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Relationship of Protein Synthesis and Energetics in Mitochondria

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Abstract

Protein synthesis in rat liver mitochondria has been studied by measuring ^{14}C-leucine incorporation into mitochondrial proteins. It has been found that addition of uncouplers of oxidative phosphorylation or cyanide inhibits leucine incorporation supported by ATP energy in the presence of oligomycin. Neither cyanide nor oligomycin used separately suppresses the ATP-supported leucine incorporation. Oligomycin arrests respiration-supported protein synthesis. These facts indicate that both ATP and membrane potential or another form of non-phosphorylated energized state are necessary for the use of extramitochondrial amino acids for protein synthesis in mitochondria.

The problem of the energy source for the mitochondrial protein synthesis has been discussed for a long time (for review see Roodyn and Wilkie [1], Pinus and Metlizkaya [2]). At present it is widely recognized that mitochondrial protein synthesis needs ATP which cannot be substituted by high-energy non-phosphorylated intermediates since oligomycin preventing utilization of these intermediates for ATP formation hinders the use of respiration energy for protein synthesis [2-5]. On the other hand, there are some indications that the rate of labelled amino acids incorporation in mitochondrial proteins depends on the state of the energy transfer chain even if ATP is used as energy source [3, 6]. This paper summarizes the results demonstrating that both ATP and a non-phosphorylated energized component(s) are indispensable for the incorporation of added amino acids in mitochondrial proteins.

Liver mitochondria of rats starved for 24 h were isolated in 0.25 M sucrose + 0.0025 M EDTA, pH 7.4, washed twice and suspended in the

isolation medium (20 mg protein/ml). The respiratory control of mitochondria with succinate was higher than 2.5. Mitochondria (3-4 mg protein) were incubated for 40 min at 30° in the mixture containing sucrose, 0.125 M; $MgCl_2$, 0.003 M; KCl, 0.02 M; potassium phosphate buffer; pH 7.4, 0.016 M; rotenone, 1×10^{-5} M, and additions indicated in legend to the tables. Moreover, each sample (2 ml) contained 80 μg of the complete amino acid mixture excluding leucine, and 1.5 μC of ^{14}C-leucine (100 μC/1 μmole).

After incubation, the equal volume of 10% TCA was added to the sample, the precipitate formed was washed with 5% TCA, solubilized by NaOH, reprecipitated by $ZnSO_4$, washed with ethanol, ethanol-ester-chloroform mixture, and ester. The resulting precipitate was solubilized by formic acid and placed on to discs for radioactivity measurements. Radioactivity was determined with a gas-flow counter, the count efficiency being 40%.

ATP was measured spectrophotometrically using hexokinase, dehydrogenase of glucose-6-phosphate and $NADP^+$.

Table I shows that the level of ^{14}C-leucine incorporation in proteins of rat liver mitochondria was the same whether succinate or ATP was used as the energy source. Oligomycin inhibited the respiration-supported leucine incorporation and slightly increased that supported by ATP, the latter result confirming the data by Lamb *et al.* [7]. Uncouplers of oxidative phosphroylation—dinitriphenol (DNP) and *p*-trifluoromethoxy-carbomyl cyanide phenylhydrazone (FCCP)-prevented almost completely both respiration and ATP-dependent leucine incorporation (Table I). Oligomycin did not prevent the system from being inhibited

TABLE I. Effect of uncouplers and inhibitors on incorporation of ^{14}C-leucine into mitochondrial proteins

Energy source	Additions	cpm/mg Protein	
		Without oligomycin	With oligomycin
–		74	–
Succinate + ADP		648	114
Succinate + ADP	DNP	138	–
Succinate + ADP	FCCP	125	–
ATP		670	827
ATP	DNP	156	135
ATP	FCCP	195	175
ATP	KCN	680	255

Incubation mixture see in the text. Concentrations of additions: ADP, 0.004 M; ATP, 0.006 M (0.002 M ATP was added thrice during incubation); succinate, 0.01 M; DNP, 1×10^{-3} M; FCCP, 1×10^{-5} M; KCN, 3×10^{-3} M; oligomycin, 25 μg/ml. Each figure is the mean of 5-8 experiments.

by the uncouplers. In the sample with ATP, KCN was without effect, when oligomycin was absent, and it inhibited the incorporation if added to the oligomycin-treated mitochondria (Table I).

