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Biosynthesis and import into the mitochondrion of L-3-glycerophosphate dehydrogenase, and the effect of thyroid hormone deficiency on gene expression

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Mitochondrial L-3-glycerophosphate dehydrogenase (EC 1.1.99.5) is synthesised in bovine kidney (NBL-1) cells treated with uncoupler as a cytosolic precursor with $M_r = 76000$ indistinguishable from the mature form. In vitro translation of rat liver mRNA also gives rise to a product of $M_r = 76000$ but when this is imported into mitochondria it is processed to a product of $M_r = 66000$. L-3-Glycerophosphate dehydrogenase activity and immunoreactive protein are greatly decreased in liver mitochondria from hypothyroid rats. Paradoxically, in vitro translation of the mRNA from such animals gives rise to large amounts of the protein, much greater than that synthesised from euthyroid mRNA and comparable with that produced from hyperthyroid mRNA.

Introduction

The mitochondrial L-3-glycerophosphate dehydrogenase (EC 1.1.99.5) is an inner membrane protein which catalyses the transfer of electrons from substrate to ubiquinone [1,2]. The enzyme has been purified from several mammalian sources and in all cases consists of a single polypeptide chain of $M_r = 76000$ (rabbit, Ref. 1; pig, Ref. 2; rat, Refs. 3 and 4). The enzyme probably contains 1 mol of FAD and in the region of 1 mol of Fe, both per 76000 g of protein, and both cofactors appear to be involved in the catalytic reaction [1,3].

The most likely role of the mammalian enzyme is in regulating the activity of the glycerophosphate 'shuttle' for the oxidation of cytoplasmic NADH [5]. In turn, the enzyme is itself regulated by Ca^{2+} [6], Mg^{2+} [7] and thyroid hormone [8,9]. The effect of thyroid hormone is very tissue-specific [9] and the changes in enzyme activity correlate well with thyroid hormone-induced oxygen consumption [10] and the potency of various thyroid hormone analogues [11]. The effect is most marked in the liver where hyperthyroidism can lead to a 20-fold increase in activity, and hypothyroidism results in a decrease in activity to barely detectable levels [9]. The thyroid hormone induced increases in enzyme activity can be ascribed to a specific increase in enzyme protein due to increased production of mRNA coding for L-3-glycerophosphate dehydrogenase [12,13].

In common with many mitochondrial proteins, L-3-glycerophosphate dehydrogenase is synthesised in the cytoplasm [12] and subsequently imported into the mitochondrion. Cytoplasmically synthesised mitochondrial proteins are characterised by an N-terminal presequence which contains the information for directing the protein to the correct mitochondrial compartment [14]. In many cases this presequence is removed during import but in other proteins, such as the ATP/ADP translocase, the presequence is not cleaved and remains as part of the mature protein [14]. L-3-Glycerophosphate dehydrogenase appears to belong to the latter group, since the protein obtained by cell-free translation of rat liver RNA comigrated with the mature protein on SDS polyacrylamide gels [13].

In this report we confirm the absence of a detectable cleavable presequence for L-3-glycerophosphate dehydrogenase using a bovine kidney cell line, and examine the ability of the precursor form to be imported into the mitochondrion. Finally, we have looked at the effect of hypothyroidism on the synthesis of the enzyme.

Abbreviations: FCCP, carbonylcyanide 4-(trifluoromethoxyl)phenylhydrazone; PMSF, phenylmethylsulfonylfluoride.

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Materials and Methods

Animals. Male Wistar rats (150 g) were made hypothyroid by the administration of 0.05% (w/v) 6-*n*-propyl-2-thiouracil in the drinking water for 21 days. Thyroidectomised rats were obtained from Charles Rivers Laboratories and were maintained for 4 weeks in a warm quiet room with 1% (w/v) D-gluconic acid (hemicalcium salt) in the drinking water. Rats were made hyperthyroid as described elsewhere [13].

SDS-polyacrylamide gel electrophoresis. This was carried out on 1.5 mm thick slab gels using the Laemmli [15] discontinuous buffer system. Protein bands were stained with Brilliant Blue R or electrophoretically transferred to nitrocellulose [16]. [^{35}S]Methionine-labelled proteins were analysed by fluorography using the fluorophor described by Chamberlain [17] and pre-flashed Hyper-film-MP (Amersham International plc) at −70 °C.

