EVIDENCE FOR A PHYSIOLOGICAL CONVERSION OF PROTEINS SYNTHESIZED IN ISOLATED MITOCHONDRIA

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1. Introduction

The mol. wt. patterns of proteins synthesized in mitochondria, estimated by SDS-polyacrylamide gelelectrophoresis after labeling in vivo in the presence of cycloheximide, range from 7000 to about 50 000 [1–6]. Almost the same pattern is obtained after labeling of isolated mitochondria [5–9]. However, a time-dependence of the labeled pattern was found by Michel and Neupert [10]. Thus, after short labeling periods, mainly proteins of mol. wts. of about 10 000 were synthesized, whereas proteins of mol. wts. above 20 000 predominate after longer incubation times [10,11]. Exclusively low mol. wt. proteins were found to be associated with the isolated mitoribosomes even after long incubation times [10,12]. If protein synthesis with mitochondrial messenger RNA is performed in a cell free system, again only proteins of 10–12 000 dalton were observed [13]. The low mol. wt. proteins are highly hydrophobic and tend to aggregate during isolation [10, 14–18].

This communication describes the conversion of the primary low mol. wt. products of mitochondrial protein synthesis into proteins of larger apparent mol. wts. during further incubation. This conversion process can be blocked by inhibitors of ATP synthesis and of protein synthesis as well. The paper summarizes some results of the Diplomarbeit of H.-D. Hofmann [19]. Similar results have been published recently by Kužela et al. [20].

Abbreviations: FCCP, carbonylcyanide-*p*-trifluormethoxy-phenylhydrazone; SDS, sodium dodecylsulfate.

2. Materials and methods

L[^{14}C] Leucine (≈ 300 mCi/mmole) and L-[4,5-^{3}H] leucine (≈ 50 Ci/mmole) were obtained from Amersham Buchler (Braunschweig). Ethidium bromide was from Serva (Heidelberg), puromycin and FCCP were from Boehringer (Mannheim). All other chemicals were of analytical grade. Male Wistar rats weighing 200–300 g were used. NCS (Nuclear Chicago solubilizer) was from Nuclear Chicago, Deutschland, GmbH (Heusenstamm).

Isolation and incubation of rat liver mitochondria were performed as described previously [21] with minor modifications. Protein concentration during incubation was 0.8–1.3 mg/ml. Two samples incubated separately with [^{3}H]leucine (5 μCi/ml) or [^{14}C] leucine (1 μCi/ml) respectively, were mixed and diluted with 30 ml ice-cold sucrose medium [21]. After centrifugation, membranes were prepared by sonication and dissolved in 10 mM Na-phosphate, pH 6.8 containing 2% SDS and 5% mercaptoethanol. Gelelectrophoresis was done according to Weber and Osborn [22] with minor modifications. 0.5–0.8 mg protein were applied onto each gel. A stacking gel (3% acrylamide in 20 mM Na-phosphate, pH 6.8, 0.1% SDS) was polymerized above the separation gel. (10% acrylamide in 75 mM Na-phosphate, pH 7.8, 0.1% SDS.) Gels were cut into slices of 1.5 mm thickness, treated with 0.7 ml NCS and counted.

3. Results and discussion

The time-dependence of the mol. wt. pattern of proteins synthesized in isolated mitochondria is shown in fig.1. After 10 min of incubation, mainly proteins of an apparent mol. wt. around 10 000 are found. After 70 min of incubation proteins of apparent mol. wts. between 20 000 and 45 000 predominate.

In order to distinguish between an altered protein synthetic activity and a conversion of primarily synthesized proteins during prolonged incubation, a pulse-chase technique was used. The results are shown in fig.2, which compares the radioactivity distribution after 10 min of incubation with that after additional 60 min of incubation in the presence of excess unlabeled leucine. During the chase period, the radioactivity within the low mol. wt. region disappears, whereas radioactivity in the region of apparent mol. wts. between 20 000 and 45 000 increases. This finding seems to indicate a conversion of low mol. wt. proteins into proteins of higher mol. wts. during the chase period. Similar conclusions were drawn from experiments on the synthesis of mitochondrial proteins in intact cells of *Neurospora crassa* [10].