The fact that oligomycin inhibits respiration-supported leucine incorporation being ineffective in the system supplemented with ATP (see Table I, the samples without uncouplers and KCN) suggests that ATP, but not non-phosphorylated intermediates of energy transfer chain is used as the energy source for protein synthesis in mitochondria. However, such simple concept fails to explain inhibitory effects of uncouplers and KCN on ATP-supported protein synthesis in samples with oligomycin.

One might think that the effect of uncouplers is due to the activation of ATPase resulting in the exhaustion of ATP in the incubation mixture. If it were the case, oligomycin would abolish the inhibitory action of uncouplers preventing the ATPase activation. The direct measurement of ATP concentration in experimental samples showed that the level of ATP is not influenced by DNP if oligomycin had been added (Table II).

TABLE II. Effect of oligomycin and DNP on ATP concentration in incubation mixture with mitochondria. Conditions as in Table I

Incubation, min	Additions	μmole of ATP/sample
0	–	4.9
40	–	2.9
40	oligomycin	3.7
40	oligomycin + DNP	3.6

Logical analysis of the above relationships leads to the conclusion that both ATP and a high-energy non-phosphorylated intermediate (or state) are of necessity for the incorporation of added amino acids in mitochondrial proteins. In this case, oligomycin inhibition of respiration-supported leucine incorporation can be explained by inhibition of ATP formation, the inhibitory effect of uncouplers by discharging the energized intermediates (state). Oxidation of added amino acids and/or endogenous substrates can be the energy source for generation of these intermediates (state) in the system containing ATP + oligomycin. Inhibition of the respiration by KCN should suppress leucine incorporation in this system, and it was the case (see Table I). KCN was ineffective in the sample with ATP and without oligomycin, since in the latter case non-phosphorylated intermediates (state) formation could be supported by ATP hydrolysis.

The role of non-phosphorylated high-energy intermediate (state) in mitochondrial protein synthesis is obscure. One of the possibilities is that it is the transmembrane electro-chemical potential of H^+ ions which is required for the use of added amino acids in mitochondrial protein

synthesis. Mitochondrial membrane potential which is formed by respiration (in oligomycin-insensitive fashion), and by ATP hydrolysis (in oligomycin-sensitive fashion) was postulated by Mitchell [8] and experimentally demonstrated recently (for review see Liberman and Skulachev [9]; Skulachev [10]). It was also shown that the membrane potential ($\Delta\psi$) and/or transmembrane pH gradient (Δ pH) can be used as a driving force for accumulation of some ions in mitochondria (for review see Klingenberg [11]; Liberman and Skulachev [9]). It is possible that $\Delta\psi$ and/or ΔpH are necessary for the transport of some added amino acids into mitochondria and, hence, for mitochondrial protein synthesis. Evidence of energy-dependent transport of dicarboxylic amino acids in mitochondria was recently furnished [12]. Some indications of the membrane potential-supported amino acid transport were obtained in experiments with microorganisms [13].

Formation of the membrane potential and alkalinization of the mitochondrial matrix space may also influence the rate of protein synthesis via changes in configuration of mitochondrial cristae. Configuration changes always occur when mitochondria are energized in the medium containing a penetrating weak acid [14, 15] (in the experiments described in this paper phosphate was present). Furthermore, it is not excluded that energization is accompanied by such a shift of intramitochondrial pH which is favourable for the maximal activity of protein-synthesizing system of mitochondria. To choose among these possibilities further investigation is required.

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