Labelling of bovine kidney NBL-1 cells. NBL-1 cells were grown in large (10 cm diameter) petri dishes in Ham's F-12 medium supplemented with 10% (v/v) newborn calf serum. When semi-confluent, the cells were transferred to a low methionine medium for 1 h at 37 °C (Ham's F-12 medium diluted 1 in 20 with 'minus-methionine' medium consisting of Glasgow-modified Eagle's medium without L-methionine, supplemented with 10% (v/v) newborn calf serum and 2 mM L-glutamine). To accumulate precursors, cells were pre-incubated for 5 min in 10 μM FCCP before addition of 200–500 μCi of L-[^{35}S]methionine for 4 h at 37 °C. In pulse-chase experiments, NBL-1 cells were initially labelled with 500 μCi of L-[^{35}S]methionine in the presence of 10 μM FCCP. After 4 h this medium was removed and the cells placed in warm, normal-growth medium (supplemented with 2 mM cold methionine) for 45 min. Cell monolayers were washed with ice-cold phosphate-buffered saline (0.15 M NaCl, 20 mM sodium phosphate, pH 7.4) before harvesting in 0.5 ml of 20 mM Hepes/KOH (pH 7.4), 0.25 M sucrose, 2 mM EDTA containing 3 mM *p*-aminobenzamidine, 2 mM 1,10-phenanthroline and 1 mM phenylmethylsulphonyl-fluoride by scraping with a rubber policeman. The resultant suspension was incubated with an equal vol. of the above buffer containing 0.2 mg of digitonin per ml of cell suspension for 2 min on ice. The mixture was centrifuged for 1 min in an Eppendorf microfuge and both the supernatant or cytosolic fraction and the particulate fraction (containing the mitochondria) were retained. The particulate fraction was treated with NET buffer (0.15 M NaCl, 5 mM EDTA, 0.05 M Tris-HCl, pH 7.4) containing 0.5% (w/v) Triton X-100 and centrifuged at 3000 r.p.m. for 5 min in a Jouan MR15 bench top centrifuge. The resulting solubilised material was diluted with 2 vol. of NET buffer containing 0.4% (w/v) SDS and clarified by centrifugation at 14000 r.p.m. for 30 min in the Jouan centrifuge. The cytosolic fraction was diluted directly with NET buffer containing 1% (w/v) Triton X-100 and 0.4% (w/v) SDS and clarified by centrifugation. The extracts from the particulate and cytosolic fractions were used for immunoprecipitation.

Analysis of the [^{35}S]methionine-labelled immunoprecipitates was achieved by fluorography after separation of the polypeptides on SDS/polyacrylamide gels.

In vitro translation. Total cellular RNA was isolated from rat liver by the guanidine isothiocyanate method (Chrigwin et al. [18]). Poly(A$^+$) RNA was prepared from total RNA using messenger affinity paper (Hybond mAP, Amersham International plc) as described by Werner et al. [19].

Translation was carried out in a mixture containing 10 μl of rabbit reticulocyte lysate (New England Nuclear), 5.5 μl of translation cocktail (New England Nuclear), 2 μl of 1 M potassium acetate, 0.5 μl of 50 mM magnesium acetate, 3 μl of water and 2 μl (15 μCi) of L-[^{35}S]methionine (New England Nuclear). Either 20 μg of total RNA or 1 μg of poly(A$^+$) RNA was added in 2 μl of water. The mixture was incubated at 30 °C for 30 min (poly(A$^+$) RNA) or 60 min (total RNA).

Import of in vitro translated precursor into isolated mitochondria. Rat liver mitochondria were prepared as described by Pedersen et al. [20] and adjusted to 25 mg of protein per ml, as determined by the Biuret method [21]. Mitochondria (2.5 mg of protein) were added to 50 μl aliquots of translation mix and incubated at 27 °C. Samples containing 0.5 mg of mitochondrial protein were removed at intervals and the mitochondria were separated from the supernatant by centrifugation for 5 min at 10000 r.p.m. in a Jouan benchtop centrifuge. Samples were then processed by immuneadsorption with the omission of SDS from the buffers.

Proteolytic cleavage of proteins. Partially purified L-3-glycerophosphate dehydrogenase or SDS-solubilised rat liver submitochondrial particles were incubated at 22 °C with varying amounts of subtilisin as described in the relevant figure legend. The samples were prepared for electrophoresis and boiled for 2 min to inhibit the protease. Radiolabelled precursors were obtained by immuneadsorption and prepared for electrophoresis. Subtilisin (see Fig. legend) was added in 0.1 of the sample volume and the mixture run into 4% (w/v) stacking gel of a 10% (w/v) polyacrylamide gel. The current was turned off for 30 min and then the samples were separated by electrophoresis.

