Fructose 2,6-bisphosphate as a signal for changing from sugar to lipid oxidation during flight in locusts

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Flight in locusts is initially powered mainly by carbohydrate but if flight is to be sustained, as in migration, the animals have to utilize fat as the predominant fuel. The molecular basis of this metabolic switch has not been identified. Fructose 2,6-bisphosphate is a potent activator of 6-phosphofructokinase (EC 2.7.1.11) purified from locust flight muscle. After the first few minutes of flight in the locust the concentration of fructose 2,6-bisphosphate in the flight muscle falls dramatically, which should lead to a decrease in the activity of 6-phosphofructokinase as part of the mechanism to conserve carbohydrate during prolonged flight.

6-Phosphofructokinase *Glycolysis* *Fructose 2,6-bisphosphate* *Fat oxidation* *(Locust flight muscle)* *Insect migration*

1. INTRODUCTION

Locusts can fly for up to 20 h non-stop covering several hundred miles. To support this activity, the respiratory rate in the flight muscles is increased about 100-fold [1]. Initially, the major fuel is carbohydrate (i.e. muscle glycogen and the blood sugar, trehalose) but the flight muscles change from carbohydrate to lipid oxidation [2–4] when the rate of carbohydrate utilisation is decreased by more than 90% [5]. This change occurs before the stores of carbohydrate are depleted [6]. This restriction in carbohydrate utilisation might be important in maintaining the carbohydrate stores for use by tissues that have an absolute requirement for glucose and also to meet a sudden increase in power output by the muscles.

A similar change in substrate for muscle occurs in mammals during prolonged exercise; in this case, exercise stimulates fatty acid mobilisation from the adipose tissue and the raised level of plasma fatty acids causes an increased rate of fatty acid oxidation by muscle which, in turn, causes inhibition of glycolysis [7]. The latter is achieved, in part, by an increased level of citrate in the muscle which inhibits 6-phosphofructokinase [8]. However, the concentration of citrate is not elevated in locust flight muscle during flight [6,9] and this metabolite does not affect the activity of 6-phosphofructokinase from a variety of insects including locusts ([6,11] and unpublished). Although considerable efforts have been made to identify the respective mechanism in insects, it is still not known what factor(s) is responsible for the switch from carbohydrate to lipid oxidation in locust flight muscle during flight (review [12,13]). As the devastating effects of locust plagues are, by more than 90%, accounted for by migrating swarms [14] identification of factors responsible for controlling the switch from carbohydrate to lipid oxidation, which is a prerequisite of prolonged flight, could therefore be of eventual importance in control of migrating locusts by man.

To identify possible regulatory factors that

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might be important in this respect, the properties of 6-phosphofructokinase from locust flight muscle have been re-investigated here.

2. MATERIALS AND METHODS

2.1. *Chemicals and enzymes*

Biochemicals, coupling enzymes and triethanolamine were obtained from Boehringer Mannheim and fructose 2,6-bisphosphate and the pyrophosphate-dependent 6-phosphofructokinase from Sigma. The other substances were purchased from Merck, Darmstadt and Bio Gel HT was from Bio-Rad, Munich.

2.2. *Flight studies and determination of fructose 2,6-bisphosphate*

Adult migratory locusts (*Locusta migratoria*) of both sexes, 20–30 days of age, were suspended in a stream of air at an ambient temperature of 25°C. At various times between 10 s and 15 min of flight, the animals were dropped into melting nitrogen (i.e. liquid nitrogen that had been cooled to its freezing point, −210°C), the flight muscles were dissected from the frozen thoraces, carefully freed from fat body material and frozen haemolymph and the tissue was then extracted as described [15]. The concentration of fructose 2,6-bisphosphate was measured on the basis of its ability to activate the pyrophosphate-dependent phosphofructokinase from potato tubers [15].

