ISOLATION AND PROPERTIES OF A STRUCTURAL PROTEIN FROM CHLOROPLASTS*

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The organized membrane system of the chloroplast has been fractionated into molecular units and a fraction similar to the structural protein of mitochondria has been isolated. Earlier investigation into the structural organization of beef heart mitochondria demonstrated the presence of a protein fraction which accounted for roughly 60% of the total mitochondrial protein (Green et al., 1961a). This fraction was designated structural protein and evidence was presented demonstrating the specific interactions of this material with the enzymes of the electron transport chain and with mitochondrial lipids (Criddle et al., 1961; Green et al., 1961b; Criddle et al., 1962). Criteria for the general nature of structural proteins from membrane systems have been established (Green et al., 1961b; Criddle et al., 1962) and membrane fractions from several sources have been investigated in light of these criteria (Richardson et al, 1963). These preparations included structural proteins from mitochondria and microsomes, the membrane protein from red blood cells and particulate acetone extracted spinach chloroplast fragments (Richardson et al, 1963). The protein fraction from chloroplasts demonstrating the characteristics of a structural protein has now been isolated in a purified form and its preparation and properties are discussed in this communication.

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Isolation of the Structural Protein

Chloroplasts were isolated by differential centrifugation of homogenized spinach leaves and were then sonicated and centrifuged to obtain the "green precipitate" fraction of the chloroplasts by the procedure of Parks and Pon (1961). An acetone powder of the fragments was then prepared to remove chlorophyl and lipid. The resultant acetone powder preparation was then suspended in 0.002 M tris (hydroxymethyl) aminomethane chloride buffer at pH 8.5 containing 0.035 M NaCl and 0.001 M sodium ascorbate. Sufficient $Na_2S_2O_4$ to reduce the cytochromes was added followed by the addition of deoxycholate (2 mg/mg protein) and cholate (1 mg/mg protein). After two hours the solution was centrifuged to remove some insoluble protein and saturated ammonium sulfate added to bring its concentration to 12-16% saturated. The resultant precipitate was collected by centrifugation and then extracted with cold acetone to remove the detergents. This product had a light yellow color due to small amounts of cytochrome b_6. A completely colorless solution was obtained by dissolving the protein in 0.1% SDS and 0.5 M urea to a concentration of 1% and slowly adding saturated ammonium sulfate until precipitation just starts. The cytochrome is precipitated free of the structural protein at this step and may be removed by centrifugation and collected in a purified form. The properties of the purified cytochrome b_6 will be discussed in a later communication. The yield of structural protein obtained is approximately 120 mg from 500 mg of acetone powder of the chloroplast fragments.

Properties of the Protein Preparation

As is required of a protein comprising a membrane system, the structural protein is insoluble under physiological conditions. It is not solubilized by sodium dodecyl sulfate concentrations as high as equal weights of the detergent and protein nor by high concentrations of urea. It can be readily solubilized, however, by a combination

of low concentration of sodium dodecylsulfate (0.1 wt. of protein) and 0.5 M urea at pH 10 in 0.01 M phosphate buffer. Physical measurements on the protein were all carried out in this solution. The protein is completely free of flavin and heme, and there is no indication of the presence of either chlorophyl or carotene pigments or lipid.

Sedimentation and Molecular Weights

Ultracentrifugation experiments were carried out in a Spinco Model E ultracentrifuge. Sedimentation velocity measurements indicated the presence of a single major sedimenting boundary with a coefficient of 2.2S, as shown in the top of Fig. 1. Molecular weights were obtained

