SEPARATION OF PROTEIN COMPONENTS OF MITOCHONDRIAL COMPLEX IV AND THEIR MOLECULAR WEIGHTS

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SUMMARY

Heme a protein separated from a purified cytochrome oxidase preparation shows an unexpectedly low molecular weight of 26,500 by gel electrophoresis. The tightly bound phospholipid, copper and iron are associated with this low molecular weight protein. This protein also retains the capacity to form membrane sheets when associated with phospholipid. The other protein separated from the oxidase has a higher molecular weight 55,000, no phospholipid, copper or iron and does not form membrane layers when phospholipid is added. It can be tentatively identified as core protein IV. Some loss of oxidase activity occurs when the two proteins are separated which can be partially restored by recombination of the fractions.

The regular preparations of the purified beef heart mitochondrial lipid-depleted cytochrome oxidase (EC1.9.3.1) (1) and the deoxycholate-cholate cytochrome oxidase (2) consistently contain two about equal bands on gel electrophoresis. Korman and Vande Zande (3) reported the isolation of a noncatalytic protein from complex IV of the electron transfer chain by treatment of the complex with acidic methanol. This procedure, however, completely inactivated the enzymatic activity of the heme a protein (the catalytic protein of the complex). Kopaczyk et al. (4) isolated a noncatalytic protein from complex IV but no electrophoretic data were shown. In the present study we report the separation of two protein components of cytochrome oxidase preparations and their apparent molecular weights determined by gel electrophoresis technique.

The regular lipid-depleted cytochrome oxidase (1) was first added with 10% Triton X100 to make 1.5 mg Triton/mg protein and adjusted with

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Table 1

Properties of Heme a Protein and Core Protein IV Preparations

Preparation	Heme a (mμmoles/ mg protein)	Phospholipid (μg P/mg protein)	Cytochrome oxidase activity	Copper mμA/mg protein	Iron mμA/mg protein
Lipid-depleted oxidase	8.3	1.55	3.7	10.0	8.2
Lipid-depleted oxidase + PL			40.0		
Heme a protein	8.0	1.95	5.2	12.0	9.5
Heme a protein + PL			28.0		
Core protein IV	not detectable	0.20	0	1.5	2.5
Core protein IV + PL			0		
Heme a protein + Core protein + PL			36.6		
BHM structural protein			0		
Structural protein + PL			0		
Heme a protein + Structural protein + PL			26.7		

Heme a protein was dialyzed against 1% Triton X100, 0.02 M Tris HCl, pH 7.4 for 4 hours after incubation and centrifugation.

BHM structural protein was prepared according to the procedure of Kopaczyk et al. (4). Structural protein and core protein were solubilized by the method of Kopaczyk et al.

Heme a content was determined by its differential spectrum using a millimolar extinction coefficient ΔA at 605 - 630 mμ as 13.1 (9): phosphorus content by the procedure of Chen et al. (10).

Enzyme activity was measured polarographically as described earlier (1). Activity of the lipid-depleted cytochrome oxidase is expressed as μmoles O_2/min/mg total protein while others are μmoles O_2/min/mg heme a protein.

0.25 M sucrose, 0.02 M Tris HCl, pH 7.4 to make a protein concentration of 6 mg/ml. The following compounds were then added to the diluted enzyme: γ-tocopherol, 0.01 mg/mg protein; potassium deoxycholate, 1.5 mg/mg protein; potassium cholate, 0.75 mg/mg protein; dithionite, 1 mg/ml of total solution (dithiothreitol could substitute for dithionite with similar results) and 12% saturation of ammonium sulfate. Core protein IV was

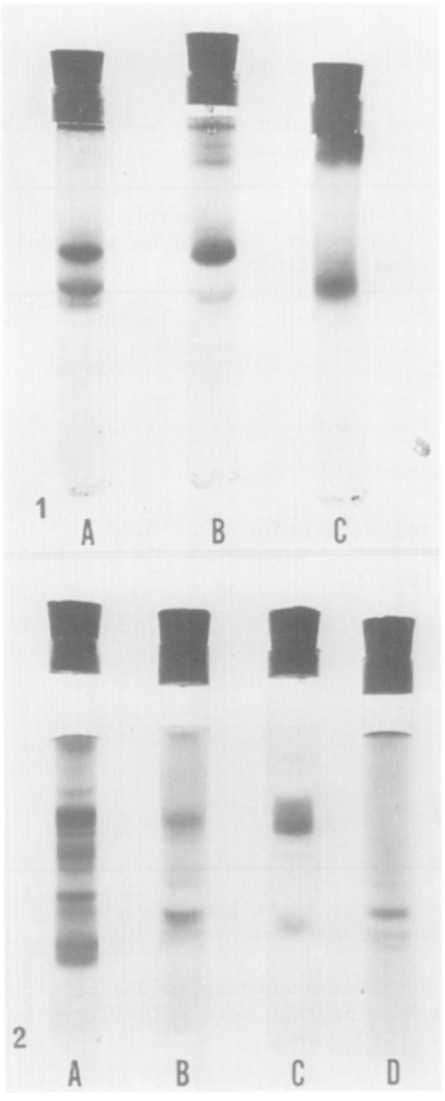

Figure 1. Gel electrophoresis of heme a protein and core protein IV according the procedure of Takayama et al. (14). A: lipid-depleted cytochrome oxidase, B: heme a protein, C: core protein IV.

Figure 2. SDS-gel electrophoresis pattern of heme a protein and core protein IV. SDS-gel electrophoresis was performed according to the method of Weber and Osborn (8) on 5 mm i.d. and 50 mm long 10% acrylamide-0.21% methylenebisacrylamide gel columns. (A) standard proteins: a, bovine serum albumin (68,000); b, pyruvate kinase (57,000); c, ovalbumin (43,000); d, glyceraldehyde phosphate dehydrogenase (36,000); e, trypsin (23,300); f, hemoglobin (15,500). (B) lipid-depleted cytochrome oxidase. (C) core protein IV. (D) heme a protein.

