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TRANSPORT OF THE PRECURSOR FOR RAT LIVER GLUTAMATE DEHYDROGENASE INTO MITOCHONDRIA "IN VITRO"

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SUMMARY Glutamate dehydrogenase of rat liver, a mitochondrial matrix enzyme, is synthesized in a larger precursor form in a rabbit reticulocyte cell-free system. When this precursor was incubated with isolated mitochondria in the absence of protein synthesis, it was processed to the mature form and rendered protease-resistant during incubation with mitochondria.

The majority of intramitochondrial proteins are initially synthesized on cytoplasmic ribosomes (1) and, therefore, must be transported across an intact outer membrane before reaching their final destination. We (2) and Mihara et al. (3) reported that glutamate dehydrogenase of rat liver is synthesized "in vitro" as a precursor larger than the mature form. Hence, pre-glutamate dehydrogenase must be transferred across both the outer and inner membranes of mitochondria to reach its final location in the mitochondria.

We further showed that a mitochondrial extract from rat liver processed the precursor synthesized "in vitro" to the mature form (2). We report here that the precursor of

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rat liver mitochondrial glutamate dehydrogenase is translocated into the mitochondria "in vitro", cleaved to its corresponding mature form and rendered insensitive to externally added proteases.

MATERIALS AND METHODS

General Previous communications (ref. 2,4-7) give full details for performing extractions of free and bound polyribosomes from rat liver, protein synthesis "in vitro", immunoprecipitations, SDS[1]-polyacrylamide gel electrophoresis and fluorography of dried gels. Purification of GDH and preparation of antisera against rat liver GDH were performed as described previously (2). Protein was determined by the method of Lowry et al. (8) with bovine serum albumin as a standard.

Incubation of pre glutamate dehydrogenase with isolated mitochondria Rat liver mitochondria were isolated by the method of Nicoletti (9). The "in vitro" import assay contained 200 µl of translated reticulocyte lysate (approximately 1.6 x 10^6 cpm protein-bound (^{35}S)) and was incubated with freshly isolated mitochondria (320 µg of protein) in medium consisting of 0.25 M sucrose, 1 mM dithiothreitol and 20 mM potassium Hepes, pH 7.4. The mixture was incubated for 30 min at 30°C with shaking, and then chilled in ice. For protease treatment, the samply was divided in half: one half received 150 µg trypsin/ml and the other half, 1 mM TLCK. After 30 min at 0°C trypsin activity was arrested by the addition of 1 mg/ml soybean trypsin inhibitor and 1 mM TLCK. Mitochondria were then reisolated by centrifugation (10,000 x g for 10 min) and the pelleted mitochondria were washed once with 0.25 M sucrose containing the protease inhibitors and lysed by the addition of 1 ml of 0.15 M NaCl; 18 mM potassium phosphate; 1% Triton X-100; 5 mM phenylmethylsulfonyl fluoride and 0.5 mM each of the four protease inhibitors (pH 7.4), then subjected to immunoprecipitation as described previously.

Trypsin treatment of mitochondria prior to import was done with 21.5 µg trypsin/ml and 3.2 mg mitochondria/ml for 10 min at 0°C and stopped by the addition of 1 mg/ml soybean trypsin inhibitor and 1 mM TLCK. The mitochondria were then reisolated by centrifugation (10,000 x g for 10 min).

RESULTS AND DISCUSSION

Import of pre-glutamate dehydrogenase into mitochondria

Intact mitochondria isolated from rat liver were incubated with products synthesized "in vitro". Half of the assay

[1] Abbreviations: SDS, sodium dodecyl sulfate; Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethane sulfonic acid; TLCK, tosyl-L-lysilchloromethylketone; GDH, glutamate dehydrogenase.

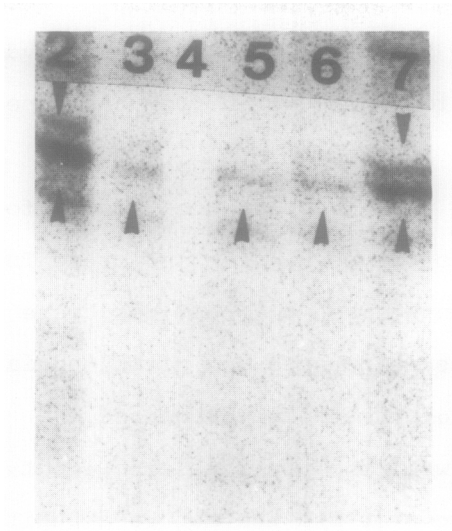

Figure 1. The precursor of glutamate dehydrogenase is imported into mitochondria and rendered protease-resistant. "In vitro" protein synthesis and its incubation with isolated mitochondria was performed as described in Materials and Methods. A photograph of the fluorogram is shown. Lane 2: precursor and mature form of GDH recovered in mitochondria which had not been treated with protease. Lane 5: GDH recovered in mitochondria after protease treatment. Lane 4: supernatant after protease treatment. Lane 6: mature GDH labeled "in vivo". Lane 7: precursor and mature form of GDH recovered in the supernatant of trypsin-treated isolated mitochondria. Lane 3: GDH recovered in mitochondria pre-treated with trypsin. Upward pointing arrows indicate mature form; downward pointing arrows indicate precursor form.

mixture was subsequently incubated with trypsin to digest (^{35}S)-labeled polypeptides accessible to protease. The mitochondria were recovered by centrifugation from each aliquot and the mitochondrial pellet was analyzed as described above. The results of the experiment are shown in Fig 1.

Mitochondria from the control aliquot contained both the precursor and the mature form of the GDH, while mitochondria from the protease treated aliquot contained only the mature form, suggesting that the precursor was left outside and the "processed" product had been imported into the organelle. The efficiency of the protease treatment in degrading any polypeptides outside the mitochondria

is clear from the fact that no glutamate dehydrogenase nor its precursor could be recovered from the supernatant after proteolysis (Fig. 1, lane 4).

Mitochondria that had been pre-treated for 10 min at $0^{\circ}C$ with 21.5 μg of trypsin per ml prior to incubation with the "in vitro" synthesized polypeptides, were then re-isolated and the supernatants and mitochondria were analyzed for rediolabeled glutamate dehydrogenase.

In contrast with the previous experiment, the supernatant contained the precursor and the mature subunit, while the mitochondria contained only the mature form. The relative amounts of mature and precursor forms recovered from the supernatant depend upon the intactness of the mitochondria, since the soluble matrix processing protease (2,10) leaks out of partially damaged mitochondria and converts p-GDH to the mature enzyme outside the mitochondria.

Most mitochondrial proteins are synthesized outside mitochondria as precursors that are usually larger than the mature forms found in mitochondria. These precursors are imported into mitochondria post-translationally (11-18) and the resulting mature polypeptides are inaccessible to externally added proteases (11,13,18,20,21).

The present data indicate that glutamate dehydrogenase of rat liver is initially made outside the mitochondria as a larger precursor which is transported into the mitochondria and processed there to the mature form which is no longer susceptible to externally added protease.

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