SOME ASPECTS OF THE REGULATION OF PROLINE METABOLISM IN THE FLIGHT MUSCLE OF THE TSETSE FLY *GLOSSINA MORSITANS*

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SUMMARY

1. Introduction

The purpose of this study was to investigate and extend knowledge of the oxidation of proline in the flight muscle of the tsetse fly Glossina morsitans. In various aspects of this work, metabolic comparison has been made with a more typical dipteran, the flesh fly Sarcophaga tibialis. Enzyme activities were assayed in the tsetse fly and compared to those of the flesh fly inorder to gain further insight into the importance of proline oxidation in these insects. NAD-linked malic enzyme was particularly active in tsetse fly muscle and for comparison, this enzyme was also measured in the flight muscle of a variety of other insect species. The enzyme was purified from tsetse fly thoraces and studied in detail, and the factors controlling its activity in vitro were investigated. Changes in metabolite levels in the thorax during early stages of flight were studied both in the tsetse fly and in the flesh fly, inorder to gain some insight into the factors that control their flight muscle metabolism in vivo.

2. A comparison of enzyme activities of the tsetse fly flight muscle with those of other insect species

High activities of alanine aminotransferase and proline dehydrogenase were found in flight muscle homo-

genates of the tsetse fly as compared to those of the flesh fly. In contrast, activities of pyruvate kinase and α-glycerophosphate dehydrogenase were higher in flesh fly flight muscle than in the tsetse fly. Investigation of the tricarboxylic acid cycle enzyme showed that activities of citrate synthase and isocitrate dehydrogenase were far lower in tsetse fly muscle homogenates than in those of the flesh fly, whereas the activities of fumarase, succinate dehydrogenase were of similar magnitude in the two flies. These findings were in keeping with the greater importance of proline metabolism and lesser dependence on carbohydrate metabolism in the tsetse fly as compared to the flesh fly.

The activity of NAD-linked malic enzyme in the tsetse fly flight muscle was over 10 times higher than that found in muscle homogenates of the flesh fly and other insect species. High activities of malic enzyme were also found in other tsetse fly species (G. austeni, G. pallidipes and G. longipennis).

NAD-linked malic enzyme was found to be largely mitochondrial, its distribution resembling that of alanine aminotransferase. A functional role for the enzyme in the pathway for the oxidative conversion of proline to alanine is postulated.

3. The properties of tsetse fly flight muscle NAD-linked malic enzyme

NAD-linked malic enzyme, EC 1.1.1.38 (L-malate:NAD^+ Oxidoreductase (decarboxylating)) was purified from flight muscle of G. morsitans to a specific activity of 15 µmoles/min per mg of protein measured at 30°C. The molecular weight as estimated by gel filtration was 230,000-255,000. SDS gel electrophoresis indicated a subunit molecular weight of about 57,540 suggesting that the enzyme was comprised of four equal subunits.

The enzyme required Mg^{2+} or Mn^{2+} for activity and K_a values of 0.28 mM and 0.007 mM respectively were obtained. At pH 7.8, the apparent K_m value for malate (with 1 mM NAD^+ and 5 mM Mg^{2+}) was 0.61 mM; that of NAD^+ (with 10 mM malate and 5 mM Mg^{2+}) was 0.25 mM. Plots of enzyme activity versus substrate concentration was sigmoidal at low concentrations of the second substrate, but hyperbolic when higher concentrations were employed. Apparent K_m values for both substrates decreased with increasing pH, whereas apparent V_{max} values were constant between pH 7.4 - 8.2.

Fumarate and succinate activated the enzyme at low substrate concentrations but did not affect maximum velocity. The apparent K_m values for the substrates were lowered and general Michaelis kinetics were observed in the presence of these activators. ATP, ADP inhibited

the enzyme significantly, being competitive with respect to malate, whereas inhibition by oxaloacetate was mixed. Pyruvate and NADH inhibited the enzyme weakly, inhibition by the latter being apparently competitive with respect to NAD^+.

