On the Structural Nature of Fraction-I Protein of Rice Leaves[1/]

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Remarkable progress has been made in recent years in clarifying the biochemical nature of the functional units in chloroplasts. There is now ample evidence that the major soluble protein of chloroplasts, designated as Fraction-I protein (Wildman and Bonner, 1947), is an enzymic entity of the CO_2 fixation reaction in the stroma. Several investigators have demonstrated the presence of ribulose-1,5-diphosphate carboxylase in Fraction-I protein (Lyttleton and Ts'o, 1958; Park and Pon, 1961). Our own experiments using rice leaf tissues provided evidence that two additional enzymes (ribose-5-phosphate isomerase and ribulose-5-phosphate kinase) are also present (Mendiola and Akazawa, 1964). Similar results were obtained by Van Noort and Wildman (1964) using tobacco leaf Fraction-I protein. The data obtained by some workers have established a relatively large molecular weight (16-18s) for the Fraction-I protein of various origins (Lyttleton and Ts'o, 1958; Kupke, 1962), and evidence is available for its granular structure in chloroplasts from electronmicroscopic observations (Park and Pon, 1961; Gross et al., 1964). It is thus of great value to elucidate the physical and chemical nature of the Fraction-I protein, which apparently functions as an enzyme complex in the photosynthetic CO_2 assimilation reactions. On the other hand, the structure of the grana in chloroplasts, which is the site of the light-phase reaction of photosynthesis, has been studied

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intensively. Park and Pon (1961) have isolated a purified preparation of the chlorophyll-bearing particulate lamellae and named it quantasomes, and Menke (1962) has reported a remarkably similar amino acid composition of the structural protein of lamellae derived from several plant species. The latter workers further demonstrated the presence of carbohydrates as a structural constituent of the protein. It is worthwhile to explore further the structural similarities which might exist between the Fraction-I protein and the lamellar structural protein of grana quantasomes and to elucidate their possible biogeneric interrelationship. Thornber et al. (1964) have undertaken the comparative studies of the two proteins with regard to amino acid composition and sedimentation analyses. In this report, experimental evidence is presented showing that Fraction-I protein as isolated from rice leaves is a glycoprotein.

Fraction-I protein was prepared from rice leaves (Taichung Native 1 variety) by essentially the same method as reported by Mendiola and Akazawa (1964). The homogeneity of the protein was ascertained by both the migration on starch-gel and acrylamide-gel electrophoresis and by the monodisperse sedimentation boundary in an analytical ultracentrifuge.

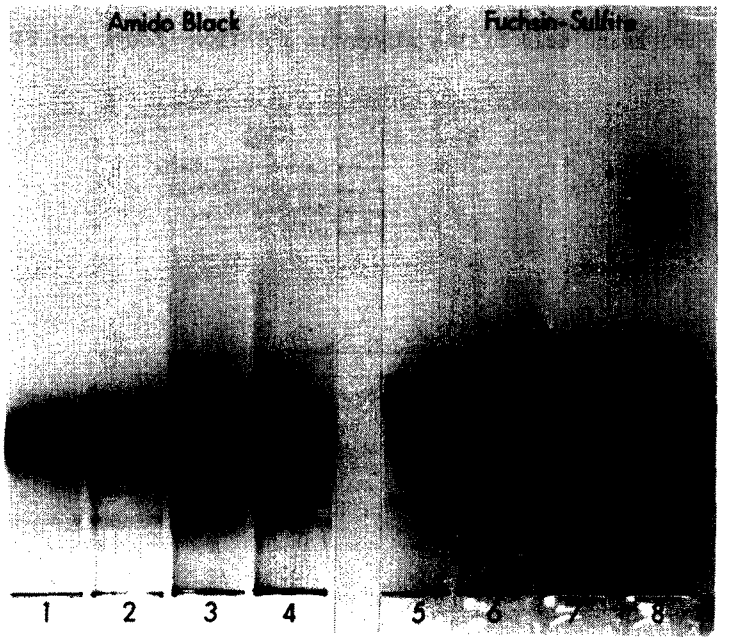

Fig. 1 Acrylamide-gel electrophoregram of the Fraction-I protein as stained by Amido Black 10B (left half) and by the fuchsin-sulfite reaction (right half).

Protein samples purified by the gel filtration on Sephadex G-75 (3,4 and 7,8) and G-200 (1,2 and 5,6) columns were applied to the gel electrophoresis after the method of Raymond et al. (1962).

The first proof for the glycoprotein nature of the protein was given by the fuchsin-sulfite staining of the gel slice after the method of Hotchkiss (1948) (Fig. 1). The location of the bright purple band coincided precisely with that of the blue band of the Fraction-I protein stained by Amido Black 10B.

