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

(Diana S. Beattie, R. E. Basford, and Seymour B. Koritz)

From the Biochemistry Department, School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania 15213

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.

EXPERIMENTAL PROCEDURE

Thirty microcuries of uniformly labeled 14C-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° to remove the water-soluble protein and were centrifuged for 10 min at 27,000 X 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

**Table I**

*Turnover of protein components of mitochondria from various tissues*

Regression coefficients and their 95% confidence limits were calculated as described by Goldstein (5). Probability values that two slopes were significantly different were calculated by the t test.

<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>
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<tbody>
<tr>
<td><strong>Liver</strong></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>8.39</td>
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<tr>
<td>Water-soluble proteins</td>
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<td>8.56</td>
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<tr>
<td>Cytochrome c</td>
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<td>Insignificant</td>
<td></td>
<td>8.56</td>
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<tr>
<td>Contractile proteins</td>
<td>-0.0360</td>
<td>Insignificant</td>
<td></td>
<td>8.37</td>
</tr>
<tr>
<td>Structural proteins</td>
<td>-0.0343</td>
<td>Insignificant</td>
<td></td>
<td>8.77</td>
</tr>
<tr>
<td>Other cytochromes</td>
<td>-0.0365</td>
<td>Insignificant</td>
<td></td>
<td>8.24</td>
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<tr>
<td><strong>Kidney</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole mitochondria</td>
<td>-0.0348</td>
<td>±0.00163</td>
<td></td>
<td>8.65</td>
</tr>
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<td>Water-soluble proteins</td>
<td>-0.0505</td>
<td>±0.00726</td>
<td>&lt;0.01</td>
<td>5.96</td>
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<td>KCl-soluble proteins</td>
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<td>±0.00463</td>
<td>&lt;0.2</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><strong>Brain</strong></td>
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<td></td>
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<td></td>
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<tr>
<td>Whole mitochondria</td>
<td>-0.0114</td>
<td>±0.00534</td>
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<td>26.3</td>
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<tr>
<td>Water-soluble proteins</td>
<td>-0.0169</td>
<td>±0.00370</td>
<td>&lt;0.1</td>
<td>17.9</td>
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<td>Water-insoluble proteins</td>
<td>-0.00959</td>
<td>±0.00362</td>
<td>Insignificant</td>
<td>31.4</td>
</tr>
</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 is attributed, in part, to the different experimental approach and perhaps, to a greater extent, to the fact that much younger rats were involved in their study than in the present one.

DISCUSSION

The results of Fletcher and Sanadi (1) indicated that the decay of various protein components of liver mitochondria occurred at the same rate and that in all probability these mitochondria were broken down as an entity. Our results with liver mitochondria confirm those of Fletcher and Sanadi (1), although the half-life obtained in these experiments was somewhat shorter i.e. 8.5 days as compared to 10.5 days. This may result from experimental differences inasmuch as the radioactive amino acid was injected intravenously in these experiments, while Fletcher and Sanadi (1) used an intraperitoneal route. This difference may also account for the difficulty of these workers to obtain reliable values within the 1st week after injection of the radioactive amino acid. As the data indicate, reliable values were obtained in this 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 KC1-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 vivo 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 3H-cysteine.

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REFERENCES

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