SPECIFIC INTERACTION OF MITOCHONDRIAL STRUCTURAL PROTEIN (S.P.) WITH CYTOCHROMES AND LIPID

by
R. S. Criddle* and R. M. Bock
Department of Biochemistry, University of Wisconsin
and
D. E. Green** and H. D. Tisdale
The Institute for Enzyme Research, University of Wisconsin

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The observed close association of mitochondrial cytochromes and lipid with an unidentified protein led us to postulate the existence of S.P. [see accompanying note by Green et al. (1961)]. Consequently, the specific combining properties of the isolated S.P. are of fundamental importance in our concept of a protein with a structural role. We shall present evidence in this communication that S.P. rapidly combines with cytochromes a, b and c_1, and with lipid to form stable complexes.

Interaction of S.P. with Cytochromes

Interactions have been demonstrated both by physical measurements and by chemical analysis. In essence the first method involved mixing a solution of S.P. in the monomeric state (see legend of Figure 1 for details of preparation) with a solution of one of the cytochromes. Ultracentrifugal or electrophoretic analysis showed (a) the disappearance of the migrating boundaries of the two molecular species that were mixed, and (b) the appearance of a new molecular species whose molecular size and electrophoretic mobility were different from those of either of the starting components. For example, by selecting the proper molecular proportions of S.P. and cytochrome c_1, it was possible to get a quantitative conversion of these two proteins to a new molecular species con-

* Predoctoral Research Fellow of Division of General Medical Sciences, United States Public Health Service.
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taining both S.P. and c_1. In this particular experiment (cf. Figure 1),

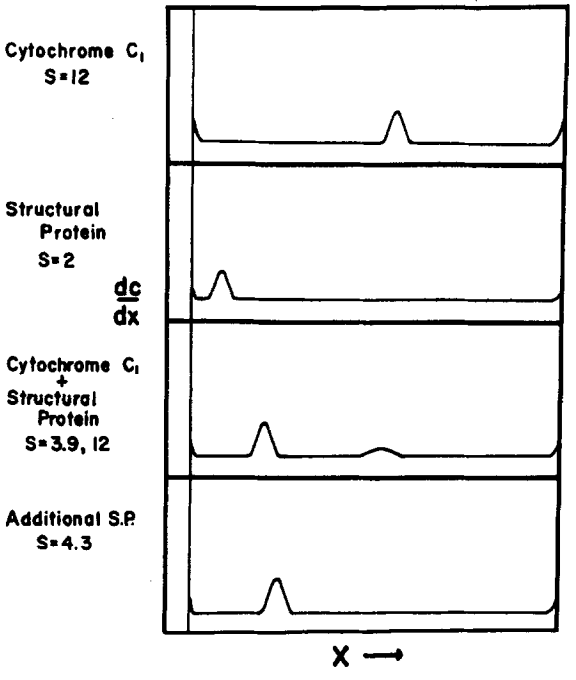

Figure 1

Sedimentation diagram after 32 min. at 59,780 RMP

in Tris buffer of pH 7.8, at 20° $\frac{\Gamma}{2} = 0.1$ (Tracings from photographs)

S.P. was solubilized by dialysis against 0.01 M phosphate buffer (pH 10.5) containing 0.2% SDS. Excess SDS was then removed by dialysis for two days against 0.01 M phosphate buffer (pH 10) containing 0.05 M NaCl. Cytochrome c_1 was added in 0.1 M phosphate solution (pH 7.4). At this pH cytochrome c_1 exists as the hexamer. When the two solutions were mixed (S.P. + c_1) the pH of the resulting mixture was 7.8 and no turbidity developed.

cytochrome c_1 [Green et al. (1959); Bomstein et al. (1960b)] was added at pH 7.4 in the form of a hexamer [$\bar{M}w$ 360,000, S=12, cf. Criddle and Bock (1959)], to a solution of structural protein existing in the monomeric form (S=2) at pH 10 (see legend for details of preparation). The sedimenting boundary of both of these species disappeared upon mixing and a new heme-containing component, migrating with a velocity of S=3.9-4.3 ($\bar{M}w$ of 75,000)

was observed. It should be noted that the S.P.-c_1 complex has a molecular weight intermediate between that of S.P. and c_1 since the formation of the complex involves the depolymerization of the c_1 hexamer. Thus S.P. seems to act in detergent fashion as a depolymerizing agent by competing for the hydrophobic binding sites of individual molecules of cytochrome c_1. The S.P.-c_1 complex is water soluble at pH 7.8 whereas S.P. alone is completely insoluble at this pH. The component with S=4.3 also moved as a single sharp boundary in electrophoresis with a mobility different from that of either S.P. or c_1.