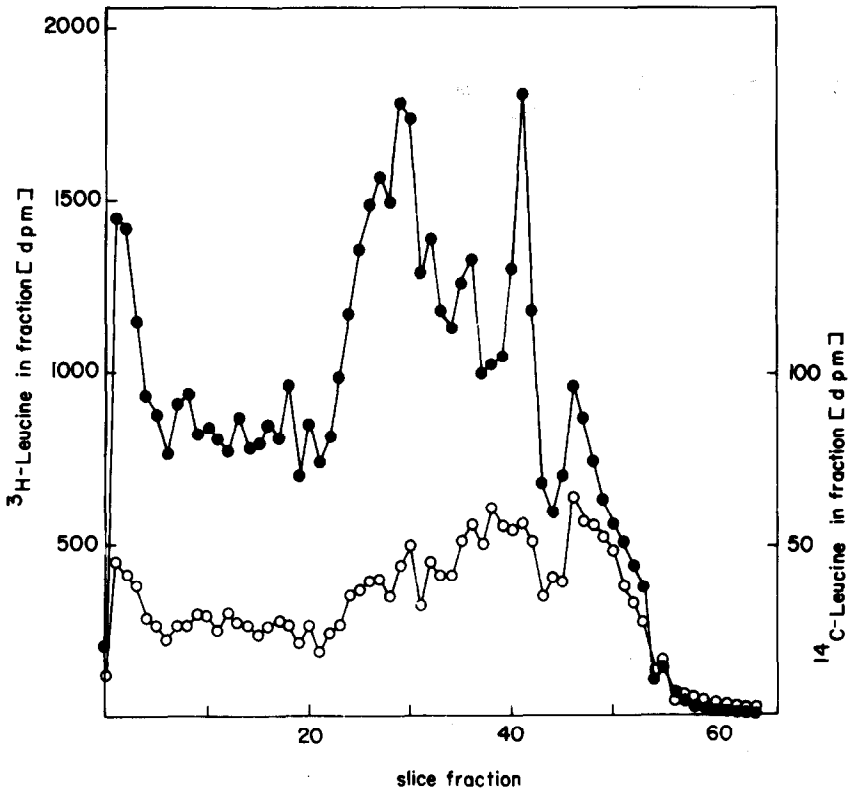

Fig.1. SDS-gelelectrophoresis of mitochondrial membrane proteins after labeling in vitro for 10 and 70 min. (o——o) 10 min ([^{14}C]leucine). (•——•) 70 min ([^{3}H]leucine). For details, see Materials and methods.

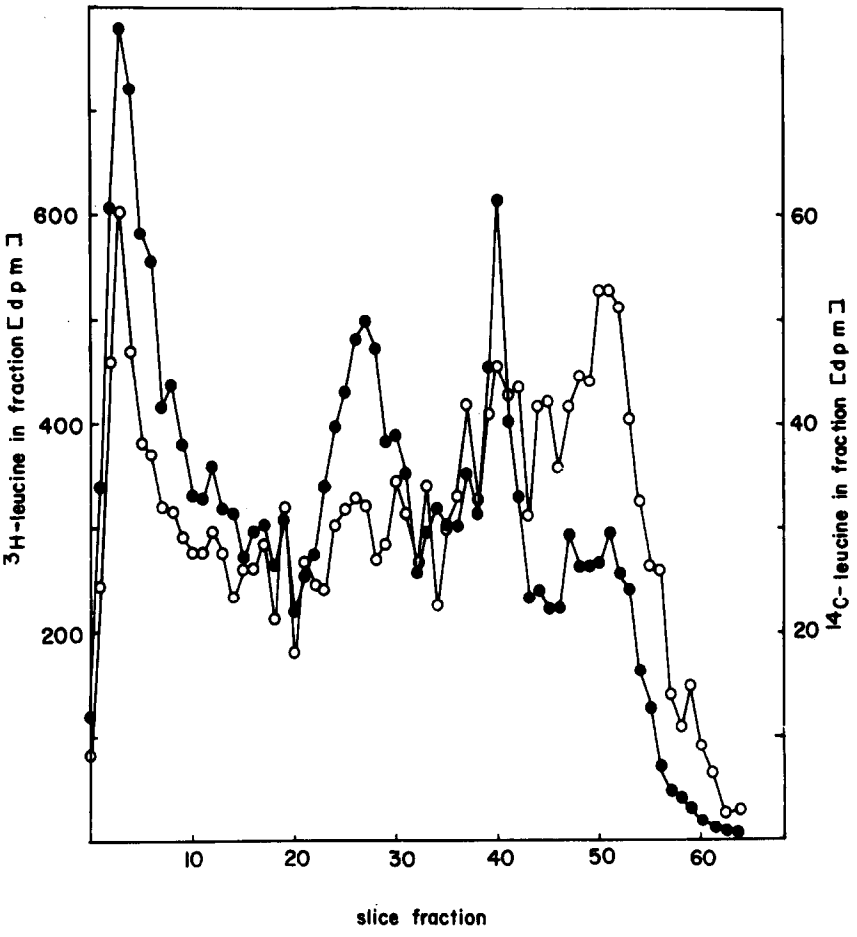

Fig.2. Mol. wt. patterns of in vitro labeled mitochondrial membrane proteins, analyzed by SDS-gelelectrophoresis. Labeling conditions (○——○) 10 min incubation with [^{14}C]leucine (●——●) 10 min incubation with [^{3}H]leucine, followed by 60 min chase in the presence of 3 mM unlabeled leucine.

