Inhibition of ATP hydrolysis by ATP (a) and reversal of ATP inhibition by Mg\(^{2+}\) (b) in \textit{R. rubrum} chromatophores

The assay medium contained 50 mM-KCl, 20 mM-Tricine, 0.83 mM-EDTA, 1 mM-succinate, 5 mM-Na\(_2\)SO\(_3\), 10\% (w/v) sucrose, 50 \(\mu\)M-carbonyl cyanide \(p\)-trifluoromethoxyphenylhydrazone, final pH 7.6, in a total volume of 2.0 ml. Reaction was started by the addition of chromatophores to give 20 \(\mu\)M-bacteriochlorophyll in assay medium. Release of phosphate was measured colorimetrically after 3 min at 30°C in complete darkness. (a) Assay medium supplemented with 2 mM-MgCl\(_2\) and ATP varied as indicated; (b) assay medium supplemented with 10 mM-ATP and MgCl\(_2\) varied as indicated.

(Fig. 1b). Also, in contrast with the situation with Mg\(^{2+}\), inhibition of ATPase activity by free ATP is not overcome by adding activating anions or by changing the membrane permeability with uncoupling agents. Significantly, however, the \(K_m\) (ATP) with CaATP\(^{2-}\) as substrate is approximately 1.0 mM in the absence of sulphite and 0.5 mM in the presence of sulphite. There seems to be a simple correlation between these values and the \(K_m\) values for CaATP\(^{2-}\) in the presence and absence of sulphite, which may indicate that ATP binds to the active site of the enzyme but cannot be hydrolysed (see also Gepshtein & Carmeli, 1974) and that ATP binding is less dependent on the presence of bivalent cation than on the catalytic state of the enzyme.


Changes in Dipteran Flight-Muscle Mitochondria after Temperature Acclimation

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Like many poikilotherms, blowflies can acclimate to high and low temperatures, within the temperature range 2–40°C. Acclimation effects are reflected in the metabolic rates.
obtained from flies kept under specific temperature regimes (Tribe & Bowler, 1968; Spencer-Davies & Tribe, 1969). These authors found that the O₂ consumption by whole Calliphora erythrocephala and their isolated flight muscle was temperature-dependent over the range 10-30°C.

The present paper examines the changes in the activity of glycerophosphate dehydrogenase in flight-muscle mitochondria of the blowfly Calliphora erythrocephala and glycerophosphate-cytochrome c oxidoreductase in a closely related species Sarcophaga bullata after temperature acclimation. Mitochondrial glycerophosphate dehydrogenase activity was measured by following the reduction of ferricyanide at 420nm in the presence of 15 mM-DL-glycerophosphate. Mitochondrial glycerophosphate-cytochrome c oxidoreductase activity was measured by following the reduction of cytochrome c at 550nm in the presence of 1 mM-KCN and 20 mM-DL-glycerophosphate.

Fig. 1 shows the effect of treating flight-muscle mitochondria with phospholipase A, which hydrolyses phosphoglycerides. The activity of glycerophosphate dehydrogenase decreases to about 25% of its initial rate within 10 min of treatment with phospholipase A. The activity of glycerophosphate-cytochrome c oxidoreductase decreases gradually, but after 60 min of treatment with phospholipase A the mitochondria have only 5% activity.

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**Fig. 1.** Effect of phospholipase A on glycerophosphate dehydrogenase and glycerophosphate-cytochrome c oxidoreductase activities

*Calliphora erythrocephala* flight-muscle mitochondria (16.9 mg of protein) were incubated at 25°C in a medium containing 130 mM-KCl, 10 mM-Tris/HC1, pH 7.2, 0.1 mM-CaCl₂ and 5 units of phospholipase A. Samples for assay were taken at the times shown. Glycerophosphate dehydrogenase (○) was assayed at 420 nm and at 25°C in a medium containing 130 mM-KCl, 10 mM-Tris/HC1, pH 7.2, 1 mM-K₃Fe(CN)₆ and 15 mM-DL-glycerophosphate. Glycerophosphate-cytochrome c oxidoreductase (●) was assayed at 550 nm and at 25°C in a medium containing 130 mM-KCl, 10 mM-Tris/HC1, pH 7.2, 1 mM-KCN, 1 mg of cytochrome c and 20 mM-DL-glycerophosphate.
of their initial activity. These results show that both enzymes are dependent on phospholipids for full activity.

It is now generally accepted that the ‘breaks’ in Arrhenius plots of many membrane-bound enzymes reflect phase changes in the lipids of the membrane (Aithal & Tustanoff, 1975), and the fatty acid moieties of the phospholipids probably control these phase transitions (Raison et al., 1971). In fish it has been shown that after temperature acclimation the fatty acids in the phospholipids of the plasma membrane are altered (Johnston & Roots, 1964); with increasing acclimation temperature the amount of saturated fatty acids increases and the amount of unsaturated fatty acids decreases. Therefore the activities of two flight-muscle mitochondrial enzymes were monitored at temperatures from 4°C to 35°C in flies acclimated at different temperatures, to determine whether the acclimation effects were a reflexion of the changes of fatty acids in the phospholipids of the mitochondrial membrane. To avoid possible variation between batches of flies, progeny from the same generation were divided at random into three different groups and each was acclimated for 4 days to different temperatures before experimentation.