Miscellaneous methods. Submitochondrial particles were prepared by the method of Pedersen et al. [20]. L-3-Glycerophosphate dehydrogenase activity was measured by the method of Cottingham and Ragan [2] using dichlorophenolindophenol as the acceptor. Immunoblotting, immunoadsorption and the partial purification of porcine L-3-glycerophosphate dehydrogenase

were as described by Taylor and Ragan [13]. Unless otherwise stated, protein was determined by a modification of the method of Lowry [22].

Results

The accumulation of the precursor of L-3-glycerophosphate dehydrogenase in a bovine kidney cell line

The observation that the precursor of L-3-glycerophosphate dehydrogenase does not contain a cleavable presequence was made using protein obtained by cell-free translation [13]. A disadvantage of the in vitro system is that the precursor may be exposed to proteases which would be compartmentalised with the cell and thus could result in the apparent absence of an N-terminal extension. To investigate this possibility we looked at precursor accumulation in vivo using the bovine kidney cell line, NBL-1. The mature protein immunoprecipitated from these cells (Fig. 1) comigrated with the largest subunit of bovine heart NADH dehydrogenase as do the pig brain and rat liver L-3-glycerophosphate dehydrogenases previously studied [13]. As expected, the mature protein (tracks 1 and 2) was entirely confined to the membrane fraction of the cells (track 2). However, in cells treated with the uncoupler FCCP to prevent import, some of the protein was present in the cytoplasm (track 3) and this could be chased completely into the membrane fraction following removal of the FCCP (tracks 5 and 6). The cytoplasmically located material did not differ detectably in M_r from the membrane-associated (mature) form. This was confirmed on 7% polyacrylamide gels (tracks 7–9) which might be expected to provide greater resolution in this M_r range.

In all experiments performed with FCCP, some L-3-glycerophosphate dehydrogenase was associated with the membrane fraction. Whether this was due to incomplete inhibition of import or association of the protein with the external surface of the mitochondrial outer membrane was not established. Nevertheless, these experiments show that the immature protein, as defined by its cytoplasmic location, was indistinguishable from the mature enzyme.

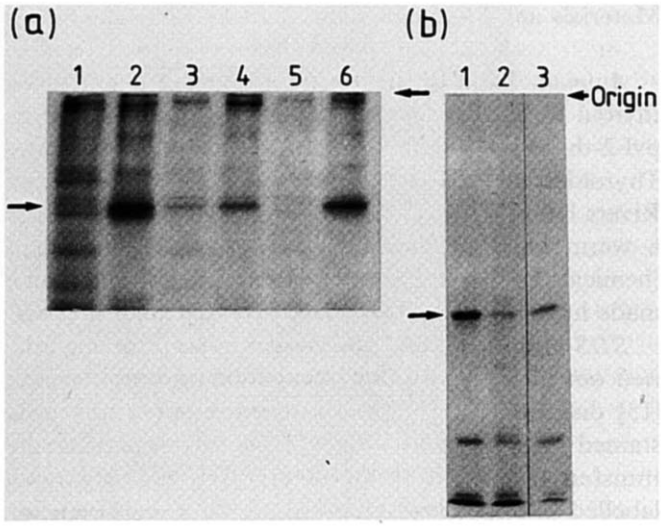

Fig. 1. Immunoprecipitation of the precursor to L-3-glycerophosphate dehydrogenase from NBL-1 cells. Cells were labelled and processed as described in the Materials and Methods section. Immunoprecipitates were analysed on (a) 12% (w/v) polyacrylamide gels or (b) 7% (w/v) polyacrylamide gels and the labelled proteins visualised by fluorography. (a) Tracks 1 and 2, cells labelled in the absence of FCCP; tracks 3 and 4, cells labelled in the presence of FCCP; tracks 5 and 6, cells labelled in the presence of FCCP then chased. Tracks 1, 3 and 5 are from the cytosolic fraction while tracks 2, 4 and 6 are from the particulate fraction. Only the upper part of the fluorograph is shown. The position of pig brain L-3-glycerophosphate dehydrogenase is shown by the arrow. (b) Tracks 1, 2 and 3 correspond to tracks 2, 3 and 6 respectively in (a). The additional protein of lower M_r in (b) is frequently present in immunoprecipitates from these cells and its identity is unknown.