2.3. *Purification of 6-phosphofructokinase*

Isolated flight muscle was slowly stirred for 15 min at 0°C in a medium containing 250 mM sucrose, 2 mM EDTA, 30 mM imidazole. After centrifugation the supernatant was fractionated with saturated ammonium sulphate. The sediment was suspended in a medium containing 1 mM K phosphate, 20 mM triethanolamine, 5 mM mercaptoethanol and desalted by Sephadex G-25. The enzyme was adsorbed to spherical hydroxyapatite and the column was eluted by a linear K phosphate gradient (5–40 mM, 5 mM mercaptoethanol). Peak fractions were again chromatographed on Bio Gel HT using a linear phosphate gradient (10–100 mM, 5 mM mercaptoethanol). The enzyme solution was concentrated about 20-fold by ultrafiltration and yielded a specific activity of about 80 $\mu mol \cdot min^{-1} \cdot mg^{-1}$ at 25°C. During the whole purification procedure the pH was kept at 7.5 and 1 mM fructose 6-phosphate and 3.5 mM glucose 6-phosphate were present to stabilise phosphofructokinase activity.

2.4. *Assay of 6-phosphofructokinase*

Enzyme activity was followed in a recording spectrophotometer at 25°C using an ADP-linked assay. The assay (total volume 500 μl) contained 50 mM triethanolamine, 50 mM KCl, 2 mM $MgCl_2$, 2 mM phosphate (sodium salt), 7.5 mM MgATP, 50 μM fructose 6-phosphate, 10 μg (2 U) pyruvate kinase, 10 μg (5.5 U) lactate dehydrogenase, 1 mM phospho*enol*pyruvate, 0.3 mM NADH, and 0.1 mM di(adenosine-5′-)pentaphosphate to inhibit any adenylate kinase activity. The final pH was 7.3. The coupling enzymes were dialyzed for 12 h prior to use, 175 μM glucose 6-phosphate and 5 μg (1.7 U) glucose-phosphate isomerase were added to maintain the concentrations of hexose phosphates close to their equilibrium value and hence buffer the concentration of fructose 6-phosphate.

3. RESULTS

3.1. *Fructose 2,6-bisphosphate and phosphofructokinase activity*

The activity of locust flight muscle 6-phosphofructokinase is activated by very low concentrations of fructose 2,6-bisphosphate but the precise magnitude of the response is highly dependent upon the assay conditions. At physiological concentrations of substrate, co-substrate, and effectors, but with the products omitted, the enzyme is virtually inactive; addition of 0.35 or 1.05 μM fructose 2,6-bisphosphate (final concentrations) increased the activity to 50% of its maximal at 140 (fig.1) and 90 μM AMP, respectively (not shown). At 50 μM fructose 6-phosphate and fructose 1,6-bisphosphate and 40 μM AMP, addition of fructose 2,6-bisphosphate increased the activity 20-fold with half-maximal stimulation occurring at 1.2 μM fructose 2,6-bisphosphate (fig.1). The effect of fructose 2,6-bisphosphate appears to be to decrease, synergistically with AMP, the $S_{0.5}$ of locust phosphofructokinase for fructose 6-phosphate: in the absence of fructose 2,6-bisphosphate and other

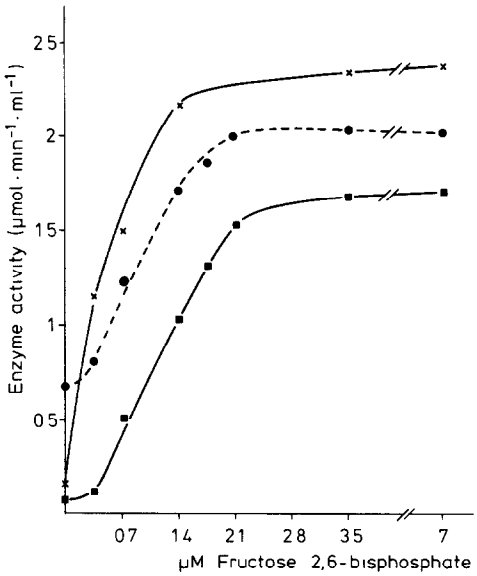

Fig.1. Effect of fructose 2,6-bisphosphate and AMP on purified 6-phosphofructokinase from locust flight muscle at near physiological concentrations of substrates and effectors. Assay conditions: 50 μM fructose 6-phosphate, 7.5 mM MgATP, pH 7.3. Other additions were as follows: (×—×) 140 μM AMP, (■—■) 40 μM AMP plus 50 μM fructose 1,6-bisphosphate, (●---●) 90 μM AMP plus 50 μM fructose 1,6-bisphosphate. For further details see text.