The characterization of the carbohydrate constituents was undertaken by means of the digestion of the protein by Streptomyces griseus protease (Pronase) as often employed in the structural studies of glycoproteins. About 15 mg of Fraction-I protein was incubated with 0.5 mg of Pronase-P (Kaken) at 40°C for 48 hours in a stoppered test tube containing 1.0 ml absolute ethanol. Nearly 95% of the protein was hydrolyzed during this incubation period as determined by the ninhydrin reaction. The whole digest was concentrated in a Kollodium Membranfilter (Göttingen) in vacuo at 2°C; the residue within the membrane was transferred to a cellophane tubing and dialyzed against distilled water for 3 hours. The dialyzed material was applied to a column of Sephadex G-25 (1.5 x 30 cm), and eluted with 0.01 M NaCl. Three milliliter fractions of the effluent were collected and aliquots were assayed for (a) hexose by the cysteine-H_2SO_4 reaction (Dische, 1955), (b) pentose by the orcinol-HCl reaction (Mejbaum, 1939), (c) amino acid by the ninhydrin reaction (Moore and Stein, 1948),

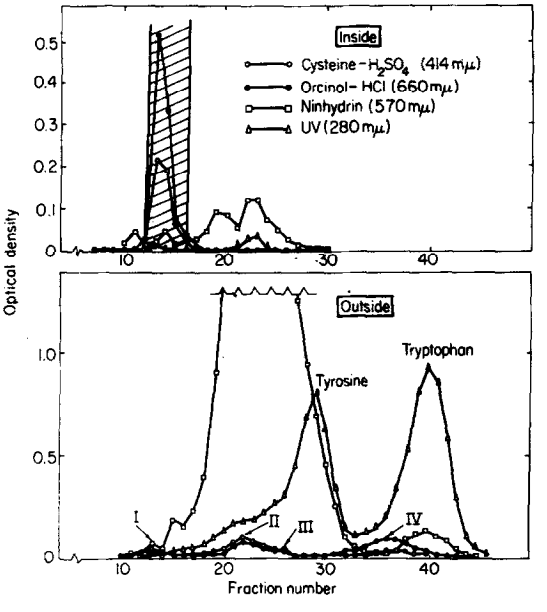

Fig. 2 Gel-filtration of the Pronase-hydrolyzate of the Fraction-I protein on Sephadex G-25.

Experimental details for the analyses of column effluents are given in text.

and (d) ultraviolet absorption at 280 mμ. The elution pattern portrayed in Fig. 2 shows that a major fraction (shaded) of the non-dialyzable digest was positive to both cysteine-H_2SO_4 and orcinol-HCl reactions but nearly negative to the ninhydrin reaction and exhibited no absorption at 280 mμ. An aliquot of the concentrated major fraction was subjected to paper electrophoresis using two different solvent systems [0.1 M borate buffer (pH 8.6); LKB veronal buffer (pH 8.6)]. A single band moving slightly to the cathode in 6 hours (200 volt/ 4 cm) stained faintly purple by the Schiff's reagent. Amino acid composition of the pooled non-dialyzable major fraction was markedly different from that of the original Fraction-I protein; of particular interest being the increased proportion of serine, threonine and alanine. These findings clearly indicated that the major fraction was a glycopeptide. The remainder of the concentrated major fraction was hydrolyzed with 2N HCl at 100°C for 2 hours. After treating the hydrolyzate with Amberlite MB-3 resin, the sugar constituents were examined by paper chromatography using three different solvent systems; (a) isopropanol: n-butanol: H_2O (140: 20: 40), (b) ethylacetate: pyridine: H_2O (120: 50: 40); and (c) n-butanol: pyridine: H_2O (80: 80: 40). The predominant sugars identified were galactose and arabinose; glucose, rhamnose and ribose (or xylose) were found to be present as minor components. A rigorous proof for the presence of galactose was given by (a) spectrophotometric determination of the chromogen produced by the cysteine-H_2SO_4 reaction of the hydrolyzate (Dische, 1955), and (b) the use of specific galactose oxidase (Worthington). The dialyzable outside fraction from the membrane filtration was treated in a similar way as that for the non-dialyzable fraction; i.e., Sephadex G-25 gel filtration, and the identification of sugars by spectrophotometry and paper chromatography. The elution pattern of the latter fraction is shown in the lower part of Fig. 2. Large quantities of peptides and both tyrosine and tryptophan were liberated, but not much sugar-bearing fractions were detectible. Pooled samples of the individual fractions, marked in the figure as I, II, III and IV, were hydrolyzed with 2 N HCl, and assayed for sugars as before. The first small fraction (I) proved to be identical with the glycopeptide retained in the membrane filtration. Glucose and ribose were the predominant sugar components in the rest of three fractions (II, III and IV), although galactose and rhamnose were detectible also. Based on the spectrophotometric analyses, the content of total sugars (hexose and pentose) in each of the inside and outside fractions was found to constitute about 4% and 1% respectively.

There has not been much experimental evidence available in

literatures concerning the biological function of glycoprotein in plant cells. In addition to the well-established studies on the glycoprotein nature of the peroxidase (Morita and Kameda, 1958; Klapper and Hackett, 1965), a recent note of Lis et al. (1964) dealing with the isolation of a mannose-containing glycopeptide from the soy-bean hemagglutinin calls our attention. However, in none of these studies the role of carbohydrates in the protein molecule has been clearly established. From the present investigation, it is highly conceivable that the glycopeptide molecule is the intrinsic structural component of the Fraction-I protein, and may have important role in orienting the molecules of the functional enzyme for the photosynthetic CO_2 assimilation in chloroplasts. Experiments for further elucidating the more complete picture of the structure of the Fraction-I protein is now in progress. It also is interesting to note that both galactose and arabinose have been reported to be the major sugar components of the lamellar structural protein of grana (Menke, 1963), and this similarity will lead us into more thorough comparative studies of the two different types of protein in chloroplasts.

Acknowledgement

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