phosphatase, which was detected in flight muscle of the insects, represented an activity that was only of functional importance during development of that muscle. Hence, the activity was measured immediately before and after the final moult of the locust (Table 4). Whereas hexokinase activity reached a maximum at the time of the moult and then declined to about 60% of its maximum activity after 10 days, glucose 6-phosphatase activity increased during the first 3 days of development, and this activity was maintained to the period when the flight muscles are capable of supporting sustained flight (10–12 days after the final moult; see Weis-Fogh, 1952). This supports the view that the enzyme plays a role in glucose metabolism in relation to the provision of energy for flight (see below).

Role of glucose 6-phosphatase in muscle

The present results demonstrate unequivocally that glucose 6-phosphatase activity is present in a wide variety of muscles across the animal kingdom. In particular, in some muscles, especially insect flight muscles, the activity is very high and similar to that present in mammalian liver and kidney. One role of glucose 6-phosphatase in liver and kidney is to release glucose from glucose 6-phosphate which has been produced from glycogen or gluconeogenic precursors. It is possible that the enzyme in muscle has a similar role. This would mean that the large store of glycogen in skeletal muscle could be made available to other tissues as glucose. This finding also raises the possibility that under some conditions skeletal muscle might be able to carry out gluconeogenesis from, for example, lactate. However, neither of these suggestions is consistent with the fact that high activities of the phosphatase are found in honey-bee and wasp flight muscles, but both fructose bisphosphatase and phosphorylase activities are very low (Crabtree *et al.*, 1972; Crabtree & Newsholme, 1975), so that the rate of glucose 6-phosphate production from either glycogenolysis or gluconeogenesis would be extremely low.

An alternative suggestion is that the role of glucose 6-phosphatase in muscle is to provide the reverse reaction to that of hexokinase, so that a substrate (or 'futile') cycle between glucose and glucose 6-phosphate is possible. The cycle may increase the sensitivity of glucose phosphorylation to changes in the concentrations of regulators of either enzyme, as proposed for the same cycle in liver (see Hue & Hers, 1974; Newsholme & Crabtree, 1973, 1976). This suggestion is supported by a strong positive correlation between the activities of hexokinase and those of glucose 6-phosphatase reported in Table 1. For the activities from 63 different muscles from 55 different animals, the correlation

Table 4. *Activities of hexokinase and glucose 6-phosphatase in locust (Schistocerca gregaria) flight muscle before and after the final moult*

Enzyme activities were measured as described in the Materials and methods section. Activities are presented as means with ranges and numbers of animals or pools of muscle from different animals given in parentheses.

Time after final moult (days)	Enzyme activities (μmol/min per g fresh wt.) Hexokinase	Glucose 6-phosphatase
−1	3.1 (2.6–3.9) (3)	0.4 (0.3–0.6)
0.1	14.0 (13.1–14.9) (3)	1.0 (0.9–1.1)
1	10.7 (9.7, 11.7) (2)	1.4 (1.3, 1.5)
2	9.9 (8.8, 11.0) (2)	1.6 (1.5, 1.7)
3	9.9 (9.0–11.3) (3)	1.8 (1.6–1.9)
5	9.2 (8.1–11.0) (3)	1.7 (1.4–1.9)
7	8.5 (8.0–9.0) (4)	1.8 (1.7–2.0)
10	7.8 (7.2–8.3) (3)	2.0 (1.7–2.3)

coefficient is 0.9. Direct measurements of cycling rates, using dual-labelled glucose, are required to test this suggestion.

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