ISOLATION AND PROPERTIES OF THE STRUCTURAL PROTEIN OF MITOCHONDRIA[1]

by
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The known oxidation-reduction proteins of the electron transport particle (succinic dehydrogenase, DPNH dehydrogenase, cytochromes a, b, c_1 and c) have been isolated in homogeneous state and their minimal molecular weights have been determined. The concentration of each of these proteins in the electron transport particle (ETP) can be calculated from the known concentrations of the prosthetic groups of these six proteins in the particle and from the molecular weight data. Such a calculation (cf. Table I) shows that the six oxidation-reduction proteins account for no more than 17% of the total protein of ETP. In the isolation of cytochromes a, b and c_1, it is relatively easy to obtain a separation of any one cytochrome from the others early in the preparation. The main impurity, the removal of which is essential for further purification, is a colorless protein (or class of protein) which is bound very tenaciously to the cytochrome in question. These considerations have led us to postulate that the mitochondrion contains structural protein which contains no oxidation-reduction components, yet which is intimately associated with both the oxidation-reduction proteins of the electron transport chain and with lipid.

For technical reasons, this communication could not be published with the two companion notes: R.S. Criddle, R.M. Bock, D.E. Green, and H.D. Tisdale "Specific Interaction of Mitochondrial Structural Protein (S.P.) with Cytochromes and Lipid," and D.E. Green, H.D. Tisdale, R.S. Criddle, and R.M. Bock "The Structural Protein and Mitochondrial Organization," which appeared in Biochem. Biophys. Res. Comm. 5, 75 and 81.

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TABLE I

Protein	Concentration of Prosthetic Group (mμM/mg protein)		% of total Protein in ETP
	in ETP	in pure enzyme	
Succinic dehydrogenase	0.32 (1)	5 (3)	6.4
DPNH dehydrogenase	0.32 (1)	13 (4)	2.5
Cytochrome a	1.30 (2)	ca. 30 (8)	ca. 4.3
Cytochrome b	0.68 (1)	36 (5)	1.9
Cytochrome c_1	0.3 (1)	27 (6)	1.1
Cytochrome c	0.3 (1)	77 (7)	0.4
		TOTAL	16.6

(1) Linnane and Ziegler (1958)
(2) Griffiths and Wharton (1961)
(3) Singer, Kearney and Massey (1956)
(4) Ziegler, Green and Doeg (1959)
(5) Bomstein, Goldberger and Tisdale (1960a)
(6) Bomstein, Goldberger and Tisdale (1960b)
(7) Theorell and Åkeson (1941)
(8) Unpublished observations of R.S.Criddle

Preparations of Structural Protein (S.P.)

When beef heart mitochondria (in a 0.25 M sucrose suspension containing 20 mg/protein/ml) are treated at 0° with deoxycholate (2 mg/mg protein), cholate (1 mg/mg protein) and 0.75 mg/mg sodium dodecylsulfate (SDS), an essentially clear "solution" is obtained. After clarification at 40,000 X G to remove a brown-green residue (protein-free), enough solid $Na_2S_2O_4$ is added to reduce the cytochromes, and then the solution is made 12% saturated with respect to ammonium sulfate (pH kept at 7.0). The copious white precipitate is sedimented and washed thoroughly with 0.25 M sucrose. The washed suspension is then treated with butanol (20% by volume) in the presence of 20% saturated ammonium sulfate and deoxycholate (1 mg/mg protein) to remove lipid. The extracted precipitate is washed in 0.25 M sucrose and then extracted twice with 10 volumes of 50% methanol at 50° to remove deoxycholate (30 second exposure time). The yield is about 33% of the total protein in mitochondria and 55% of the particulate protein (total protein minus readily extractable protein*).

* The protein moiety which becomes soluble when a mitochondrial suspension in 0.25 M sucrose is mixed with 0.33 Vol. of 0.12 M KCl and 0.11 Vol. of t-amyl

The supernatant fluid, after removal of the S.P., contains the four cytochromes. At higher ammonium sulfate concentrations (20-30%) these are precipitated out at a purity level some five times higher than that of the same components in the mitochondrion. The cytochromes do not show any spectral evidence of structural modification.

S.P. can also be prepared from particulate fractions of the electron transport chain or from preparations of purified cytochromes by similar procedures. The S.P. which is obtained from the cytochrome oxidase segment of the chain appears (from sedimentation and solubility measurements) to be the same as that isolated from the segment of the chain containing cytochromes b and c_1. This suggests that a single species of S.P. is implicated in the entire electron transport chain.