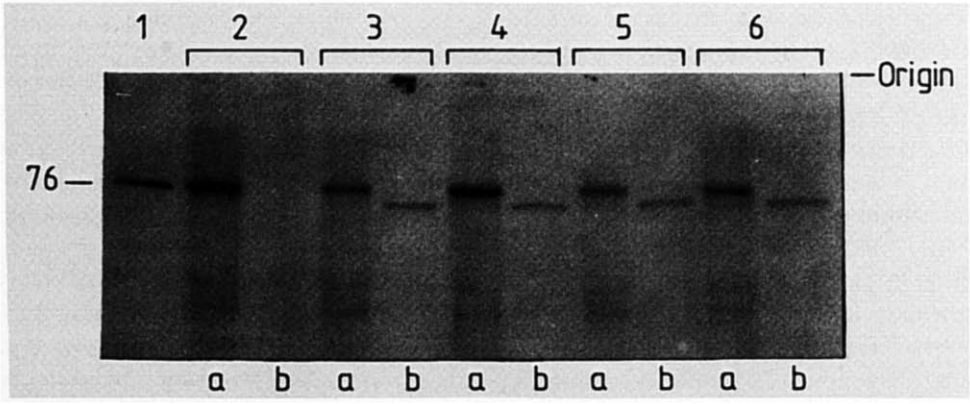

Fig. 2. The import of L-3-glycerophosphate dehydrogenase into isolated rat liver mitochondria. Cell-free translation was carried out using total rat liver RNA prepared from a hyperthyroid animal. Aliquots (50 μl) were incubated with 2.5 mg of mitochondrial protein for 0 (tracks 2), 15 (tracks 3), 30, (tracks 4), 60 (tracks 5 and 6) min. Samples were separated into supernatant (a)and pellet (b) and subjected to immunoprecipitation. The products were analysed on a 12% SDS polyacrylamide gel and visualised by fluorography. Only the upper part of the fluorograph is shown. Track 1 shows the position of porcine L-3-glycerophosphate dehydrogenase, located by Brilliant Blue R staining and marked with radioactive ink. In tracks 6, the incubation buffer additionally contained 0.8 μM FAD. The M_r of the mature enzyme is indicated (in thousands).

The import of L-3-glycerophosphate dehydrogenase

Experiments on the import of the $M_r = 76000$ in vitro translated product into mitochondria gave results apparently at odds with the experiments done in intact cells. After 60 min, 30–40% of the enzyme present in a cell-free translation mix became associated with the mitochondrial pellet (Fig. 2). However, the protein was cleaved suggesting that it had been imported into the mitochondria and was now accessible to an intramitochondrial protease, since no breakdown occurred in the enzyme remaining in the supernatant. Inclusion of FAD in the incubation did not affect uptake or cleavage. There are two possible explanations as to why the imported protein is smaller than the mature protein. One is that the L-3-glycerophosphate dehydrogenase does have an N-terminal extension, but that during synthesis in vitro an equivalent fragment was lost from the C-terminus, leaving a truncated protein after import. Whilst this is a possibility using the cell-free translocation, it is unlikely that the loss of an identical fragment from the precursor would have occurred in the cell line. An alternative suggestion is that the protein has been incorrectly assembled to give an unstable form, accessible to an intramitochondrial protease. The truncated protein has an apparent M_r of 66000 by comparison with standard proteins and we have observed that in mitochondria stored at $-20\,^\circ$C, the enzyme degrades with time to a product of this size. This could be prevented to a certain extent by storing the membranes as submitochondrial particles. This degradation could be mimicked in vitro by treating partially purified enzyme with trypsin or chymotrypsin. Finally, Beleznai and Jancsik [4] found that unless PMSF was present in the isolation buffer, the rat liver enzyme purified with an apparent M_r of approx. 60000. All these observations point to a tendency of the enzyme to degrade if not protected by an intact membrane (in freshly prepared mitochondria, the enzyme is resistant to trypsin digestion). Support for the above explanation was provided by partial proteolytic digestion in SDS.

Subtilisin digestion of the pig brain or rat liver enzymes in SDS gave rise to several proteolytic degradation products. In particular, rather stable 45 kDa and 30 kDa fragments were formed, presumably by a single cleavage of the native protein. Further degradation of the 45 kDa fragment was accompanied by formation of several smaller polypeptides, in particular those of $M_r = 22000$ and $M_r = 17000$ as shown in Fig. 3 for the pig enzyme.

