

Amino acid incorporation into the protein of mitochondria and mitochondrial fragments from beef heart

KALF AND SIMPSON¹ found a high incorporation of labelled amino acids into the protein of isolated heart mitochondria. HULSMANS² also obtained the highest rate of amino acid incorporation in the mitochondrial fraction of rat-heart cytoplasm. More detailed investigations of the experimental conditions and energy requirements for the incorporation of radioactive amino acids into the protein of beef-heart mitochondria will be published elsewhere. This report gives some preliminary results on the incorporation of [¹⁴C]leucine into submitochondrial particulate fractions, obtained from heavy-layer beef-heart mitochondria by sonic oscillation and digitonin treatment.

TABLE I

[¹⁴C]LEUCINE INCORPORATION INTO THE PROTEIN OF FRACTIONS OBTAINED
FROM BEEF-HEART MITOCHONDRIA BY SONIC OSCILLATION

The mitochondria were disrupted by sonic oscillation (M.S.E. sonic disintegrator, 60 W, 60 kHz/sec) for 2 min in a medium containing 0.25 M sucrose, 5 mM EDTA, 1 mM MgCl₂ and 6 mg protein/ml, and the suspension was fractionally centrifuged. Incorporation activity was measured by incubating at 37° for 2 h under shaking in a medium containing 50 mM Tris, 50 mM KCl, 5 mM MgCl₂, 1 mM EDTA, 30 mM potassium phosphate, 2 mM ADP, 50 µg of a synthetic amino acid mixture³ and 0.04 mM [¹⁴C]leucine (176000 counts/min) in a final volume of 1 ml. The final pH was 7.4. The reactions were terminated by the addition of trichloroacetic acid, the mixture was heated at 90° for 15 min, and the precipitate was washed with alcohol and ether and plated and counted in a gas-flow counter. Corrections were made for background and self-absorption.

Fraction	Specific activity (counts/min/mg protein)	Recovery of incorporation activity (%)	Recovery of protein (%)
Intact mitochondria	220	100.0	100
Particulate fraction from sonicate after 12 min at 15000 × g	209	74.3	77
Particulate fraction from 15000 × g supernatant after 45 min at 105000 × g	1145	30.5	6

The beef-heart mitochondria were prepared according to CRANE *et al.*³, the layers being separated as described by HATEFI AND LESTER⁴. Table I shows the distribution of protein and incorporation activity in various fractions obtained from the mitochondria after sonic disruption. It can be seen that the fraction containing the lightest particles has the highest specific activity. Table II shows the distribution pattern after digitonin treatment according to COOPER AND LEHNINGER⁵, the smallest particles again having the highest specific activity. However, the larger particles also show much more activity than the original mitochondria. Apparently the detergent action of digitonin not only brings about the liberation of small submitochondrial fragments, but also causes the removal of inactive protein from the larger fragments. The interpretation of the results is somewhat complicated by an apparent stimulation of the incorporation process as such, since the total recovery of incorporation activity exceeds 100 %.

All fractions tested required energy, Mg²⁺ and the added amino acid mixture for the incorporation of [¹⁴C]leucine. There was no beneficial effect of added soluble

fractions from mitochondria nor of pH-5 enzymes from rat liver. Ribonuclease (EC 2.7.7.16) caused only 5 % inhibition, if added in amounts that totally block microsomal systems (*cf.* ROODYN *et al.*⁶). With chloramphenicol (50 μ g/ml) an inhibition was obtained of at least 95 % (*cf.* RENDI⁷). Transpeptidation in the sense recently described by SUTTIE⁸ appears to be excluded, since the incorporation was inhibited at least 90 % by 0.5 mM 2,4-dinitrophenol.

TABLE II

DISTRIBUTION OF [¹⁴C]LEUCINE-INCORPORATION ACTIVITY AND PROTEIN IN DIGITONIN FRAGMENTS FROM HEAVY-LAYER BEEF-HEART MITOCHONDRIA

The mitochondria were pretreated at 0° for 30 min in a medium containing 1 % digitonin (E. Merck A.G.), 50 mM sucrose and 5 mg protein/ml. The conditions of incubation and treatment of the radioactive precipitates are given in Table I

Fraction	Specific activity (counts/min/mg protein)	Recovery of incorporation activity (%)	Recovery of protein (%)
Intact mitochondria	225	100.0	100
Particulate fraction from the digitonin-treated mitochondria after 25 min at 75000 \times g	652	95.5	33
Particulate fraction from 75000 \times g supernatant after 30 min at 105000 \times g	996	26.6	6

In conclusion, it is clear that it is possible to obtain fractions from heart mitochondria which show a much higher specific activity with respect to amino acid incorporation than the intact particles, even higher than the activities generally reported for mammalian ribosomal and microsomal systems. The properties of the incorporation system in these submitochondrial particles are essentially the same as those in intact mitochondria.

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