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

By EDITH KOLB and J. IEUAN HARRIS (*Medical Research Council Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, U.K.*)

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

By RONALD J. GATES, M. I. HUNT, R. SMITH and NORMAN R. LAZARUS (*Diabetes Research Unit, The Wellcome Foundation, Temple Hill, Dartford, Kent U.K.*)

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

insulin concentration of 12½% (112 to 128 μ units/ml). (b) Thirteen animals, with chambers with islets from New Zealand obese mice, showed increases in body weight, blood glucose concentration and plasma insulin concentration essentially similar to those in group (a). (c) Eleven animals, with chambers with islets from normal albino mice, showed a 19% increase in body weight (27 to 32g), a decrease in blood glucose concentration of 49% (207 to 106mg/100ml) and a decrease in plasma insulin concentration of 65% (152 to 54 μ units/ml). There was no significant difference between groups (a) and (b). Group (c) differed from groups (a) and (b) with a significance of $P < 0.001$ on all three variables monitored.

After 45 days chambers were removed from three animals of group (c) and reimplanted into three other New Zealand obese mice. The body weight, blood glucose concentration and plasma insulin concentration of the former group reverted to values characteristic of New Zealand obese mice. The latter group normalized. It is concluded that the implanting of normal islets into New Zealand obese mice normalizes these animals, suggesting that the basic genetic defect in these obese mice lies in the islets. When normal islets were implanted into *ob/ob* mice, similar results were obtained, confirming the work of Strautz (1970).

NZO mice when given glucose orally respond by producing a typical diabetic tolerance curve. After implantation with normal islets there is an improvement in the diabetic tolerance curve after 1 week. After 3 weeks the glucose tolerance curve is normal and this is maintained for at least 7 weeks.

In an effort to identify a functional difference between the islets of albino and New Zealand obese mice, the activity of adenylate cyclase was examined. No difference was found between the islets of the two groups when they were stimulated with tolbutamide, glucose and glucagon. This suggests that the defect seen by Larkins & Martin (1972), where no response of plasma insulin concentration to glucose, glucagon and tolbutamide *in vivo* was observed, lies beyond the adenylate cyclase system.

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The Synthesis of a Discrete High-Molecular-Weight Ribonucleic Acid Species by Isolated Chloroplasts

By MARTIN R. HARTLEY and R. JOHN ELLIS (*Division of Biological Sciences, University of Warwick, Coventry CV4 7AL, U.K.*)

Chloroplasts contain DNA, but so far no specific gene product of chloroplast DNA has been unambiguously identified. Hybridization studies have shown that chloroplast rRNA has complementary sequences in both chloroplast and nuclear DNA (Tewari & Wildman, 1968; Ingle *et al.*, 1970). Although there is indirect evidence that the chloroplast cistrons function in the synthesis of chloroplast rRNA (Surzycki *et al.*, 1970), investigations on RNA synthesis by isolated chloroplasts have failed to demonstrate the synthesis of any discrete species of RNA (Spencer *et al.*, 1971). We now report that intact isolated spinach chloroplasts synthesize a discrete high-molecular-weight RNA species of similar size to rapidly labelled chloroplast RNA synthesized *in vivo*.

Chloroplasts were isolated from the first-formed leaf pair of 14–16-day-old spinach plants (*Spinacea oleracea*) by the method of Ramirez *et al.* (1968). Incorporation of [³H]uridine into RNA showed an absolute requirement for light, and did not occur in lysed chloroplasts. Fractionation of the product synthesized *in vitro* by polyacrylamide-gel electrophoresis revealed a major product of mol.wt. 3.0×10^6 and two minor products of mol.wt. 1.25×10^6 and 0.5×10^6 . Chloroplasts isolated from excised leaves labelled via their petioles with [³²P]P_i for 1 h contained three major radioactive RNA species of mol.wt. 3.0×10^6 , 1.25×10^6 and 0.67×10^6 and a minor component of mol.wt. 0.5×10^6 . With increasing times of labelling, radioactivity appeared in the chloroplast rRNA species (mol.wt. 1.1×10^6 and 0.56×10^6), and during this transition the radioactivity in the rapidly labelled RNA species became a progressively smaller proportion of the total. The RNA species of mol.wt. 3.0×10^6 synthesized *in vivo* and *in vitro* coincided exactly with each other on co-electrophoresis. When actinomycin D was applied after a 1 h pulse-label with [³²P]P_i *in vivo*, further incubation resulted in an increase in the specific radioactivities of the chloroplast rRNA species with a decrease in the radioactivity of the rapidly labelled RNA species. This is consistent with the latter being the precursors of the former. We suggest that the rapidly labelled chloroplast RNA of mol.wt. 3.0×10^6 is a likely candidate for a large precursor to chloroplast rRNA, and is transcribed from chloroplast DNA.