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The Activities of Proline Dehydrogenase, Glutamate Dehydrogenase, Aspartate–Oxoglutarate Aminotransferase and Alanine–Oxoglutarate Aminotransferase in some Insect Flight Muscles

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Many insects contain a high concentration of proline in the haemolymph (see Wyatt, 1961) and, as this amino acid had been shown to be oxidized by homogenates of flight muscle from the housefly (Sacktor, 1955), it was suggested that proline oxidation could provide energy for insect flight (Sacktor, 1961). In the initial period of flight in the tsetse fly Bursell (1963) observed a decrease in the thoracic content of proline and an increase in that of alanine. Further work by Bursell (1965, 1966, 1967) indicated that proline is converted into alanine via oxidation to glutamate, transamination of the latter with pyruvate to form alanine and oxoglutarate (catalysed by alanine–oxoglutarate aminotransferase, EC 2.6.1.2) and the conversion of this oxoglutarate into pyruvate via the latter portion of the tricarboxylic acid cycle followed by decarboxylation of oxaloacetate.

Despite the importance of this pathway in the tsetse fly and possibly other insects (see Kirsten, Kirsten & Arese, 1963; Sacktor & Wormser-Shavit, 1966), little work has been carried out on the enzymes of this metabolic pathway. One possible reason for this is the small amount of muscle available from the one insect in which the pathway appears to be of quantitative importance, namely the tsetse fly. The flavoprotein-linked enzyme proline dehydrogenase, which catalyses the oxidation of proline to Δ^1-pyrroline-5-carboxylate (see Brosemer & Veerabhadrappa, 1965; Sacktor & Childress, 1967), is the first enzyme of the proline oxidation pathway and therefore its activity should be limiting for the operation of this pathway (see Krebs, 1964). Thus the maximum activity of this enzyme should provide some indication of the quantitative importance of proline oxidation for the provision of energy in muscle, and to this end its activity has been measured in the flight muscles of various insects. The activities of glutamate dehydrogenase (EC 1.4.1.2), aspartate–oxoglutarate aminotransferase (EC 2.6.1.1) and alanine–oxoglutarate aminotransferase (EC 2.6.1.2) have also been measured and are reported in this paper.

Insects used in this investigation were obtained from the sources given by Newsholme & Taylor (1969); tsetse flies were obtained from the Tsetse Research Laboratory, School of Veterinary Science, Langford, Bristol, U.K. Freshly dissected flight muscles were homogenized (ground-glass homogenizer) in 10–100 vol. of 50mM-triethanolamine containing 30mM-mercaptoethanol, 2mM-$MgCl_2$ and 1mM-EDTA at pH 7.4. Homogenates were assayed within 20min of preparation without further treatment; preliminary experiments established that sonication was unnecessary for obtaining maximum enzyme activities.

Proline dehydrogenase was assayed by the reduction of INT;* the method was based on the assay for succinate dehydrogenase described by Pennington (1961). The incubation medium contained (final concentrations) 50mM-sodium phosphate, 1mM-KCN, 100mM-L-proline, 1.7mM-acetaldehyde and 200μg of yeast alcohol dehydrogenase at pH 7.5. To this was added 10–100μl of muscle homogenate and the reaction was initiated by addition of 100μl of phenazine methosulphate solution (10mg/ml) followed by 150, 200 or 250μl of a saturated aqueous solution of INT (approx. 5mg/ml). The final volume of the incubation was 1.5ml and the times of incubation varied from 5 to 15min at 25°C; preliminary experiments established that the amount of INT reduced was directly proportional to the time of incubation during this time-interval. Proline was omitted from control incubations. The reactions were terminated by the addition of 1.5ml of 10% (w/v) trichloroacetic acid. A 4ml volume of ethyl acetate was added to each tube, the contents were shaken vigorously for approx. 30s and the tubes were centrifuged at 300*g* for 1min. The ethyl acetate layer was removed with a Pasteur pipette and its extinction at 490nm measured against ethyl acetate; the molar extinction coefficient of reduced INT in ethyl acetate was assumed to be 20.1×10^3 (Pennington, 1961). Maximum enzyme activities were obtained by

* Abbreviation: INT, 2-*p*-iodophenyl-3-*p*-nitrophenyl-5-phenylmonotetrazolium chloride.

