The Turnover of the Protein Components of Mitochondria from Rat Liver, Kidney, and Brain*

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SUMMARY

The turnover rates of the protein components of rat liver, kidney, and brain mitochondria were determined. In liver mitochondria, all protein components had an identical half-life of 8.5 days. In kidney mitochondria, the water-soluble proteins had a half-life of 5.9 days which was significantly shorter than that of the whole mitochondria, 8.6 days. The other protein components of kidney mitochondria had half-lives which did not differ significantly from that of the whole mitochondria. A similar situation was obtained with brain mitochondria in which the water-soluble proteins had a half-life of 17.9 days as compared to 26.3 days for the whole mitochondria. It is of interest that these water-soluble proteins appear to be synthesized extramitochondrially and then subsequently integrated into the mitochondrial structure.

The protein and lipid components of rat liver mitochondria have been found by Fletcher and Sanadi (1) to have an identical turnover rate. This suggested to these workers that liver mitochondria were labile and were broken down as an entity. In kinetic studies on the biosynthesis in vivo of rat liver and kidney mitochondria, Beattie, Basford, and Koritz (2) reported that at short time intervals after the injection of radioactive leucine the specific activities of the water-soluble proteins and the fraction containing cytochrome c were significantly lower than the specific activity of the unfractionated mitochondrial protein from both tissues. At 2 hours, the specific activity of the water-soluble proteins from kidney mitochondria had increased 300% over that of the unfractionated mitochondrial protein. By 8 hours, the specific activities of all fractions approximated that of the whole mitochondria. These results indicated that certain mitochondrial proteins may be synthesized outside the mitochondria and subsequently incorporated into the mitochondrial structure.

Two possible mechanisms for the turnover of mitochondrial protein components are suggested by these data. One, as the results of Fletcher and Sanadi (1) indicate, is that despite the differential rates of synthesis of certain mitochondrial components, all proteins would turn over at the same rate. A second is that certain protein components of the mitochondrion turn over at a rate different from other proteins. This second possibility was made attractive by the variation of specific activity with time of the water-soluble proteins of the kidney mitochondria (2). To test the second case the experiments of this paper were performed. In agreement with the results of Fletcher and Sanadi (1), it was observed that the protein components of liver mitochondria turned over at the same rate; however, the water-soluble proteins of kidney and brain mitochondria had turnover rates significantly greater than those of the other protein components.

EXPERIMENTAL PROCEDURE

Thirty microcuries of uniformly labeled $^{14}$C-L-leucine (specific activity, 250 mC per mmole) were administered intravenously to adult male rats weighing 150 to 180 g and the animals were killed at the indicated times after injection. Liver and kidney mitochondria were isolated and fractionated as previously described (2). Brain mitochondria were prepared in 0.3 M mannitol containing 0.1 mM EDTA by the method of Ozawa et al. (3) and washed two times with the above medium. The brain mitochondria were then extracted with water for 5 min at 30°C to remove the water-soluble protein and were centrifuged for 10 min at 27,000 × g. The resulting pellet was resuspended in mannitol and designated water-insoluble protein. This simplified fractionation procedure was used because of the small amount of mitochondria obtained from brain.

Water-soluble and KCl-soluble proteins, cytochrome c, structural proteins, and contractile proteins were extracted from mitochondria and prepared for counting by methods described previously (2, 4).

RESULTS

Fig. 1 shows the rate of decline of the radioactivity of liver mitochondrial protein. Specific radioactivity is plotted on a logarithmic scale against time in days. The specific activity obtained for all liver submitochondrial fractions did not differ significantly from that of the whole mitochondria at all times studied. The regression coefficients per day, statistically evaluated, were identical for each fraction (Table I), and the average half-life was calculated to be 8.5 days.

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In contrast to the liver mitochondria, the water-soluble proteins of kidney mitochondria were found to have a half-life of 5.96 days as compared to that of 8.65 days for the whole mitochondria (Fig. 2; Table I). This difference was statistically significant with a p value less than 0.01. There is also a suggestion (p < 0.2) that the KCl-soluble proteins of kidney mitochondria have a shorter half-life than that of the whole mitochondria. The half lives of structural protein and the fraction corresponding to the other cytochromes were not significantly different from that of the whole mitochondria.