If the in vitro translated protein contained a cleavable presequence of approx. 10 kDa and was truncated at the C-terminus by an equivalent amount, then it follows that at least one of the 45 and 30 kDa fragments should be absent following digestion of this polypeptide. In the experiment of Fig. 4, the in vitro translated protein was isolated by immunoprecipitation and subjected to subtilisin digestion. Both the 45 and 30 kDa products were found along with others including prominent 22 and 17 kDa fragments. The resolution achievable in this experiment was insufficient to claim complete identity between the mature and in vitro translated proteins, and indeed quantitative comparison was not possible, since the products were visualised in Fig. 3 by their antigenicity and in Fig. 4 by their methionine contents. However, the experiment serves to demonstrate that the in vitro translated protein is not artefactually truncated at the C-terminus.

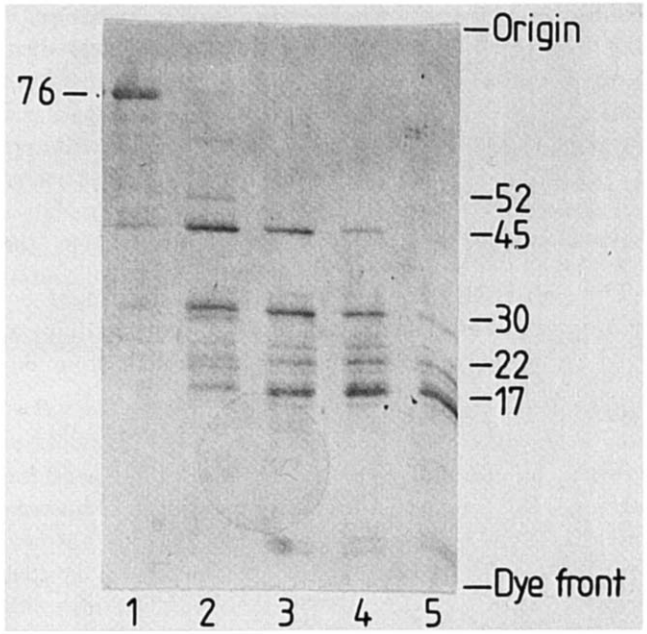

Fig. 3. Subtilisin digestion of porcine L-3-glycerophosphate dehydrogenase. Immunoblotting was carried out on nitrocellulose transfers from 12% SDS polyacrylamide gels, using anti L-3-glycerophosphate dehydrogenase antiserum and the biotin-streptavidin detection system. Partially purified porcine enzyme (6 μg of protein at a final concentration of 2 mg/ml was digested for 30 min at 22° C with subtilisin at concentrations of 0 (track 1), 2.5 (track 2), 5 (track 3), 7.5 (track 4) and 10 μg of protein/ml (track 5). M_r values of major components are indicated (in thousands).

The effect of hypothyroidism on the level of L-3-glycerophosphate dehydrogenase

In agreement with other workers [9] we found that the L-3-glycerophosphate dehydrogenase activity of rat liver mitochondria was very low in the absence of thyroid hormone. When the amount of the active form of thyroid hormone, T_3 [23], was reduced by treatment with 6-*n*-propyl-2-thiouracil, the activity was 0.62 ± 0.07 S.E.M. ($n = 4$) nmol per min per mg of mitochondrial protein. This was further reduced to an activity of 0.065 ± 0.04 S.E.M. ($n = 4$) nmol per min per mg of mitochondrial protein in rats where both the T_4 and T_3 forms were decreased by thyroidectomy. By comparison, the activity in mitochondria from untreated rats

