THE MITOCHONDRIAL STRUCTURAL PROTEINS FROM WILD-TYPE AND RESPIRATORY-DEFICIENT YEASTS

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Slonimski and his collaborators (Slonimski, 1953; Sherman et al., 1964) have remarked that respiration-deficient strains of yeast defective in the cytoplasmic gene, ρ , lack, among the components of the respiratory system, cytochromes a, c_1 and b, which are considered to be tightly bound to the organized membrane matrix of mitochondria. On the basis of this observation they have proposed a postulate that the normal yeast mitochondria involve in the membrane structure, as do mammalian mitochondria, the specific protein which can form polymer complexes with those cytochromes, and that respiration-deficient strains (ρ^-) lack the ability of forming the competent mitochondrial membrane matrix. It, then, is interested whether there is a difference in the protein component of mitochondrial membrane between the normal and the respiration-deficient strains. This paper reports that the structural protein isolated from the mitochondrial fraction of a normal strain was found identical in various respects with that from the corresponding fraction of a respiration-deficient strain.

The organisms used were a diploid strain of Saccharomyces cerevisiae, D206, and a respiration-deficient strain, D206P, derived from it by p-nitrophenol treatment (Yanagishima, 1957). Biochemical characters of the respiration-deficient mutant used here were in accord with a criterion of cytoplasmic respiration-deficient mutant (Sherman et al., 1964). The composition of the nutrient medium was: peptone 3.5 g, yeast extract 2.0 g, KH_2PO_4 2.0 g, $MgSO_4 \cdot 7aq$ 1.0 g, sucrose 40 g and tap water up to 1 litre. Cells cultured for 30 hrs under continuous, vigourous aeration at 30°, were

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harvested, washed and then subjected to the enzymatic treatment of snail gut juice to prepare intact mitochondria according to the method of Duell et al. (1964). Intact mitochondria fraction obtained by mild maceration of spheroplasts in 0.7 M sorbit, pH 7.4, was then subjected to centrifugation in a density-gradient of Urografin solution to be freed from contaminating particles (Schatz et al., 1964). The major, red-brown colored layer, clearly separated from other minor layers after 5 hrs' spin at 25,000 rpm in the rotor SW 25.1 of the Spinco ultracentrifuge, showed markedly high specific activities of mitochondrial enzymes, and was according to electron microscopic observation composed exclusively of typical mitochondria without particulate contamination.

Electron transport particles, prepared according to Mackler et al. (1962) with slight modifications, were found to be equally useful for isolating the mitochondrial structural protein. The cells suspended in 0.7 M sorbit buffered with phosphate (0.1 M) to pH 6.8 were disrupted with French press instead of glass homogenizer. From the electron transport particles thus prepared, the structural protein can be isolated in larger quantity, and in the analyses described below its purity was found closely identical to that obtained from intact mitochondria.

Just the same procedures as above were followed for respiration-deficient cells in order to collect the organized membrane system which corresponded to the mitochondria or to the electron transport particles of normal cells. Yield of the membrane system was little less than that from the wild-type cells.

The procedure for preparing the structural protein was virtually that devised by Criddle et al. (1962). The mitochondrial fraction (intact mitochondria or electron transport particles) from wild-type cells and the corresponding fraction from the mutant were solubilized by a mixture of deoxycholate, cholate and dodecylsulfate (2, 1, 0.75 mg/mg protein, respectively) with addition of dithionite. After keeping the solution at pH 7.3 for 16 hrs and removing insoluble materials, ammonium sulfate was added to 12% saturation and the resulting white precipitate was collected and washed with butanol and 50% methanol to remove bound phospholipid and the detergents. The fraction thus obtained did not show slightest spectral

absorptions due to cytochromes and flavoproteins, and was free of indications of iron, phosphorus, carbohydrate and nucleic acids. The yield of the purified protein was 35 to 45 % of the protein contained in the intact mitochondria from wild strain, and the same was true with the corresponding membrane fraction from the mutant.