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obtained by centrifugation after 2.5 hours incubation at room temperature in the dark, following by three washes with 1% Triton X100, 0.02 M Tris HCl, pH 7.4. Green heme a protein remained in the supernatant after 12

hours incubation to remove residual core protein IV. Table 1 shows phosphorus heme a, copper and iron content of the core protein IV and heme a protein and cytochrome oxidase activity. It is interesting to note that phospholipid, Cu and Fe were predominantly present in the heme a protein fraction. Low recovery of heme a content and enzymatic activity may be due respectively to detergent effect after long time incubation or to removal of core protein IV. After re-introduction of core protein IV to the heme a protein, the activity is increased from 28.0 to 36.6 μmoles O_2/min/mg of heme a protein in the presence of mitochondrial phospholipids (Table 1). Electrophoretic data illustrate the separation of two proteins (Figures 1 and 2). Proteins of cytochrome oxidase preparation of Fowler et al. (2) could also be fractionated by the same procedure with pretreatment of Triton X100.

Molecular weight of cytochrome oxidase has been reported to be 100,000 - 150,000 (5), 530,000 (6) and 72,000 (7). Sodium dodecyl sulfate-acrylamide gel electrophoresis has been shown to be a reliable method for protein molecular weight determination (8). Figure 3 shows a calibrated graph of electrophoretic mobility of known molecular weight proteins. Band positions of core protein IV and heme a protein on SDS-gel electrophoresis are shown in Figure 2. A molecular weight of 26,500 was obtained for the

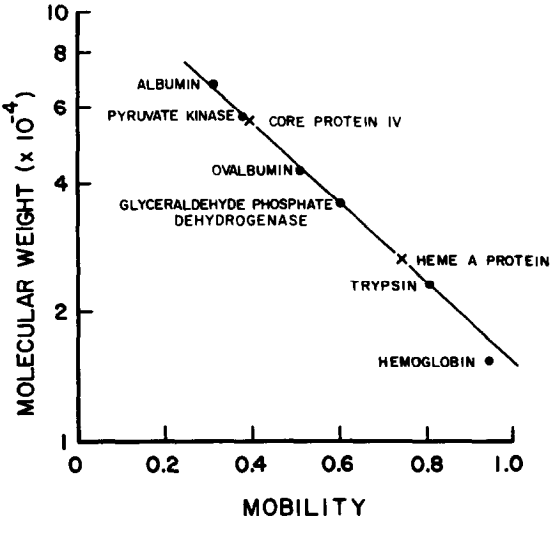

Figure 3. Calibration of electrophoretic mobility.

Table 2

Molecular Weights of Noncatalytic and Catalytic Proteins

Protein	# of component	Mol. weight	Ref.
SP from HBHM (purified)	4	60,000 - 65,000	11
SP from HBHM (outer membrane)	2	62,000 - 63,000	11
SP from HBHM ATPase	1	60,000 - 65,000	11
Core protein III	1	50,000 - 51,000	11
Core protein IV	1	55,000 $\pm$ 6,000	this study
Cytochrome b		28,000	12
Cytochrome c_1		38,000	13
Cytochrome oxidase		100,000 - 150,000	5
		530,000	6
		72,000	7
Heme a protein		26,500 $\pm$ 1,200	this study

SP, structural protein; HBHM, heavy beef heart mitochondria.

heme a protein, and 55,000 for the core protein. Molecular weight of core protein IV obtained by this method is in the range of reported noncatalytic proteins (Table 2). Value for the heme a protein may be the smallest unit for this protein and it is in the same order of other cytochromes (Table 2). Values reported by other workers for cytochrome oxidase might be for the complex of heme a protein and core protein (7) or the polymer of the complex (5,6).

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REFERENCES

1. T. F. Chuang, F. F. Sun and F. L. Crane, J. Bioenergetics 1, 227 (1970).
2. L. R. Fowler, S. H. Richardson and Y. Hatefi, Biochim. Biophys. Acta 64, 170 (1962).
3. E. F. Korman and H. Vande Zande, Federation Proc. 27, 526 abs. (1968).
4. K. Kopaczyk, J. Perdue and D. E. Green, Arch. Biochem. Biophys. 115, 215 (1966).
5. K. Okunuki, in Comprehensive Biochemistry (ed., C. M. Florkin and E. H. Stotz, Vol. 14, Elsevier, Amsterdam, 1966, p. 232.
6. S. Takemori, I. Sekuzu and K. Okunuki, Biochim. Biophys. Acta 51, 464 (1961).
7. R. S. Criddle and R. M. Bock, Biochem. Biophys. Res. Commun. 1, 138 (1959).
8. K. Weber and M. Osborn, J. Biol. Chem. 244, 4406 (1969).
9. W. H. Vannesta, Biochim. Biophys. Acta 113, 175 (1966).
10. P. S. Chen, T. Y. Toribara and H. Warner, Anal. Chem. 28, 1756 (1956).
11. D. E. Green, N. F. Haard, G. Lenaz and H. I. Silman, Proc. Nat. Acad. Sci., U. S. 60, 277 (1968).
12. R. Bomstein, R. Goldberger and H. Tisdale, Biochim. Biophys. Acta 50, 527 (1961).
13. R. Goldberger, A. L. Smith, H. Tisdale and R. Bomstein, J. Biol. Chem. 236, 2788 (1961).
14. K. Takayama, D. H. MacLennan, A. Tzagoloff and C. D. Stoner, Arch. Biochem. Biophys. 114, 223 (1966).