IMMUNOLOGICAL SIMILARITY BETWEEN NADH-CYTOCHROME c REDUCTASES OF MITOCHONDRIAL OUTER MEMBRANE AND MICROSOMES

Sachiko Takesue and Tsuneo Omura

Institute for Protein Research, Osaka University, Osaka, Japan

Received June 10, 1970

By using an antibody against microsomal NADH-cytochrome b_5 reductase, the rotenone-insensitive NADH-cytochrome c reductase system in the outer membrane of mitochondria has been confirmed to be immunologically very similar to the microsomal NADH-cytochrome c reductase system. The rotenone-sensitive NADH-cytochrome c reductase activity of mitochondrial inner membrane was not affected by the antibody.

Microsomes from various animal tissues contain an NADH-cytochrome c reductase system consisting of NADH-cytochrome b_5 reductase and cytochrome b_5 (1). Raw et al. (2,3) reported the presence of a similar enzyme system in liver mitochondria. Sottocasa et al. (4) and Parsons et al. (5) demonstrated that the rotenone-insensitive NADH-cytochrome c reductase activity of mitochondria is localized in the outer membrane. NADH-cytochrome c reductase activities of microsomes and mitochondrial outer membrane are apparently similar to each other, but no definite evidence for their identity has been presented so far.

In a previous paper (6), the preparation of an antibody against rat liver microsomal NADH-cytochrome b_5 reductase was described. The antibody reacts with the microsome-bound reductase as well as with the purified enzyme resulting in strong inhibition of the reductase activity (7). In this paper, the NADH-cytochrome c reductase systems of mitochondrial outer membrane and microsomes are compared by the use of this antibody. The results suggest

their immunological similarity or identity.

METHODS

Adult male Sprage-Dawley rats were used in preparing mitochondrial and microsomal preparations. The inner and outer membrane fractions of liver mitochondria were prepared according to the method of Parsons et al. (8) except that 0.28 M sucrose solution containing 1 mM Tris-HCl (pH 7.2) and 0.1 mM EDTA was used as the homogenizing medium (5). The post-mitochondrial supernatant of the homogenate was further centrifuged at 12,000 x g for 20 minutes. The precipitate was discarded, and the supernatant was centrifuged at 105,000 x g for 90 minutes to sediment microsomes, which was washed once with 0.25 M sucrose solution.

NADH-cytochrome c reductase activity of microsomes and mitochondrial subfractions was assayed as described in a previous paper (6). 1 mM KCN was included in the assay mixture, and, when necessary, 1 μM rotenone was also added. NADH-cytochrome b_5 reductase and NADPH-cytochrome c reductase activities were assayed as described previously (6). Succinate-cytochrome c reductase activity was assayed at 25° by measuring the initial rate of reduction of cytochrome c spectrophotometrically at 550 mμ. The reaction mixture contained 5 mM sodium succinate, 20 μM yeast (Candida krusei) cytochrome c, 1 mM KCN, and enzyme in 2 ml of 0.1 M potassium phosphate buffer (pH 7.5). Monoamine oxidase was assayed according to the method of Schnaitman et al. (9).

The antiserum against NADH-cytochrome b_5 reductase was obtained by immunizing a rabbit with the enzyme purified from rat liver microsomes (6). The immunoglobulin fraction was prepared by ammonium sulfate fractionation and used as the antibody.

RESULTS AND DISCUSSION

Purity of mitochondrial inner and outer membrane fractions

and microsomal fraction was examined by measuring the activities of succinate-cytochrome c reductase, monoamine oxidase, and NADPH-cytochrome c reductase as the marker enzymes for mitochondrial inner membrane, outer membrane, and microsomes, respectively. Contamination of each fraction by the other two fractions was about 10 % or less (Table I).

TABLE I

Distribution of Succinate-Cytochrome c Reductase, Monoamine Oxidase and NADPH-Cytochrome c Reductase among Three Membrane Fractions

Enzyme activities	Relative specific activity		
	Mitochondria		Microsomes
	Inner membrane	Outer membrane	
Succinate-cytochrome c reductase	100	12.4	1.3
Monoamine oxidase	13.7	100	9.9
NADPH-cytochrome c reductase	1.0	13.0	100

TABLE II

Effect of Rotenone on NADH-Cytochrome c Reductase Activities of Three Membrane Fractions

Fractions	NADH-cytochrome c reductase (mμ moles cyt. c reduced/min./ mg protein)	
	- rotenone	+ rotenone
Mitochondrial inner membrane	610	264
Mitochondrial outer membrane	2,700	2,650
Microsomes	1,860	1,890

Table II shows NADH-cytochrome c reductase activities of inner and outer membrane fractions and microsomal fraction. In confirmation of previous findings (4,5), the reductase activity of inner membrane fraction was inhibited by rotenone, whereas those of outer membrane and microsomal fractions were not inhibited. The residual reductase activity of the inner membrane fraction observed in the presence of rotenone may be explained by the contamination with about 10 % of the outer membrane fraction. The same extent of contamination was indicated from monoamine oxidase activities of the inner and outer membrane fractions (Table I). Judging from NADPH-cytochrome c reductase assay, the microsomal contamination into the outer membrane fraction was 13 % (Table I). Since the outer membrane fraction had a higher rotenone-insensitive NADH-cytochrome c reductase activity than the microsomal fraction as shown in Table II, the contribution of contaminating microsomes to the reductase activity of the former fraction should be less than 13 %.