Each of the purified protein fractions, obtained from the wild type strain and from the mutant, proved to fulfill a general criterion of structural protein from membrane systems such as beef heart mitochondria (Criddle et al., 1962): namely, it polymerized at neutral pH to form a water-insoluble aggregate, which was readily solubilized by dilute alkali (pH 11-12), acetic acid (66 %) or by a dilute solution of sodium dodecylsulfate (SDS), but not by triton X-100, 0.6 M KCl, or urea (2 M).

Each of the two protein preparations, dissolved in 1 % SDS at pH 8.0, 9.7 and 11.5, was analysed for the homogeneity by means of Tiselius Electrophoresis Apparatus. At these pH's, each preparation gave only one major boundary not accompanied by any detectable minor boundaries. The structural protein of the wild-type strain and that of the mutant strain were compared each other by ultracentrifuge analysis, amino acid analysis and immunological reactions as follows.

Ultracentrifuge Analysis: When the protein solution in 0.1 % SDS, pH 7.0, were spun by Spinco Model E ultracentrifuge, each gave a single sedimentation boundary which proved to be 1.9 S (Fig. 1, top and middle). This value is in the range of the

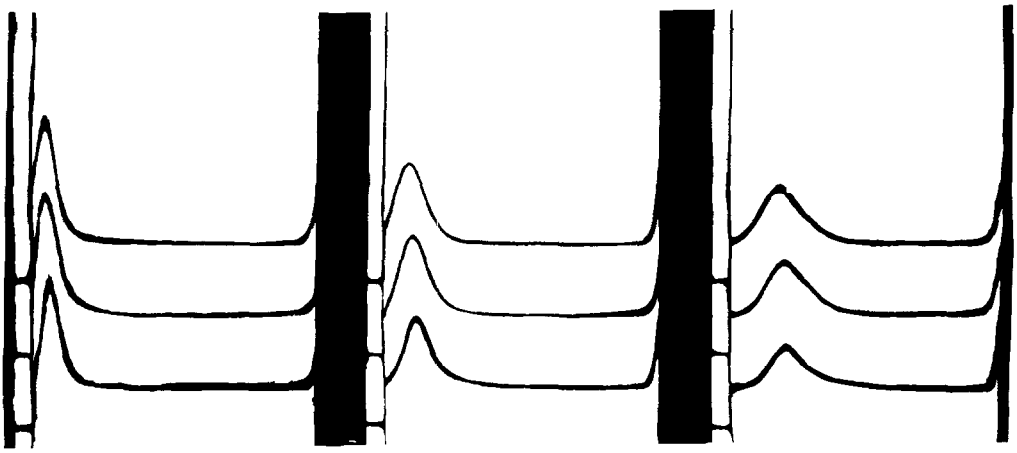

Fig. 1. Sedimentation of mitochondrial structural protein isolated from normal yeast (top), respiration-deficient yeast (middle) and beef heart (bottom). The centrifuge was run at 59,780 rpm at 20°. The time interval between photographs is 24 minutes.

sedimentation coefficients generally found for the structural proteins from other sources (Criddle et al., 1962). The structural protein isolated from beef heart mitochondria showed essentially the same sedimentation pattern as those from the yeast strains (Fig. 1, bottom).

Amino Acid Analysis: The results of amino acid analysis on the structural proteins from two yeast strains are reproduced in Table 1. A remarkable agreement is realized between the two. It is noted that they are devoid of tryptophan residue. The proportion of amino acids carrying hydrophobic side chains is as high as 43 % . This is coincident with the value for the structural protein of beef heart mitochondria, in spite of considerable dissimilarity in the proportion among hydrophobic amino acids.

