TURNOVER OF OUTER AND INNER MEMBRANE PROTEINS OF RAT LIVER MITOCHONDRIA

G.BRUNNER and W.NEUPERT
Institut für Physiologische Chemie der Universität München

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1. Introduction

Recent reports on the properties and composition of outer and inner mitochondrial membranes suggest that outer and inner membranes of mitochondria are derived from different sources of the cell [1-3]. *In vitro* experiments [4-6] indicate that the protein synthesizing system of isolated rat liver mitochondria contributes only to the synthesis of the inner and not to that of the outer mitochondrial membrane. From these findings it seemed possible that a difference in the biogenetic origin of outer and inner mitochondrial membrane might be reflected in the turnover of these mitochondrial components. The question whether all components of the mitochondrion turn over at the same half life has already been raised by Fletcher and Sanadi [7]. These authors concluded from their data that the mitochondrion turns over as an entity. More recently Bailey et al. [8] have shown evidence for the presence of mitochondrial phospholipid components with large differences in half life. Data presented in this paper indicate a half life of mitochondrial outer membrane proteins (4.2 ± 1.1 days) which significantly differs from the half life of the inner membrane proteins (12.6 ± 1.3 days).

2. Experimental

Male rats of Wistar stock weighing 200 g were injected intraperitoneally with 0.5 ml 0.9% NaCl solution containing 60 μC of 1-(^{35}S)-methionine (138 mC/mMole). The animals were fed ad libitum on a normal diet. The rats were killed in groups of two at intervals up to 4 weeks after injection. The preparation of mitochondria and of outer and inner membranes, measurement of radioactivity, and estimation of enzyme activities were carried out as previously described by Neupert et al. [5]. Cytochrome P 450 was determined according to Omura and Sato [9]. Microsomes were prepared by centrifuging the liver homogenate for 20 min at 35 000 × *g*. The resultant supernatant was centrifuged for 60 min at 100 000 × *g* to sediment the microsomal fraction. 'Soluble proteins' were prepared by 3 min sonication (Branson Sonifier, step 6) of mitochondria in 0.1 M phosphate buffer, pH 7.2, followed by centrifugation for 60 min at 144 000 × *g*. The supernatant was taken as soluble protein fraction.

3. Results

Fig. 1 shows the decline of log (specific radioactivity) of rat liver mitochondrial and microsomal proteins after *in vivo* labelling with 1-(^{35}S)-methionine. For microsomes a half life of 5.7 ± 0.7 days can be calculated assuming first order kinetics. In mitochondria a linear decline was not observed. During the period investigated the steepness of the breakdown curve slows down. For the 2nd to 15th day after injection a half life of 6.7 ± 0.4 days can be calculated and for the 15th to 27th day a half life of 12.2 ± 0.8 days. This leads to the conclusion that mitochondria of rat liver contain protein components which turn over at different rates.

In fig. 2 the decline of log (specific radioactivity) of mitochondrial subfractions is demonstrated. Mito-

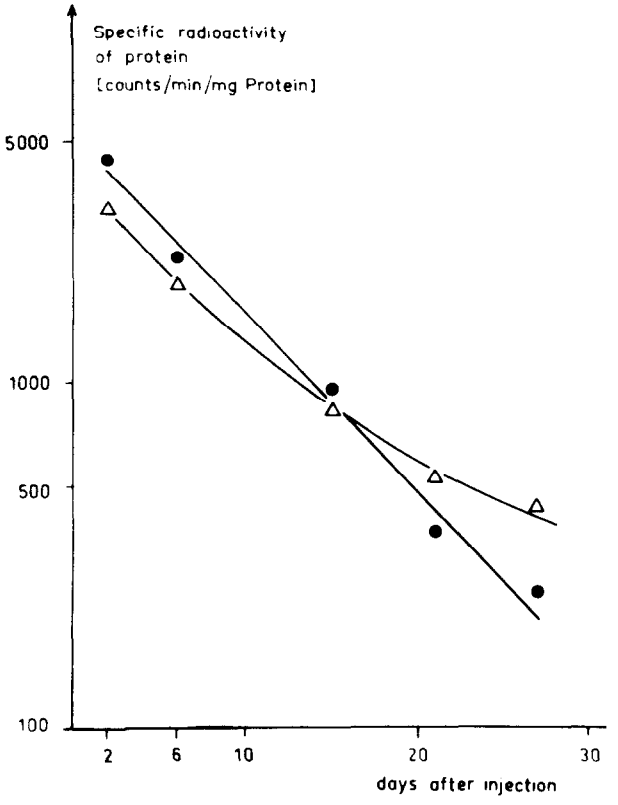

Fig. 1. Changes of the specific radioactivity of mitochondrial △——△ and microsomal ●——● proteins with time. Ordinate in logarithmic scale.

