Tyler, D. D., Gonze, J. & Estabrook, R. W. (1966). *Archs Biochem. Biophys.* **115**, 373.

Localization of the L-Glycerol 1-Phosphate-Flavoprotein Oxidoreductase on the Outer Surface of the Inner Membrane of Insect Flight-Muscle Mitochondria

By J. F. DONNELLAN, M. D. BARKER and R. B. BEECHEY. (*Shell Research Ltd., Woodstock Agricultural Research Centre, Sittingbourne, Kent, U.K.*)

The oxidation of L-glycerol 1-phosphate by insect flight-muscle mitochondria follows Michaelis-Menten kinetics in the presence of $0.1 \mu\text{M-Ca}^{2+}$ or $10 \mu\text{M-Sr}^{2+}$ or 1.0 mM-Mg^{2+} (Estabrook & Sacktor, 1958; Hansford & Chappell, 1967; Donnellan & Beechey, 1969). In the presence of ethanedioxybis(ethylamine)tetra-acetate to effectively remove all multivalent metal ions the velocity-substrate concentration curve is sigmoidal. We have examined the effects of the following compounds on the oxidation of L-glycerol 1-phosphate in the presence and absence of $0.1 \mu\text{M-Ca}^{2+}$: DL-glyceraldehyde 3-phosphate, 2-deoxyglycerol 1-phosphate (propane-1,3-diol 1-phosphate), dihydroxyacetone phosphate, DL-3-phosphoglycerate, DL-2-methylglycerol 1-phosphate, DL-2-propylglycerol 1-phosphate, propyl phosphate and 2-chloroethyl phosphonate. In the presence of Ca^{2+} DL-glyceraldehyde 3-phosphate, 2-deoxyglycerol 1-phosphate, dihydroxyacetone phosphate and D-3-phosphoglycerate are competitive inhibitors, the K_i values being $70 \mu\text{M}$, 12 mM , 7 mM and 12.4 mM respectively. In the absence of Ca^{2+} DL-glyceraldehyde 3-phosphate and dihydroxyacetone phosphate still inhibit the oxidation of L-glycerol 1-phosphate. However, all the other compounds in the concentration range $5\text{--}30 \text{ mM}$ increase the rate of oxidation of L-glycerol 1-phosphate, and the sigmoidicity of the substrate-velocity curve is decreased and in most cases becomes hyperbolic. None of these substances is oxidized by flight-muscle mitochondria. These results imply different specificity requirements for the enzymic and modifier sites on the oxidoreductase molecule.

Thus many phosphate or phosphonate esters affect the oxidation of L-glycerol 1-phosphate. However, the flight-muscle mitochondrial membrane is peculiarly impermeable (Van den Bergh & Slater, 1962). If the L-glycerol 1-phosphate-flavoprotein oxidoreductase (EC 1.1.99.5) is located on the inside of the inner mitochondrial membrane it would imply that the L-glycerol 1-phosphate porter has a very low specificity. The location of the oxidoreductase on the outside of the inner membrane would more satisfactorily explain the ease with which these substances have access to the enzyme and exert their effect.

The latter location of the L-glycerol 1-phosphate-flavoprotein oxidoreductase is supported by the following observations. Antimycin A inhibits the oxidation of pyruvate but not that of L-glycerol 1-phosphate with cyanoferrate as electron acceptor in cyanide-treated mitochondria. This implies that the electron-transport chain from NADH-flavoprotein oxidoreductase to cytochrome *b* is not available to react with cyanoferrate. The latter is a non-permeant anion in rat liver mitochondria (Mitchell & Moyle, 1969). Thus either the L-glycerol 1-phosphate-flavoprotein oxidoreductase or its associated non-haem-iron protein is available to react with cyanoferrate. Also, flight-muscle mitochondria do not swell in iso-osmotic solutions of the ammonium salt of DL-glycerol 1-phosphate, and swelling is noted in iso-osmotic ammonium pyruvate. From this result we conclude that L-glycerol 1-phosphate is an impenetrant anion. This renders it improbable that the L-glycerol 1-phosphate-flavoprotein oxidoreductase is located on the inside of the inner mitochondrial membrane.

The cyanoferrate technique employed in this work was based on information supplied by Professor M. Klingenberg. Donnellan, J. F. & Beechey, R. B. (1969). *J. Insect Physiol.* **15**, 367.

Estabrook, R. W. & Sacktor, B. (1958). *J. biol. Chem.* **233**, 1014.

Hansford, R. G. & Chappell, R. B. (1967). *Biochem. biophys. Res. Commun.* **27**, 686.

Mitchell, P. & Moyle, J. (1969). *Eur. J. Biochem.* **9**, 149.

Van den Bergh, S. G. & Slater, E. C. (1962). *Biochem. J.* **82**, 362.

Effects of Calcium Ion and Electron-Acceptor Concentrations on the Activity of Mitochondrial Glycerol 1-Phosphate Dehydrogenase from Insect Flight Muscle

By H. VAUGHAN and E. A. NEWSHOLME. (*Agricultural Research Council Unit for Insect Physiology, Department of Zoology, University of Oxford, Oxford OX1 3PS, U.K.*)

Mitochondrial glycerol 1-phosphate dehydrogenase (EC 1.1.2.1) can be considered a regulatory enzyme of the glycerol 1-phosphate cycle, and the work of Hansford & Chappell (1967) has suggested that Ca^{2+} controls its activity. The enzyme activity was measured by following the oxygen uptake of mitochondria provided with glycerol 1-phosphate as substrate. In the present investigation the enzyme was assayed more directly by following the reduction of an electron-acceptor dye, INT.* Activation of the enzyme by raising the Ca^{2+} concentration from 1 mM to $10 \mu\text{M}$ was confirmed. In these assays the enzyme activity is limited by the concentration of

* Abbreviation: INT, 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyltetrazolium chloride.