Antigenic Property: In order to examine if the structural proteins from the two yeast strains are immunologically distinct from each other, the structural protein isolated from the highly purified wild-type mitochondria was emulsified in the Adjuvant Complete (Freunds') and injected on the sides of vertebral column of three adult rabbits. The serum was obtained after 2 weeks following 3 weekly injections.

TABLE I. Amino acid composition of mitochondrial structural proteins

(in μmoles amino acid per 100 mg protein)

Amino acid	Wild-type	RD-mutant	Beef Heart	Amino acid	Wild-type	RD-mutant	Beef Heart
Asp	78	78	58	Leu	70	71	68
Thr	40	41	37	Tyr	20	21	28
Ser	44	44	37	Phe	39	39	39
Pro	33	33	20	Lys	64	61	56
Glu	55	56	55	His	18	18	11
Gly	58	60	69	Arg	46	43	36
Ala	58	60	74	Try	0	0	29
Val	50	51	48	Amide-N	86	73	90
Met	13	13	18	Cys (as cysteic acid)			
Ileu	44	44	44		6	7	68

RD-mutant: respiration-deficient mutant
Beef Heart: cited from Criddle et al. (1962)

The reaction of this antiserum with proteins was tested by the two methods; by the precipitation in the agar-diffusion plate and by the passive cutaneous anaphylaxie on the back of guinea-pigs.

It was revealed by either method that the antibody induced by the structural protein from the wild-type mitochondria reacted with the structural protein from the mutant strain as distinctly as with the former structural protein. On the other hand, the serum used did not react with any other proteins thus far tested, including yeast cytochrome c and the structural protein of beef heart mitochondria. In the agar plate, single slur of precipitation was formed between antiserum-well and a well containing either of the structural proteins from the two yeast strains. The homogeneity of each preparation was thus confirmed again. When the proteins from the two yeast strains were placed in neighboring wells near the antiserum-well in the agar plate, the two slurs did not intersect each other, but made a continuous arc, indicating the serological identity of the two proteins.

Discussion: The experimental results reported above have shown that the structural protein isolated from the mitochondrial fraction of wild-type yeast is indistinguishable in physical, chemical and serological examinations from the structural protein isolated from the corresponding membrane fraction of respiration-deficient mutant strain. According to Yotsuyanagi's electron-microscopical studies (1962), mitochondria of respiration-deficient cells, though characteristically modified from the normal ones in the inner cristate structures, increase in number and size at the early stationary phase of aerobic growth, namely, at the phase when the development of mitochondria is conspicuous in normal cells. Thus, the respiration-deficient cells seem to contain the structure homologous with the mitochondria of wild-type cells. And it may be intelligible that these organelles, even though morphologically different, contain the identical structural protein. The mutation of ρ to ρ^- does not seem to imply a change in the informations on the structural protein studied in the present work, nor on the capacity to organize these organelles.

The fact that the cytochromes to be bound to the membrane matrix are missing in

the respiration-deficient mutant makes one expect some difference in the membrane structure. The structure here studied constitutes only about 40 % of the protein of the mitochondrial fraction. Hence there remains a possibility that the wild-type and the respiration-deficient mitochondria differ in some proteins not presently studied, including other "structural" proteins, such as contractile protein.

The structural protein dealt with in the present paper is devoid of tryptophan. Kattermann et al. (1960) have reported that the aerobic adaptation of anaerobically grown yeast is markedly inhibited by 5-methyltryptophan, an analogue of tryptophan. It has been confirmed in our laboratory (Katoh, unpublished) that azatryptophan is also a potent inhibitor of the same reaction. Hence it seems that the protein synthesis which requires tryptophan is essential for the aerobic adaptation of anaerobically grown yeast, a complex process in which active synthesis of the structural protein is an important part (Katoh, unpublished). As for phospholipids, which are generally believed to combine with the structural protein and from active membrane matrix of mitochondria, we have found no significant difference between those extracted from the mitochondrial fractions of the wild-type and the mutant yeasts strains (Gohgi et al., in preparation).

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