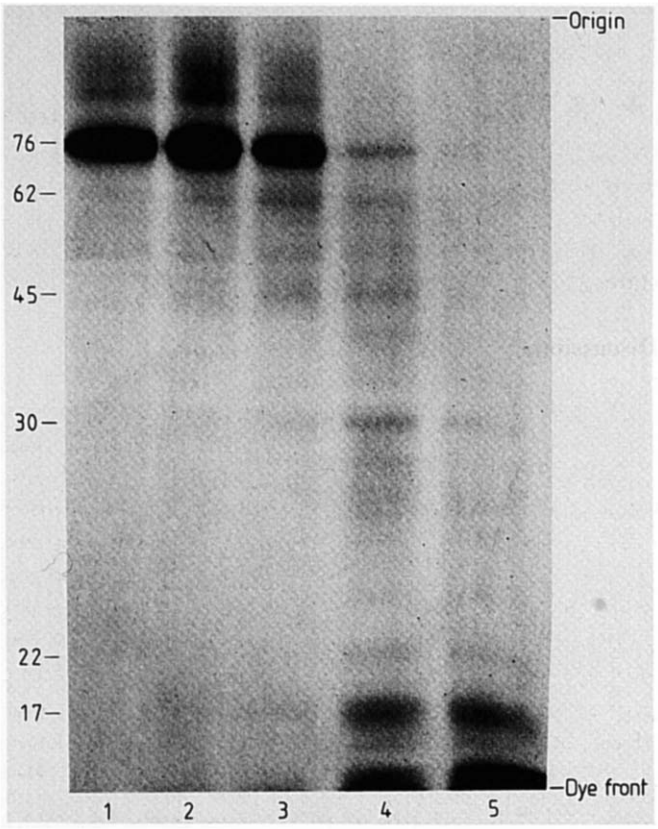

Fig. 4. Subtilisin digest of the $M_r = 76000$ cell-free translation product from euthyroid rat liver RNA. Translation was carried out on total liver RNA prepared from euthyroid animals. Aliquots (35 μl) of immunoprecipitated protein were mixed with 0.1 vol of subtilisin to give final protease concentrations of 0 (track 1), 0.2 (track 2), 0.8 (track 3), 2 (track 4), and 4 μg of protein per ml (track 5). Samples were run into the stacking gel and the current switched off for 30 min. Electrophoresis into the 12% SDS-polyacrylamide gel was continued and the products visualised by fluorography. M_r values of major components are indicated (in thousands).

was 2.4 ± 0.4 S.E.M. ($n = 8$) nmol/min per mg of mitochondrial protein, rising to 15.7 ± 2.9 S.E.M. ($n = 19$) nmol/min per mg of mitochondrial protein in hyperthyroid animals [13]. As expected, the low enzyme activity in the hypothyroid rats was paralleled by the inability to detect enzyme protein in these samples by immunoblotting (Fig. 5). When the same preparations were immunoblotted using an antiserum to bovine heart Complex I (Cleeter and Ragan [24]) the NADH dehydrogenase subunits were unaffected by the absence of thyroid hormone, showing that the effect on L-3-glycerophosphate dehydrogenase was specific.

In vitro translation of liver mRNA from hypothyroid rats

The increase in L-3-glycerophosphate dehydrogenase activity and protein seen in hyperthyroid rats is due to an increase in mRNA levels [13]. Consequently, we expected to see a decrease in mRNA levels in hypothyroid rats to correlate with the very low enzyme activity and protein levels in these animals.

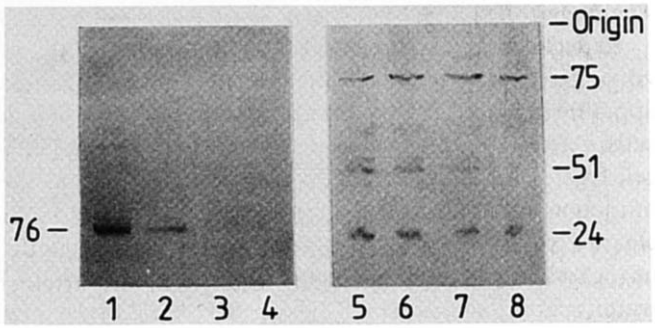

Fig. 5. The effect of hypothyroidism on the mitochondrial content of L-3-glycerophosphate- and NADH-dehydrogenases. Immunoblotting was carried out on nitrocellulose transfers from a 10% SDS polyacrylamide gel (tracks 1–4) or a 12–16% SDS polyacrylamide gel (tracks 5–8). In tracks 1–4 blotting was with an antiserum against porcine L-3-glycerophosphate dehydrogenase, and in tracks 5–8, with an antiserum against bovine NADH dehydrogenase. Each track contained 200 μg of liver submitochondrial particle protein from a hyperthyroid rat (tracks 1 and 5), an untreated rat (tracks 2 and 6), a thyroidectomised rat (tracks 3 and 7) and a rat treated with 6-*n*-propyl-2-thiouracil (tracks 4 and 8). Only the upper part of the blot is shown. M_r values of major polypeptides are indicated (in thousands).