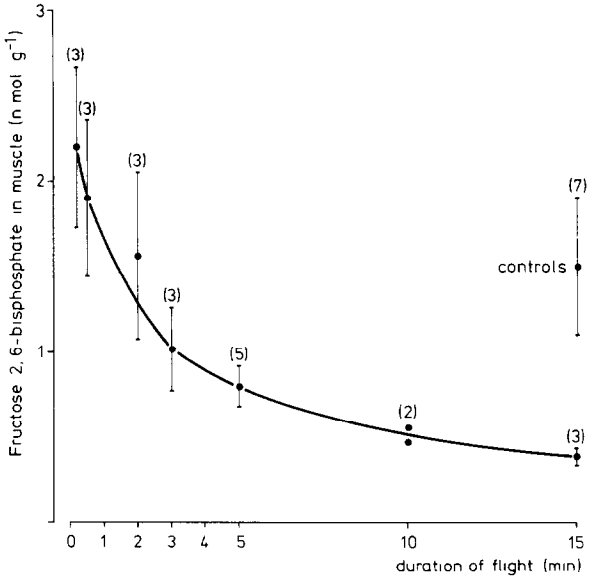

Fig.2. Effect of the duration of flight on the concentration of fructose 2,6-bisphosphate in flight muscle of the locust. Tethered flight was stopped at the times indicated (from 10 s to 15 min) by dropping the insects into melting nitrogen. Each observation is presented as the mean ± SE and the number of observations on separate animals is given in parentheses. Controls were kept in the apparatus for 15 min but were not allowed to fly.

activators, half-maximal activity can be attained only at unphysiologically high concentrations of fructose 6-phosphate (e.g. $S_{0.5}$ = 9 mM).

3.2. *Fructose 2,6-bisphosphate concentration in locust flight muscle during flight*

During flight the concentration of fructose 2,6-bisphosphate was rapidly decreased in the flight muscle, the level at 10 s of flight was 2.2 ± 0.47 and this was decreased to 0.39 ± 0.04 nmol/g at 15 min of flight (fig.2). The variation in the concentration of fructose 2,6-bisphosphate between insects was considerably higher in those that had flown for 2 min or less in comparison to those that had flown for more than 3 min: this suggests precise control of the concentration of this regulator during sustained flight.

4. DISCUSSION

Fructose 2,6-bisphosphate is a potent activator of 6-phosphofructokinases from a variety of sources and it has been found in all animal tissues studied so far [16,17] including insect flight muscle [18–20]. In mammalian liver, the function of fructose 2,6-bisphosphate in controlling the rates of glycolysis and gluconeogenesis is well established [16,17]. In all other animal tissues, its function is unclear [17]. Apart from liver, this would be the first example of fructose 2,6-bisphosphate being involved in changing from one metabolic pathway to another in animal tissues.

The marked stimulation of locust phosphofructokinase activity by fructose 2,6-bisphosphate (fig.1) and the dramatic decrease in its concentration during the first few minutes of flight (fig.2) suggests a mechanism for the inhibition of glucose utilisation and hence glucose oxidation as the haemolymph concentration of diacylglycerol increases during flight. In the locust flight muscle at rest, the activity of phosphofructokinase will be low because of the high concentration of the in-

hibitor ATP and the low concentrations of the de-inhibitors AMP and phosphate [7]. Upon initiation of flight, the decrease in the concentration of ATP and the increases in those of AMP and phosphate will increase the rate of glycolysis and this will be part of a mechanism to increase the rate of glucose utilisation [7,9]. It is now further suggested that the decrease in sugar utilisation that occurs early in flight [5] is, in part, due to inhibition of phosphofructokinase by the marked decrease in the concentration of fructose 2,6-bisphosphate (fig.2). This inhibition could then permit the oxidation of haemolymph diacylglycerol [4,5,21] by the flight muscle to provide the energy for flight. In this way fat could gradually replace carbohydrate as the major fuel for the flight muscle. At any time, however, an increase in AMP could override the effect of a decrease in the concentration of fructose 2,6-bisphosphate (unpublished), thus activate glycolysis and secure a sufficient ATP production to maintain flight.

Hence it can be considered that the decrease in concentration of flight muscle fructose 2,6-bisphosphate in the locust plays a similar role to the increase in citrate concentration in vertebrates as part of a mechanism to decrease the glycolytic rate when lipid oxidation is increased. The present findings raise the important question of how the decrease in the concentration of fructose 2,6-bisphosphate is brought about in locust flight muscle. This could be of potential importance in developing chemicals to control the migration of locusts.

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