Apparent K_m value for each substrate, determined at pH 7.8 in the presence of 2 mM fumarate was dependent on the concentration of the second substrate. That for malate tended towards zero as the NAD^+ concentration became high, while that for NAD^+ decreased with increasing malate concentration to a limiting value of 0.15 mM.

Other activities associated with the purified enzyme included:

a) A Ca^{2+} stimulated oxaloacetate decarboxylase

b) A reductive pyruvate carboxylase (reverse of normal enzyme activity) which was stimulated by either Mg^{2+} or Mn^{2+}, and required very high concentrations of pyruvate and bicarbonate.

c) A malate dehydrogenase (oxaloacetate reductase) which was independent of Mg^{2+} or Mn^{2+}.

d) An NADP-dependent malic enzyme whose activity was far less than the normal activity with NAD^+

The above properties of NAD-linked malic enzyme are discussed in relation to its postulated function in proline metabolism.

4. Some kinetics of other tsetse fly flight muscle enzymes involved in the control of its flight metabolism

Alanine aminotransferase (EC.2.6.1.21) and malate dehydrogenase (EC.1.1.1.37) were partially purified from the flight muscle of the tsetse fly. The apparent K_m values of alanine aminotransferase for glutamate (with 5 mM pyruvate) and pyruvate (with 70 mM glutamate) were 12 mM and 0.28 mM respectively at pH 7.4. The apparent K_m value of malate dehydrogenase for malate was 0.3 mM in the presence of 1 mM NAD^+, and that for NAD^+ was 0.12 mM in the presence of 5 mM malate.

5. Metabolite levels in the thorax of the tsetse fly during early stages of flight compared with those at rest

Proline decreased from 72 µmoles/g wet wt at rest to 30 µmoles/g wet wt after 2 min of flight, whereas alanine increased from 32 µmoles/g wet wt to 62 µmoles/g wet wt. The concentration of glutamate decreased at the onset of flight. The concentration of ammonia also decreased and no evidence was found for a significant initial contribution of deamination towards glutamate utilization.

Levels of pyruvate, malate, 2-oxoglutarate increased while those of citrate and isocitrate dropped on flight initiation. Succinate levels showed little change. The

level of ATP decreased, while those of ADP and AMP rose slightly at the onset of flight. Concentrations of sugar phosphates were very low; those of fructose-1,6-diphosphate appeared to increase slightly on initiation of flight. These metabolite changes in the tsetse fly are discussed in relation to the control of proline oxidation at the onset of flight.

6. Metabolite levels in the thorax of the flesh fly during early flight and at rest

Patterns of changes in metabolite levels in the thorax of the flesh fly during early stages of flight were similar to those observed in the tsetse fly. The resting concentrations of proline and alanine were more than 10 times lower in the flesh fly than in the tsetse fly. However, a decrease in alanine was observed during the first 2 min of flight, similar to that observed in the tsetse fly, although to a much smaller scale.

Levels of sugar phosphates were much higher in the flesh fly than in the tsetse fly. The fructose-1,6-diphosphate/fructose-6-phosphate ratio increased six times during the first 10 sec of flight. Concentrations of acetyl-CoA and acetyl carnitine were several fold higher in the flesh fly than in the tsetse fly, and were found to decrease somewhat on flight initiation.

Metabolic changes in the thorax of the flesh fly are discussed within the context of control of flight energy metabolism. No evidence was found to support the theory that proline is required at the onset of flight to augment the level of tricarboxylic acid intermediates. An energy yielding role for the proline-alanine oxidative pathway is suggested in this insect, supplementing carbohydrate metabolism at least during early stages of flight. The difference between the flesh fly and the tsetse fly as regards the energy metabolism of the flight muscle appears to be one of emphasis i.e. quantitative rather than qualitative.