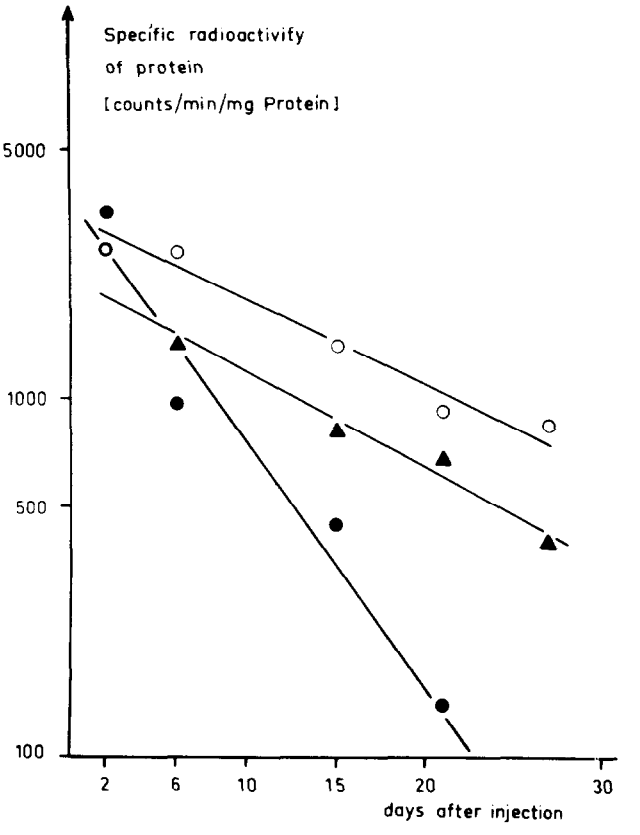

Fig. 2. Changes of the specific radioactivity of mitochondrial subfractions with time. ●——● outer membrane, ○——○ inner membrane, ▲——▲ soluble protein. Ordinate in logarithmic scale.

chondria were subfractionated into inner and outer membrane fractions and into a soluble protein fraction. The fractions were followed with marker enzymes and corrected for pure membrane content [5]. Microsomal contamination of the mitochondrial fractions was estimated by measuring cytochrome P450. It appeared to be below 10% in outer membrane fraction and could not be detected in the inner membrane fraction.

Table 1 shows the half lives of outer and inner mitochondrial membrane proteins and of the 'soluble' mitochondrial proteins as calculated from the linear decline of log (specific radioactivity). This linearity however does not exclude the existence of components with a very fast or slow break down, especially if they are present in a low amount. Preferentially this might be the case in mitochondrial outer membrane since the limit of error in this case is relatively high. The half lives of the mitochondrial subfractions found in this experiment are 4.2 ± 1.1 days for outer

Table 1
Half life values of microsomes and mitochondrial components.

Fraction	$t_{\frac{1}{2}}$ (days)	Standard error of $t_{\frac{1}{2}}$ (days)
Microsomes	5.7	±0.7
Mitochondria		
2nd-15th day	6.7	±0.4
15th-27th day	12.2	±0.8
Outer membrane	4.2	±1.1
Inner membrane	12.6	±1.3
Mitochondrial soluble protein	11.6	±1.0

membrane, 12.6 ± 1.3 days for inner membrane, and 11.6 ± 1.0 for the soluble protein.

4. Discussion

The non-linear decline of log (specific radioactivity) of protein of whole mitochondria after *in vivo* labelling with a radioactive amino acid as shown in these experiments is in contrast to previous findings of other authors. Part of the differences can be explained by different experimental conditions. Fletcher and Sanadi [7] have calculated the half life of mitochondrial fractions (total soluble and insoluble protein) from the period of the 14th to 28th day after injection of radioactive amino acid. Their data do not exclude a faster decay during the first fourteen days and especially not one during the first week after application of label.

There are also experimental differences in the experiments of Bailey et al. [8], who observe a linear decline of the total insoluble mitochondrial protein and a non-linear decline of the 'soluble' mitochondrial proteins. Bailey et al. have distributed whole rat liver mitochondria in cold distilled water and taken the sediment of a 10 min 15 000 × *g* centrifugation as the insoluble protein fraction and the supernatant as the 'soluble' protein fraction. However from experiments of Schnaitman et al. [10] we know that this procedure leads to a separation of outer and inner membranes. In our hands the 'soluble' protein fraction prepared by this method contained a 25% higher monoamine oxidase activity than the resulting sediment. This shows that this type of fractionation leads to a 'soluble' protein fraction highly contaminated by outer membrane and a mitochondrial membrane fraction largely depleted of outer membrane.

Our findings with whole mitochondria are consistent with the observation of significantly different half lives of outer and inner membrane proteins, that of the outer membrane being much faster than that of the inner membrane, whereas the soluble proteins turn over at a rate similar to the inner membrane proteins. The fast component in the half life of whole mitochondria however cannot be fully explained only by the shorter half life of the outer membrane, for if one considers the protein content of the outer membrane to amount to about 5-8% of the total mitochondrial proteins, a slower decline in the specific radioactivity of whole mitochondria during the first fourteen days must be expected. The half life of the microsomal fraction calculated from our data is in agreement with the data of Omura et al. [11] who found an average value of four days.

We cannot conclude from our data that all proteins of one fraction have identical half lives. The values shown in table 1 may be those for main components. Furthermore we have not made corrections for reutilisation of breakdown products. Consequently the calculated half lives may be overestimated. However it should be noted that the radioactivity in the blood plasma 3 days after the first point of estimation was only 0.35% of the radioactivity at the day of injection.

The data which are presented here provide strong evidence that the mitochondrion does not turn over as an entity. They are in agreement with the view that outer and inner mitochondrial membranes are under control of different protein-synthesizing and breaking-down systems.

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