Table 1. *Activities of proline dehydrogenase, alanine–oxoglutarate aminotransferase, aspartate–oxoglutarate aminotransferase and glutamate dehydrogenase in insect flight muscles*

Each activity represents the mean of at least two determinations for each insect; the variation between separate determinations was less than 20%.

Insect	Enzyme activity (μmol of product formed/min per g wet wt. of muscle at 25°C)			
	Proline dehydrogenase	Alanine–oxoglutarate aminotransferase	Aspartate–oxoglutarate aminotransferase	Glutamate dehydrogenase
Cockchafer (*Melolontha melolontha*)	60	113	8	3.9
Tsetse fly (*Glossina austeni*)	40	402	—	—
Locust (*Locusta migratoria*)	1.7	34	48	4
Cockroach (*Periplaneta americana*)	6.7	45	28	4.8
Waterbug (*Lethocerus cordofanus*)	0.5	4.5	15	7.7
Honey bee (*Apis mellifera*)	1.5	7.2	—	—
Bumble bee (*Bombus hortorum*)	2.9	8	10	4.5
Small tortoiseshell butterfly (*Vanessa urticae*)	2.8	—	—	—
Poplar hawk moth (*Laothoe populi*)	2.4	7.5	18	6.4
Fleshfly (*Sarcophaga barbata*)	3.0	45	12	4.6
Blowfly (*Calliphora erythrocephala*)	2.0	40	16	—

plotting the reciprocal of ΔE_{490} against the reciprocal of the INT concentration and extrapolating this linear plot to infinite INT concentration (see Singer, 1963). The acetaldehyde–alcohol dehydrogenase system was present in the assay to buffer any NADH in the homogenate at a low concentration, thus minimizing the effect of enzymic reduction of INT by NADH.

Glutamate dehydrogenase was assayed by the method of Schmidt (1963) except that 1mM-KCN was present in the assay buffer. Aspartate–oxoglutarate aminotransferase and alanine–oxoglutarate aminotransferase were assayed by the methods of Bergmeyer & Bernt (1963*b*) and Bergmeyer & Bernt (1963*a*) respectively, except that the reactions were initiated with amino acid rather than keto acid; the concentration of the amino acid (i.e. alanine or aspartate) was varied and the maximum rate of the reaction estimated from a double-reciprocal plot; 1mM-KCN was also present in the aminotransferase assay mixtures.

Table 1 shows that the highest activities of proline dehydrogenase and alanine–oxoglutarate aminotransferase are found in the flight muscles of the cockchafer and tsetse fly; however, the activities of glutamate dehydrogenase and aspartate–oxoglutarate aminotransferase are much lower than that of proline dehydrogenase in the cockchafer. These results support the existence of the pathway of proline oxidation as proposed by Bursell (1967). The number of molecules of ATP that can be produced from the conversion of one molecule of proline into alanine (assuming conventional P/O ratios) by the pathway proposed by Bursell (1967) is 14, and this value is greater than that produced by the complete oxidation of one molecule of acetyl-CoA via the tricarboxylic acid cycle. Since the maximum activities of proline dehydrogenase in these two insects are similar to those of two important enzymes of the tricarboxylic acid cycle (NAD-linked isocitrate dehydrogenase and succinate dehydrogenase) in the flight muscles of many other insects (B. Crabtree & E. A. Newsholme, unpublished work), this suggests that proline oxidation could provide most of the energy for a period of flight in these insects.

The demonstration that the activities of proline dehydrogenase in the flight muscles of insects other

than the cockchafer and tsetse fly are very low may support the suggestion that the physiological significance of this enzyme in such muscles is the provision of tricarboxylic acid-cycle intermediates during the initial period of flight (Childress & Sacktor, 1966; Sacktor & Wormser-Shavit, 1966; Sacktor & Childress, 1967). However, the physiological significance of a quantitatively important energy-providing pathway from proline to alanine in insect flight muscle remains unanswered. For example, proline could have been converted into a more conventional storage form such as glycogen or fat and the energy released by glycolysis, β-oxidation and the tricarboxylic acid cycle to support flight.

The discovery of a high activity of proline dehydrogenase and the probable existence of the proline oxidation pathway in the flight muscle of the cockchafer may permit a more detailed study of the enzymes of this pathway, because comparatively greater amounts (300–400mg) of muscle can be obtained from this insect. Moreover, large numbers of this insect appear in late spring in the British Isles and can be caught easily. Such an investigation may throw some light on the physiological significance of this pathway in the cockchafer and the tsetse fly.

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