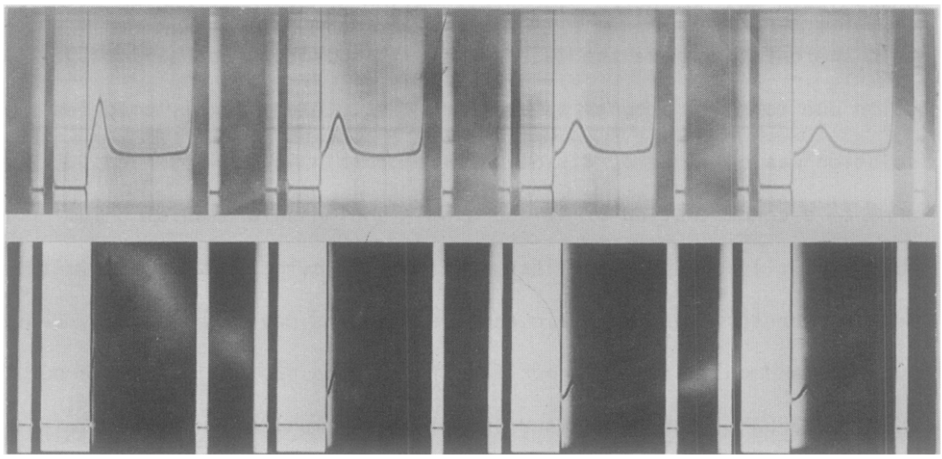

Fig. 1. Sedimentation of chloroplast structural protein (top) and the structural protein-chlorophyl complex (bottom). The centrifuge was run at 59,780 rpm and the temperature controlled at 20°C. The time interval between photographs is 16 min (upper) and 8 min (lower). Note the movement of the chlorophyl color with the sedimenting protein.

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by the short column equilibrium procedure of Van Holde and Baldwin (1958). The weight average molecular weight was found to be 23,000 grams/mole. The partial specific volume of the protein was assumed to be 0.76 cm^3/

gram, which is in the range determined for mitochondrial structural protein. The detergent was assumed to be completely bound by the protein under these conditions and the contribution of detergent to the molecular weight of the protein-detergent complex was subtracted out using a value of 0.87 cm^3/gram for the $\bar{v}$ of the detergent. The observed value of the molecular weight is in the range normally found for structural proteins from other sources.

Interaction of Structural Protein with Other Components of the Chloroplast

As part of the functional description of a structural protein depends upon the ability of that protein to form specific complexes with the functionally active components of the system, a preliminary investigation of these interactions has been made. Binding of ATP and phospholipid by the acetone extracted green particulate fraction of chloroplasts has been demonstrated (Richardson et al., 1963). To demonstrate ATP binding by the soluble structural protein, the sedimentation of the protein-ATP complex was studied by dialysis equilibrium and by using a UV absorption optics system in the ultracentrifuge. In this system, the ATP and structural protein were mixed and dialyzed against 1000 fold excess of tris buffer, the concentrations determined by UV spectra, and the solution centrifuged. The resultant ATP-protein complex moved with a 2.2S sedimentation coefficient.

Complex formation with larger components was followed by both centrifugation and by chromatography on sephadex columns. The binding was studied by adding each of the components to the solubilized structural protein solution and then dializing the mixture overnight against pH 8.5 tris buffer. Sedimentation velocity or molecular weight measurements were then used to demonstrate complex formation and the results correlated with elution volume of the components and complexes off Sephadex G200. A summary of the binding studies is shown in Table I. Cytochrome C has been demonstrated to have little or no affinity for

TABLE I

Binding Experiments with Structural Protein

	Amount bound	Method
ATP	0.95 moles ATP/mole protein	centrifuge, dialysis equilibrium
Phospholipid*	3.2 μg P/mg protein	centrifuge, Sephadex-Pi analysis
Bovine serum albumin	0	centrifuge, Sephadex
Cytochrome C	0	centrifuge
Chlorophyl	0.93 mole/mole structural protein	OD of protein-chloropl complex
Myoglobin**	1 mole/mole structural protein	centrifuge, molecular Sephadex

* Phospholipid micells prepared from sample of soybean azolectin by the method of Fleischer (1962). The value obtained for the binding of phospholipid was based on determination of protein and lipid phosphate associated after elution of Sephadex G200.

** Sperm whale myoglobin has been shown to complex stoichiometrically with mitochondrial structural protein and was therefore tested in this study.

mitochondrial structural protein under these conditions and behaved similarly with the chloroplast preparations. Bovine serum albumin was also unreactive. Myoglobin, which is tightly bound to the mitochondrial protein is also readily complexed in the chloroplast system. The phospholipid mixture, azolectin, and chlorophyl are both readily bound. The latter binding was studied by the method of Wolken (1940) and also by dialysis and centrifugation in the same manner as with ATP binding. The sedimentation of the chlorophyl-structural protein complex is shown in the lower portion of Fig. 1.

The properties of the chloroplast protein are very analogous to those of the structural protein from mitochondria. Based on the solubility and chemical properties of this enzyme it is also suggested that it may be the major protein subunit of the protein-chlorophyl complex isolated by Smith (1940) and Smith and Pickels (1940).

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