A similar situation was obtained with brain mitochondria (Fig. 3; Table I) where it was found that the water-soluble proteins had a half-life of 17.9 days compared to one of 26.3 days for the whole mitochondria. The water-insoluble proteins did not differ significantly from the whole mitochondria. While the

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**Fig. 1.** Decline in radioactivity of liver mitochondria. Each point represents the mean activity of the samples, while the standard error of the mean is indicated by the vertical line. The number of animals used for the points are five at 1, 7, 21, and 28 days and seven for 2, 4, and 14 days. The regression line was drawn using all points by the method of least squares (5).

**Fig. 2.** Decline in radioactivity of kidney mitochondria. O---O, whole mitochondria; O---O, water-soluble proteins. The number of animals used for each point are five at 1, 7, 21, and 28 days, six at 2 days and seven at 4 and 14 days. Statistical treatment of the data was as described in the legend to Fig. 1.

**TABLE I**

<table>
<thead>
<tr>
<th>Fraction corresponding to</th>
<th>Regression coefficient per day</th>
<th>95% confidence interval</th>
<th>p</th>
<th>t</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole mitochondria</td>
<td>-0.0361</td>
<td>-0.00492</td>
<td></td>
<td></td>
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<tr>
<td>Water-soluble proteins</td>
<td>-0.0352</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytochrome c</td>
<td>-0.0352</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contractile proteins</td>
<td>-0.0360</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Structural proteins</td>
<td>-0.0343</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other cytochromes</td>
<td>-0.0365</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole mitochondria</td>
<td>-0.0348</td>
<td>-0.00163</td>
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<td>Water-soluble proteins</td>
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<td>-0.00726</td>
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<td>KCl-soluble proteins</td>
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<td>-0.00463</td>
<td>&lt;0.2</td>
<td>7.63</td>
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<td>Structural proteins</td>
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<td>-0.00239</td>
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<td>Other cytochromes</td>
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<td>0.00687</td>
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<td>Brain</td>
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<tr>
<td>Whole mitochondria</td>
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<td>Water-soluble proteins</td>
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<td>-0.00370</td>
<td>&lt;0.1</td>
<td>17.9</td>
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<tr>
<td>Water-insoluble proteins</td>
<td>-0.0059</td>
<td>-0.00362</td>
<td></td>
<td>31.4</td>
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</tbody>
</table>

*The 95% confidence intervals for the remaining liver fractions were not calculated, since it is apparent that the regression coefficients for these fractions do not differ from that of the whole mitochondria by a factor greater than 0.00492.*
specific activity of the water-soluble proteins 4 days after injection was higher than that of the whole mitochondria, the 1-day value was lower and hence not used in calculation of the regression line. Using an experimental design which involved continuous exposure of rats and their mothers to tritiated drinking water from conception to the time of weaning, Khan and Wilson (6) found a half-life of brain mitochondria of 16.4 days. The difference between this value and that obtained in the present study at all times, including the shortest time studied, 1 day after injection. This is in accord with our previous observations (2) that in liver mitochondria maximum specific activities were obtained 30 min after injection of the radioactive amino acid.

The conclusion that mitochondria are degraded as an entity cannot be extended to those of kidney and brain. It is apparent from this study that the water-soluble proteins of both kidney and brain mitochondria turn over at a rate greater than that of the other mitochondrial proteins and there is a suggestion that the KCl-soluble proteins of the kidney mitochondria may also have a faster turnover rate. The results with water-soluble proteins are of particular interest, since kinetic studies on the biosynthesis in vitro of mitochondria (2) had indicated that these proteins are synthesized extramitochondrially and transported into the mitochondria in a subsequent step.

A possible model consistent with these observations would involve an initial synthesis of the membrane structures of the mitochondria, including requisite lipid components, followed by an appropriate integration of proteins synthesized in extramitochondrial sources to form the completed mitochondrion. In accord with this model is the observation that mitochondria incorporate amino acids, in vitro, preponderantly into insoluble membranous proteins (7-9) and, specifically, into those same elements of the inner membrane (10, 11). These results would also account for the relatively small amount of DNA present in mitochondria which has been calculated by Sinclair and Stevens (12) to contain information sufficient to code for only a fraction of the proteins of the mitochondrion.

It would appear that in kidney and brain mitochondria proteins synthesized extramitochondrially have unique turnover rates. This may also be true in liver mitochondria but the differences in the half-life are too short to be detected. Of possible significance in this respect is the preliminary report of Gonzalez Cadavid and Campbell (13) that the specific activity of a purified cytochrome c component of liver mitochondria increased 3-fold over that of intact mitochondria 30 min after the intravenous injection of $^{14}$C-lysine.

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REFERENCES

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