In the experiments shown in Fig. 6, rabbit reticulocyte lysate was programmed with mRNA and the conditions for translation were chosen such that the level of incorporation of [^{35}S]methionine into total protein was linearly dependent on the amount of mRNA used [13]. Total incorporation into protein was similar for all samples of mRNA. The translation was carried out with

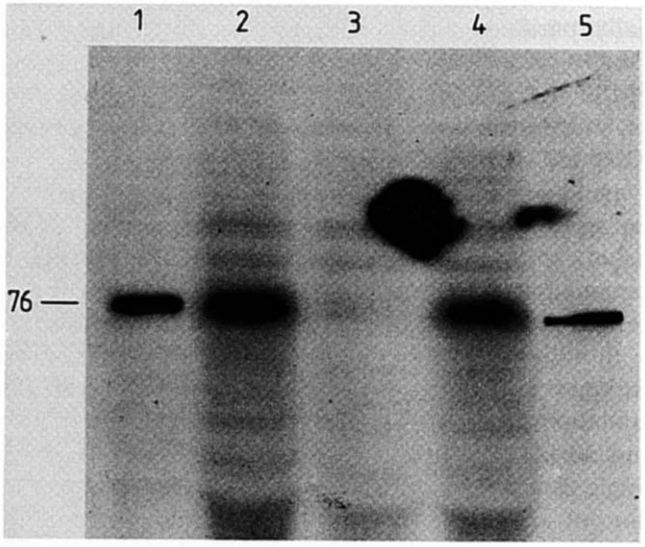

Fig. 6. The effect of hypothyroidism on the cell-free translation of L-3-glycerophosphate dehydrogenase. Translation was carried out on a message fraction from rat liver RNA. Immunoprecipitates were analysed on a 12% SDS polyacrylamide gel and visualised by fluorography. Tracks 1 and 5 show the position of porcine L-3-glycerophosphate dehydrogenase located by staining with Brilliant Blue R and marked with radioactive ink. The messenger RNA was prepared from a hyperthyroid rat (track 2), a euthyroid rat (track 3) and a rat treated with 6-*n*-propyl-2-thiouracil (track 4). Only the upper part of the fluorograph is shown.

mRNA from hyperthyroid and euthyroid animals and animals treated with 6-*n*-propyl-2-thiouracil. The mRNA from the hypothyroid rat directed the synthesis of enzyme to the same extent as did mRNA from the hyperthyroid rat, both being severalfold higher than the amount of enzyme produced by mRNA from the euthyroid rat [13]. The same result was obtained using mRNA from a thyroidectomised rat (not shown), thus removing fears that the first result was an artefact caused by the chemical treatment.

Proteolytic digestion of L-3-glycerophosphate dehydrogenase translated from hypothyroid mRNA

Although the specificity of the antiserum is evidence that the 76 kDa protein produced by translation of mRNA from hypothyroid rat liver is L-3-glycerophosphate dehydrogenase,we have confirmed its identity by comparing the proteolytic digestion patterns of the protein from both hypothyroid and euthyroid animals.