Fig. 1 shows the effects of anti-reductase immunoglobulin on NADH-cytochrome c reductase activities of these three fractions. The NADH-cytochrome c reductase activity of microsomes was strongly inhibited by the antibody, and the extent of inhibition was more than 95 % at the highest concentration of the antibody used. The reductase activity of the outer membrane fraction was also strongly inhibited, and the extent of inhibition at various concentrations of immunoglobulin was identical with that of microsomes. Since the contribution of contaminating microsomes to the reductase activity of the outer membrane fraction is lower than 13 %, this result clearly indicates that the reductase in mitochondrial outer membrane is also sensitive to the antibody, and immunologically very similar to its microsomal counterpart. Control immunoglobulin

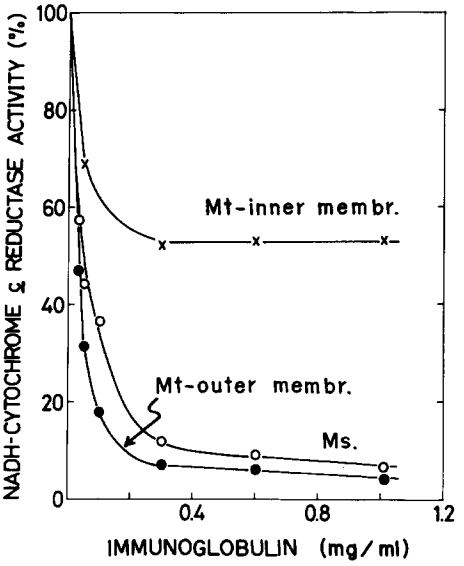

Fig. 1 Inhibition of NADH-cytochrome c reductase activities of three membrane fractions by the antibody.; NADH-cytochrome c reductase of mitochondrial inner membrane fraction was assayed in the absence of rotenone and those of mitochondrial outer membrane fraction and microsomes were assayed in the presence of rotenone as described in Methods. The antibody was preincubated at 25° with the membrane preparations for 10 minutes in the reaction mixture.
(—x—) mitochondrial inner membrane (0.02 mg protein/ml)
(—●—) mitochondrial outer membrane (0.05 mg protein/ml)
(—○—) microsomes (0.02 mg protein/ml)

obtained from normal rabbits did not inhibit the reductase activities of these fractions.

When assayed in the absence of rotenone, NADH-cytochrome c reductase of the inner membrane fraction was also partially inhibited by the antibody (Fig. 1). The maximum extent of inhibition attained at 0.3 mg/ml antibody was about 40 - 45 %, and the inhibition was not increased even by further addition of the antibody. The amount of antibody-sensitive reductase activity in the inner membrane fraction agrees well with that of rotenone-insensitive reductase, which is explained by the presence of contaminating outer membrane fragments and microsomes in this fraction. In fact, the activity surviving the antibody inhibition was completely

abolished by rotenone. It is concluded that rotenone-sensitive NADH-cytochrome c reductase associated with mitochondrial inner membrane does not react with the antibody, and is immunologically different from the microsomal NADH-cytochrome c reductase system.

The immunological similarity or identity of microsomal NADH-cytochrome b_5 reductase with the corresponding reductase of mitochondrial outer membrane is the first evidence for the intimate relationship between the components present in these two separate membrane systems. Furthermore, it has been found that the outer membrane fraction catalyzes the reduction by NADH of purified cytochrome b_5 from liver microsomes, and the ratio of cytochrome b_5 reductase to cytochrome c reductase activities of the outer membrane fraction is identical with that of microsomes. The NADH-cytochrome c reductase system of mitochondrial outer membrane is thus very similar in many respects to that of microsomes, although their molecular identity is yet to be established in future.

We wish to thank Prof. R. Sato for his interest and advices during this work.

REFERENCES

1. Strittmatter, P., and Velick, S. F., J. Biol. Chem. 228, 785 (1957)
2. Raw, I., Molinari, R., do Amaral, D. F., Mahler, H. R., J. Biol. Chem. 233, 225 (1958)
3. Mahler, H. R., Raw, I., Molinari, R., and do Amaral, D. F., J. Biol. Chem. 233, 230 (1958)
4. Sottocasa, G. L., Kuylenstierna, B., Ernster, L., and Bergstrand, A., J. Cell Biol. 32, 415 (1967)
5. Parsons, D. F., Williams, G. R., Thompson, W., Willson, D., and Chance, B., Mitochondrial Structure and Compartmentation, ed. by E. Quagliariello, S. Papa, E. C. Slater, and J. M. Tager, Adriatica Editrice, Bari (1967) p. 29
6. Takesue, S., and Omura, T., J. Biochem. 67, 267 (1970)
7. Takesue, S., and Omura, T., in preparation
8. Parsons, D. F., Williams, G. R., and Chance, B., Ann. N. Y. Acad. Sci., 137, 643 (1966)
9. Schnaitman, C., Erwin, V. G., and Greenawalt, J. W., J. Cell Biol. 32, 719 (1967)