Precisely the same type of experiment has been carried out with S.P. and cytochrome a [Griffiths and Wharton (1961)]. In this experiment the cytochrome a was dispersed mostly in the monomeric or dimeric form [Criddle and Bock (1959)] at pH 10 and in the presence of 0.05% SDS. Some 50% of the original cytochrome oxidase activity survived this treatment. The cytochrome a solution was added to an equal quantity of S.P. prepared as described in the legend for Figure 1. The resulting mixture was allowed to stand at room temperature (24 hours) and was then dialyzed against buffer of pH 8 (0.01 M Tris acetate, 0.05 M NaCl). Sedimentation and electrophoretic experiments once again indicated the formation of a complex between S.P. and cytochrome a. A single, symmetrical boundary was observed to sediment with a coefficient of S=3.5. The green color of the a heme moved with the peak. Sedimenting boundaries for both S.P. and a were absent.

The interaction of cytochrome b [solubilized with cetyldiethylmethylammonium bromide according to Bomstein et al. (1960a)] with S.P. is demonstrable by sedimentation and electrophoretic studies as are the following additional complexes: (1) a complex of a, c_1 and S.P.; (2) a complex of b, c_1 and S.P.; and (3) a complex of a, b, c_1 and S.P. In the formation of the complex containing a, c_1 and S.P. the starting molecular proportions were 1:1:2 and the proportions in the resulting complex were the same.

When a particulate suspension of S.P. is mixed at neutral pH with a solution of any of the three cytochromes (a, b and c_1), rapid interaction takes

place even at 0°. If the S.P. is in large excess most of the cytochrome becomes particle-bound and little protein remains in solution. At lower ratios of S.P. to cytochrome, part of the cytochrome becomes particle-bound and part of S.P. becomes soluble. Under the conditions of the experiments, two types of complexes are formed between S.P. and the cytochromes: (1) insoluble complexes (when S.P. is in large excess); and (2) soluble complexes (when the reacting partners are of comparable concentration.)

Interaction of S.P. with Lipid

When S.P. is incubated at 0° with solubilized mitochondrial lipid [Fleischer and Klouwen (1960)] or with a purified phospholipid at pH 10.5 and then the solution is neutralized to pH 7.0, the precipitated particles of S.P. are found to contain lipid. The amount of lipid bound to the particles depends upon (1) the time of incubation and (2) the state of the S.P., i.e., whether soluble or particulate. In the particulate state the interaction of S.P. with lipid is minimal. Under optimum conditions (24 hour incubation time at pH 10.5), in excess of 4 mg of lipid is bound to 1 mg of S.P. This lipid is not extractable by prolonged washing of the particles in water. The lipid is readily released when the particles are exposed to a mixture of deoxycholate, butanol (20% by volume) and ammonium sulfate (20% saturation).

Cytochrome b can be shown to bind mitochondrial lipid under the same conditions and to about the same degree as S.P. In fact, all the cytochromes which have been isolated have been found to contain variable amounts of lipid (up to 50%) except when specific measures such as the procedure outlined above have been taken to remove lipid.

Figure 2 summarizes the scope of the binding experiments which have been carried out thus far and emphasizes the central role of S.P. in these interactions.

Specificity of Binding

Some fourteen different soluble proteins have been tested for their ability to react with the monomeric form of S.P. at pH 10.5 and no evidence for combin-

MITOCHONDRIAL PROTEIN COMPLEX FORMATION

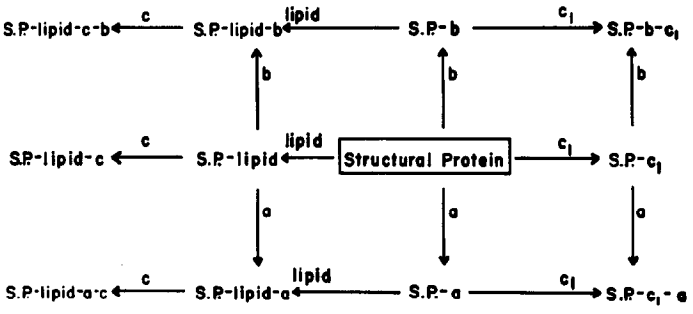

Figure 2

ation has been found. The list includes bovine serum albumin, protamine, hexokinase, phosphatase, trypsin, "Nagarse", β-casein, catalase, xanthine oxidase, glutamic acid decarboxylase, lysine decarboxylase, cytochrome c and hemoglobin. The failure of S.P. to interact with cytochrome c is of interest in view of the fact that cytochrome c is the only one of the four cytochromes that can be readily extracted from the mitochondrion. Myoglobin was also tested because of its tight association with heart mitochondria. Unlike hemoglobin, myoglobin readily forms a complex with S.P.

As yet we have not considered systematically the changes in enzymatic properties which may accompany the binding of cytochromes to S.P. Our colleague, Dr. R. Goldberger, has shown that at least in one instance there is a significant change in properties. Cytochrome b when linked to S.P. in a soluble complex is reducible by various electron donors which point to a relatively high E'_o at pH 7.0 (about 0.0V) whereas the uncomplexed cytochrome is not reducible by these same reductants and the estimated E'_o is relatively low (ca.-0.3V).

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