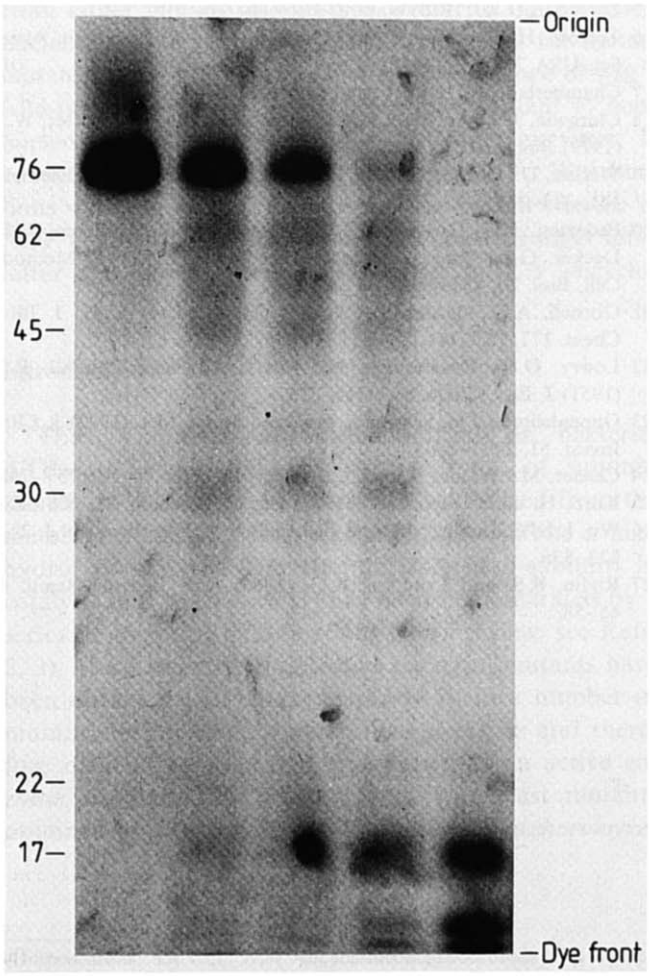

Fig. 7. Subtilisin digest of the $M_r = 76000$ cell free translation product from hypothyroid rat liver RNA. Conditions were as in the legend to Fig. 5 except that RNA from a hypothyroid rat was used and the subtilisin concentrations were (from left to right): 0, 0.01, 0.05, 0.1 and 0.5 μg of protein per ml.

As shown in Fig. 7, digestion of the protein produced from translation of hypothyroid rat liver RNA with subtilisin gave rise to exactly the same pattern of products as before (Fig. 4). Thus the protein produced from hypothyroid mRNA is indeed L-3-glycerophosphate dehydrogenase and contrary to expectation, L-3-glycerophosphate dehydrogenase mRNA is elevated not only in the hyperthyroid, but also hypothyroid states.

Discussion

We have demonstrated that the primary translation product of L-3-glycerophosphate dehydrogenase does not have a detectable presequence. Although a cleavable presequence is a common feature of proteins imported into the inner mitochondrial membrane, some proteins, such as the adenine nucleotide translocator [14] do not share this characteristic. The precursors in this category tend to differ from the mature protein in other ways. For example, precytochrome *c* lacks the haem group present in the functional protein [25]. L-3-Glycerophosphate dehydrogenase is a flavoprotein and contains some Fe therefore the pre-protein may lack one or other of the prosthetic groups.

The precursor was imported into isolated mitochondria, but the level of import was low and the precursor was incorrectly processed to a protein of $M_r = 66000$. Wu et al. [26] found that the import of the in vitro translated precursor of pyridine nucleotide transhydrogenase into isolated mitochondria occurred with low efficiency, around 20–40%. They proposed several factors that may contribute to this, including insufficiently intact mitochondria, incorrect folding and aggregation of the precursor and the possible absence of additional translocation factors. In addition, for L-3-glycerophosphate dehydrogenase, we anticipate that FAD and/or Fe may be involved in insertion of the enzyme into the inner membrane. When added to the in vitro system, FAD did not improve either the yield or intactness of the mitochondrially associated protein, but little is known about insertion of the prosthetic group. Other factors may be required for its correct incorporation.

The requirement for FAD may provide an explanation for the results obtained when we examined the synthesis of L-3-glycerophosphate dehydrogenase in hypothyroid animals. In agreement with Lee and Lardy [9], we found that enzyme activity was virtually absent in the liver of these animals, and this was paralleled by the absence of detectable enzyme protein in the mitochondria. In contrast, the NADH dehydrogenase was unaffected by the reduction in thyroid hormone levels. An mRNA fraction prepared from the liver of hypothyroid animals showed an increase in the synthesis of L-3-glycerophosphate dehydrogenase. It can be argued that in vitro translation is an indirect and there-

fore imprecise assay of mRNA level. However, the employment of subsaturating mRNA concentrations, the quantitative and qualitative similarity between the total translation products from the different mRNA preparations and the very large increases in L-3-glycerophosphate dehydrogenase translation with mRNA from hyper- and hypothyroid animals support the idea that the effects are due to different levels of specific mRNA. Therefore, the hypothyroid animals must be deficient in either the translation of the mRNA or import and processing of the protein, including the incorporation of its prosthetic group(s). Rivlin and Langdon [27] found that the livers of hypothyroid animals had a decreased FAD content due to a reduction in activity of the thyroid-hormone sensitive flavokinase (the enzyme which converts riboflavin to FMN). When they deprived normal rats of riboflavin, L-3-glycerophosphate dehydrogenase activity fell to the levels found in hypothyroid animals and this drop was not reversed by T_3 administration. Thus, the availability of FAD, itself a function of thyroid status, can control enzyme activity. The parallel changes in enzyme protein can be explained by instability of the apoflavoprotein compared with the holoenzyme, and it is therefore unnecessary to propose direct transcriptional control of the L-3-glycerophosphate dehydrogenase gene by thyroid hormone to explain the effects of thyroid status on this enzyme. The unusual response of the mRNA levels to thyroid status remains unexplained, but it suggests that multiple factors are involved in transcription of the gene.

Acknowledgements

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