The effect of inhibitors of ATP synthesis and of protein synthesis on this conversion is shown in fig.3. The figure represents differences of radioactivity patterns of two samples respectively. The two samples, incubated under different conditions, as indicated in the legend, were mixed and run together on the same gel. Fig.3A clearly shows the transfer of radioactivity from the low mol. wt. range to higher mol. wt. ranges. In control experiments it was made sure that the total radioactivity did not change during the chase period. In the presence of FCCP during the chase period, the conversion of proteins is completely blocked (fig.3B). In fig.3C the difference between chase experiments in the presence and absence of ethidium bromide is shown. A similar pattern as that of fig.3A is obtained, indicating the inhibition of the conversion process by ethidium bromide. No marked difference of radioactivity pattern is found if the effects of KCN and FCCP during the chase period are compared (fig.3D). Puromycin also prevents the formation of high mol. wt. proteins (not shown).

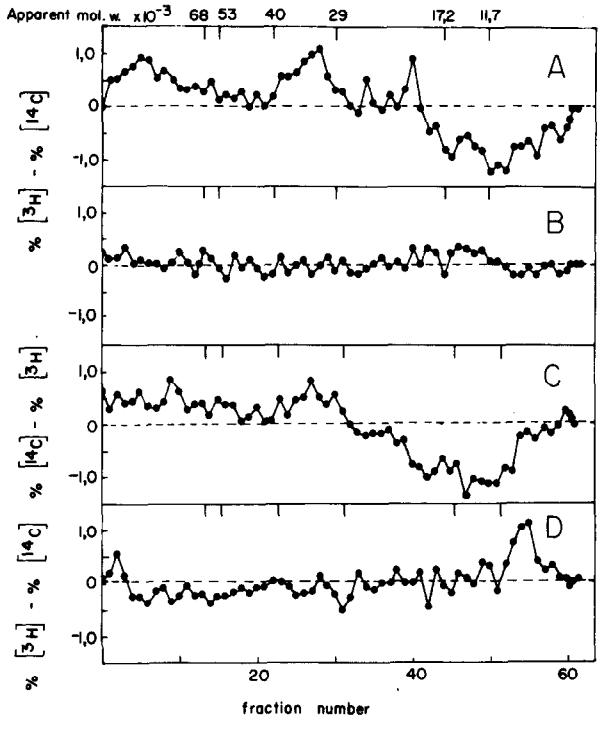

Fig.3. Effect of inhibitors on the increase of apparent molecular weights of in vitro labeled proteins during the chase period. The percentage radioactivity of [^{3}H] respectively [^{14}C] in each fraction was calculated, by taking the total radioactivity of [^{3}H] respectively [^{14}C] applied to the gel as 100%. Total radioactivities recovered from the gels were: [^{3}H] = 19 480, 22 500, 7650 and 6150 counts/min and [^{14}C] = 1750, 1683, 1714 and 1778 counts/min for gels A, B, C and D respectively. Incubation conditions: Gel A, (10 min [^{3}H]leucine + 60 min chase) – (10 min [^{14}C] leucine); Gel B, (10 min [^{3}H]leucine + 60 min chase with 0.5 μM FCCP) – (10 min [^{14}C]leucine); Gel C, (10 min [^{14}C]leucine + 60 min chase) – (10 min [^{3}H]leucine + 60 min chase with 1 μg/ml ethidium bromide); Gel D, (10 min [^{3}H]leucine + 60 min chase with 1 mM KCN) – (10 min [^{14}C]leucine + 60 min chase with 0.5 μM FCCP).

The inhibition of the conversion process by various kinds of inhibitors seems to indicate a physiological conversion of the primary products of mitochondrial protein synthesis, occurring after synthesis in an additional step. This conversion process obviously depends on the supply of energy and a functioning protein synthesis. It seems that the proteins are still attached to the ribosome during the conversion. Our data are in accordance with those from Kužela et al. [20] who showed an inhibition by chloramphenicol of the protein conversion during the chase period.

Work is in progress to study the nature of binding within the converted proteins.

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