Physical Properties of the Structural Protein

S.P. contains no flavin, heme, non-heme iron, copper or lipid. It is completely insoluble in water, but, as are the structural coat proteins from many viruses, ribosomes, etc., is readily soluble in aqueous media that contain reagents which induce major charge repulsion between subunits or which attack hydrophobic bonds. Table II lists the solubility of S.P. in various media, and the results of sedimentation and molecular weight measurements which have been made on the solubilized species obtained by exposing S.P. to some of these media. Detergent solutions and extremes of pH are most effective in the solubilizations. The protein is soluble only to the extent of about 0.5% in sodium hydroxide solutions of pH 11. Urea probably aids in the solubilizing of S.P. mainly by rupturing the tertiary configuration of the individual protein molecules which is necessary for stable and specific protein-protein interaction.

Sedimentation Studies

Sedimentation velocity measurements, carried out in the media listed in Table II which lead to solubilization, indicated the presence of one major sed-

alcohol and the mixture warmed to 20° for 10 min. Sedimentation for 10 min. at 110,000 X G.

TABLE II

Medium	Solubility*	Sedimentation Coefficient (in S)	$\bar{M}w$--
Dilute NaOH (pH 11)	+		
Dilute NaOH (pH 10.5), 0.1% SDS	++	2.1	22-28,000
0.1% SDS	+		
0.3% SDS	++	1.8	
67% Acetic acid	++	2.2	25-30,000
8 M Urea, 0.1% SDS	++	2.2	20-30,000

S.P. was insoluble in the following media: 8 M urea, 3 M thioglycollate, 4 M guanidinium hydrochloride, 0.1% Triton-X-100, formamide, pyridine-water and dilute mineral acid.

* + = partly soluble, < 0.5%
++ = very soluble, > 0.5%

imenting boundary in each case with coefficients of 1.9 to 2.2 S. This is an indication that each of these treatments gives rise to the same molecular species, or at least to a molecular species of similar size.

Molecular weight determinations were carried out by Ehrenberg's (1957) modification of the Archibald method of approach to sedimentation equilibrium. All runs were made in a double sector synthetic boundary cell so that corrections for the raised base line at the meniscus due to the extreme solvent systems could readily be made. From experiments of this type, the weight average molecular weight of the S.P. at the meniscus can be calculated. The variation of the weight average molecular weight with time in such experiments is a measure of the heterogeneity of molecular size within the sample. These experiments indicated molecular weights of 20,000 to 30,000 for the structural protein. Part of this apparent heterogeneity might be accounted for by a relatively slowly approached equilibrium between monomer and higher aggregates of S.P. Evidence for such a readily reversible dissociation of S.P., which our picture of mitochondrial structure predicts, has been obtained from an experiment on the solubility of the S.P. When S.P. is dissolved at pH 10.5 in presence of

0.1% SDS and the solution is then dialyzed against a solution of slowly changing pH, it is possible, at low ionic strength, to go as low as pH 8.5 and still have the S.P. remain in solution. The sedimentation coefficient, however, has not remained constant. Instead of a single peak of 1.8 S, we observe a number of peaks of sedimentation coefficients in the range of 10 to 20 S. Dilution of this system with the buffer used in the dialysis, which would tend to favor a lower degree of association in a system at equilibrium, does in fact reduce the S values of the system which now fall in the range of 4 to 10.

Electrophoresis of S.P.

A solution of S.P. in 0.3% SDS was brought to pH 10.5 with sodium hydroxide and then dialyzed against a buffer of pH 9.5 containing 0.05 M diethylbarbiturate and 0.05 M sodium chloride. In free electrophoresis in the Tiselius apparatus all of the protein migrated with one peak which had a mobility of -9.5×10^{-5} cm^2 $volt^{-1}$ sec^{-1} measured on the descending boundary under a potential gradient of 5.9 volts/cm. No resolution of S.P. into more than one component has been achieved by chromatography on DEAE cellulose or on Dowex-1 resin.

Amino Acid Composition

The amino acid analysis by T. Gerritsen of the Department of Pediatrics of the University of Wisconsin Medical School showed that 51% of the total amino acids had non-polar side chains while the combined glutamic and aspartic acid content was low (18% of the total amino acids). By comparison of these values with corresponding values for representative proteins compiled by Waugh (1954) and allowing for any differences in the selection of non-polar amino acids, the content of non-polar side chains in S.P. falls in the upper range for proteins generally while the aspartic and glutamic content falls in the lower range.

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