Purification and Properties of Glycolytic-Cycle Enzymes from Coelacanth (*Latimeria chalumnae*) Muscle

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Comparative structural studies show that enzymes of the glycolytic pathway have existed in their present form from a very early stage in evolution (cf. Jones & Harris, 1972). It was therefore decided to investigate the molecular and enzymic properties of such enzymes from the coelacanth, a rare and primitive deep-water fish believed to have been extinct for over 75 million years until a specimen was recently rediscovered off the Comores Islands in the Indian Ocean (see Smith, 1939*a,b*). Four enzymes, glyceraldehyde 3-phosphate dehydrogenase, lactate dehydrogenase, triose phosphate isomerase and fructose 1,6-diphosphate aldolase have so far been isolated in pure crystalline form from coelacanth muscle.

Initial fractionation of the muscle extract was by addition of $(\text{NH}_4)_2\text{SO}_4$ to 0.7 saturation. Lactate dehydrogenase and fructose 1,6-diphosphate aldolase, which were insoluble, were subsequently eluted as separate peaks from hydroxyapatite and crystallized from about 1.8M- $(\text{NH}_4)_2\text{SO}_4$. Glyceraldehyde 3-phosphate dehydrogenase and triose phosphate isomerase were precipitated together by $(\text{NH}_4)_2\text{SO}_4$ between 0.7 and 1.0 saturation and were eluted as separate peaks from DEAE-cellulose. The pure enzymes were crystallized from $(\text{NH}_4)_2\text{SO}_4$ solution after additional chromatography on hydroxyapatite and after gel filtration on Sephadex G-100 respectively. The crystalline enzymes were homogeneous by gel filtration on Sephadex G-200, as well as by electrophoresis on Cellogel at different pH values and on polyacrylamide gel in 0.1% sodium dodecyl sulphate. On the basis of these results the molecular weights of the native enzymes, and of their subunits in sodium dodecyl sulphate, were generally similar to those of their counterparts from mammalian muscle. Glyceraldehyde 3-phosphate dehydrogenase, lactate dehydrogenase and fructose 1,6-diphosphate aldolase (mol.wt. approx. 140000) are tetramers whereas triose phosphate isomerase (mol.wt. approx. 50000) is a dimer. The results of gel electrophoresis in sodium dodecyl sulphate indicate, however, that the subunits of the coelacanth enzymes, in particular those of triose phosphate isomerase, are slightly smaller than those of the corresponding enzymes from mammalian muscle.

The specific activities of the coelacanth enzymes are similar to those of their mammalian counterparts, and for the lactate dehydrogenase substrate inhibition and K_m (for pyruvate) were characteristic

of muscle-type lactate dehydrogenase. Moreover, electrophoretic analysis of muscle, heart and testis extracts showed only one lactate dehydrogenase-active band, indicating the absence of isoenzymes. Nevertheless coelacanth lactate dehydrogenase was successfully hybridized *in vitro* (in 4M-urea for 30min) to give five active electrophoretically distinct bands with both bovine heart and muscle lactate dehydrogenase.

Crystalline coelacanth glyceraldehyde 3-phosphate dehydrogenase contains 3–4mol of bound NAD^+ /mol, and the binding of NAD^+ to the charcoal-treated apoenzyme (studied by spectrophotometric and fluorimetric methods) exhibits negative cooperativity [K_1 and $K_2 < 10^{-8}\text{M}$, K_3 $1.5 \times 10^{-6}\text{M}$ and K_4 $50 \times 10^{-6}\text{M}$ at pH7.0; cf. Conway & Koshland (1968) and De Vijlder *et al.* (1969)]. These results provide additional evidence for the highly conserved nature of the glycolytic pathway and of its constituent enzymes.

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Conway, A. & Koshland, D. E. (1968) *Biochemistry* **7**, 4011

De Vijlder, J. J., Boers, W. & Slater, E. C. (1969) *Biochim. Biophys. Acta* **191**, 214

Jones, G. M. T. & Harris, J. I. (1972) *FEBS Lett.* **22**, 160

Smith, J. L. B. (1939*a*) *Trans. Roy. Soc. S. Afr.* **27**, 47

Smith, J. L. B. (1939*b*) *Nature (London)* **143**, 748

Studies on Implanted Islets of Langerhans: Normalization of Blood Glucose Concentration, Blood Insulin Concentration and Weight Gain in New Zealand Obese Mice

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Inbred New Zealand obese mice, derived from the original stock of Bielschowsky & Bielschowsky (1953) and 5–8 weeks old, were divided into three groups, (a), (b) and (c), and implanted intraperitoneally with Millipore diffusion chambers (pore size $0.45\mu\text{m}$). Chambers of groups (b) and (c) contained islets of Langerhans (200) isolated by the collagenase technique. It was found that islets are viable for up to 10 weeks in implanted chambers. Body weight, blood glucose concentration and plasma insulin concentration were monitored in all three groups at intervals over a 45-day period.

(a) Nine animals, with chambers only, showed a mean increase in body weight of 40% (25 to 35g), a mean increase in blood glucose concentration of 2% (163 to 166mg/100ml) and mean increase in plasma