Table 1 shows the transition temperatures of the Arrhenius plots for the two enzymes at different acclimation temperatures. The activation energies above and below the transition temperature are also given. The transition temperatures for glycerophosphate dehydrogenase activity in Calliphora erythrocephala acclimated at 10, 22 and 30°C are very similar; these range from 18.3 to 20°C. The transition temperature for glycerophosphate-cytochrome c oxidoreductase activity in Sarcophaga bullata is very similar for flies acclimated at 10 and 17°C, but it falls to 10.8°C in flies acclimated at 27°C. The activity of this enzyme at this acclimation temperature also shows a large increase in activation energy below the transition temperature.

The results with glycerophosphate-cytochrome c oxidoreductase suggest that acclimation at 27°C causes a difference in the fatty acid composition of phospholipids in the mitochondrial membrane as compared with that in the 10°C- and 17°C-acclimated groups. Guarnieri et al. (1976) have shown that fatty acids in the neutral lipids and phospholipids of Phormia regina mitochondrial membranes are mainly palmitoleic acid (32%) and oleic acid (29%), with some palmitic acid (11%) and linoleic acid (13%). It is tentatively suggested that there may be an increase in the palmitic acid and stearic acid and a decrease in the proportion of palmitoleic acid, oleic acid and linoleic acid in the flight muscle mitochondrial membrane during acclimation to 27°C.

The fact that there is no change in the transition temperatures for glycerophosphate dehydrogenase activity in Calliphora erythrocephala acclimated at 10, 22 and 30°C would tend to contradict this hypothesis. However, glycerophosphate dehydrogenase is assayed by direct interaction of ferricyanide with the dehydrogenase on the outside of the inner mitochondrial membrane (Donnellan et al., 1970; Klingenberg, 1970).

Table 1. Transition temperatures and activation energies of flight-muscle mitochondrial enzymes after acclimation

<table>
<thead>
<tr>
<th>Species (enzyme assay)</th>
<th>Acclimation temperature (°C)</th>
<th>Transition temperature (°C)</th>
<th>Activation energy (a) (kJ·mol⁻¹·K⁻¹)</th>
<th>Activation energy (b) (kJ·mol⁻¹·K⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calliphora erythrocephala (glycerophosphate dehydrogenase)</td>
<td>10</td>
<td>19.8</td>
<td>37.1</td>
<td>67.1</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>18.3</td>
<td>41.9</td>
<td>71.7</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>20.0</td>
<td>46.5</td>
<td>73.2</td>
</tr>
<tr>
<td>Sarcophaga bullata (glycerophosphate-cytochrome c oxidoreductase)</td>
<td>10</td>
<td>17.4</td>
<td>31.7</td>
<td>69.1</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>18.7</td>
<td>26.9</td>
<td>63.9</td>
</tr>
<tr>
<td></td>
<td>27</td>
<td>10.8</td>
<td>35.1</td>
<td>97.5</td>
</tr>
</tbody>
</table>
Therefore, although Fig. 1 shows that phospholipids are essential for glycerophosphate dehydrogenase activity, the fatty acids attached to these phospholipids may not be important. Glycerophosphate–cytochrome c oxidoreductase is a much larger segment of the respiratory chain, with the fatty acids of the phospholipids probably occupying key positions in the membrane, and therefore the actual fatty acids present will be important for full activity.

Johnston, P. V. & Roots, B. I. (1964) Comp. Biochem. Physiol. 23, 303-309

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Spectroscopic Studies on the Folding and Unfolding of α-Lactalbumin in the Presence of Small Ligands

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Bovine α-lactalbumin is a globular single-chain protein (mol.wt. 14 176) with four intra-chain disulphide bonds, no free thiol groups, four tyrosine and four tryptophan residues. It is thus well suited to difference spectroscopy, and we chose this method to follow conformational change.

We decided to study folding in the presence of smaller molecules, since the concentration of metabolites in vivo is potentially sufficient to affect folding. For example there is enough free amino acid in liver for one molecule (on average) to be in the domain of a typical folding protein (Singh et al., 1975).

When we first attempted to measure the unfolding and folding caused by a change in the urea concentration, we noted some unusual difference spectra. They could be interpreted, at low urea concentration, as a combination of positive contributions from tyrosine residues (286nm) and negative ones from tryptophan residues (292nm). The latter means that conformational change and exposure of tryptophan residues to the solvent occurred at urea concentrations as low as 0.1 M. Tyrosine residues followed a more normal course and began to emerge into the solvent at about 3 M-urea. Thus the tryptophan-emergence curve was biphasic, with about 0.3 tryptophan residue emerging between 0 and 3 M-urea, and another two between 3 M- and 8 M-urea. The remaining 1.7 residues were exposed in the native protein and remained so during unfolding. Also, small tryptophan difference spectra could be produced by changing the solvent at pH 7.0 from aq. NH₃ to sodium phosphate.

The biphasic tryptophan transition was confirmed by circular-dichroism measurements at 297.5 nm (Cowburn et al., 1972). The collected normalized denaturation curves are shown in Fig. 1. They were completely reversible and suggested that our preparation of α-lactalbumin contained a labile tryptophan environment (cf. Kronman et al., 1972). We found very little pH-dependence between pH 4.5 and 9.2, nor did removal of the C-terminal amino acid with carboxypeptidases A and B have any effect. The C-terminal leucine residue is believed to lie close to tryptophan-118 (Warne et al., 1974), but this result suggests that, in our preparation, residue 118 is already exposed to the solvent. Tryptophan-26 is buried near the centre of the molecule, so that the labile region was probably in the cleft, and involved residues 104 and 60. Fig. 1 includes the circular-dichroism curve for helix disappearance, and it is noteworthy that the helix apparently persisted well after all the aromatic residues had emerged, though none of