BBA 43185

Isolation and properties of two digitonin-soluble pigment–protein complexes from spinach

Previously we have reported[1] that by isodensity centrifugation of the 80000 × *g* supernatant of digitonin-treated chloroplasts three coloured bands are obtained. From the top downwards there appeared a yellowish-green band with an absorption maximum at 671 mμ, a light pink band containing the cytochromes *f* and b_6, and a blue-green band with an absorption maximum at 678 mμ. The blue-green band was found to be able to reduce $NADP^+$ in the light in the presence of ascorbate–dichlorophenolindophenol (DCIP), ferredoxin and ferredoxin–$NADP^+$ reductase, provided that plastocyanin was added[2]. The ratios chlorophyll *a*:chlorophyll *b* (7) and chlorophyll:P700 (200) supported the view that this fraction represents a purified pigment system I preparation. In this paper the purification and some of the properties of the two green fractions are described.

The further resolution and analysis of the blue-green fraction was hampered by its sensitivity to various agents and its insolubility in water. Any agent that shifts the red absorption maximum to shorter wavelengths was found to effect an inactivation of the preparation. A significant purification without loss of $NADP^+$-photoreducing activity was accomplished by repeated isodensity centrifugation, and chromatography of the particles on a DEAE-cellulose column in the presence of 0.2% digitonin. After gradient elution (with NaCl) from the column, the ratio of the absorbance at 679 mμ to that at 270 mμ, which may be representative of the purity of the preparation, increased to 2.0, and the ratio chlorophyll:P700 decreased to 140. The fraction was found to be weakly fluorescent, but at 77 °K a strong emission band was observed at about 730 mμ. A low fluorescence yield and an enrichment in P700 have also been found recently in "light" particles prepared by differential centrifugation of detergent-treated chloroplast preparations[3–7].

Digitonin was removed from the particles by precipitation of the chlorophyll–protein complex with 40% ethanol, which leaves the digitonin in solution. The precipitate could be solubilized to an active preparation again by sonication in the presence of digitonin. Chemical analysis of the washed sediment showed it to contain about 75% protein and 25% lipid. The lipid portion was found to be made up of 15% chlorophyll, 1.6% carotenoids, and 8.4% non-pigment lipids, including 0.5% phospholipid. The pigment composition is summarized in Table I. For comparison the pigment composition of chloroplast lamellae[8,9] and of light particles obtained from Triton[3]-, digitonin[11]-, and sodium dodecyl sulfate[10]-solubilized chloroplasts is given (*cf.* also refs. 12, 13). As compared to chloroplast lamellae the purified blue-green fraction has been enriched in chlorophyll *a* and β-carotene. The content of lutein has been decreased, whereas violaxanthin and neoxanthin are completely absent. The low protein content may indicate that the preparation has been highly purified in comparison with other chloroplast fragments. The pigment composition corresponds most closely to that of one of the two pigment proteins (Component I) obtained by electrophoresis from sodium dodecyl sulfate-solubilized spinach chloroplasts[10]. However, the lower sedimentation constant of the sodium dodecyl sulfate-solubilized

Abbreviation: DCIP, dichlorophenolindophenol.

TABLE I

PIGMENT COMPOSITION OF THE BLUE-GREEN DIGITONIN PARTICLES AS COMPARED TO THAT OF CHLOROPLAST LAMELLAE[8,9], LIGHT DETERGENT PARTICLES[3,11], AND COMPONENT I OF SODIUM DODECYL SULFATE-SOLUBILIZED CHLOROPLASTS[10]

Pigments were extracted with 80% acetone and separated by thin-layer chromatography, using the method of HAGER AND BERTENRATH[14]. The separated compounds were eluted with appropriate solvents, and the concentration of each pigment was determined spectrophotometrically. The absorbance coefficients reported by GOODWIN[15] and MACKINNEY[16] were used for carotenoids and chlorophylls, respectively. The contents of chlorophylls *a* and *b* in the fractions were also determined by the method of OGAWA AND SHIBATA[17]. Lipids were extracted into chloroform–methanol by the method of FOLCH, LEES AND STANLEY[18]. The washed extract was dried and weighed. The lipid was dissolved in chloroform–methanol and aliquots were used for the determination of total lipid[19] and phospholipid[20]. The protein value corresponds with the dry weight of the lipid-free residue. By multiplying the nitrogen content of this material, as determined by a micro-Kjeldahl procedure[21], by the factor 6.3, a 10% lower value was found for the protein content. Data are presented in mμmoles and are normalized to 100 mμmoles total chlorophyll. Protein is given in mg/100 mμmoles total chlorophyll.

Pigment	*Chloroplast lamellae*	*Blue-green digitonin particles*	*Light Triton particles*	*Light digitonin particles*	*Component I of sodium dodecyl sulfate-solubilized chloroplasts*
Chlorophyll *a*	70	88	85	85	88
Chlorophyll *b*	30	12	15	15	12
Chlorophyll *a*:chlorophyll *b*	2.4	7.3	5.7	5.7	7.3
β-Carotene	6.3	12.5	16	14	14
Lutein	9.6	4.5	6	6	5
Violaxanthin	2.8	0	2	5	0
Neoxanthin	2.5	0	1	2	0
Protein	0.4	0.45	4.7	1.2	1.3

preparation and the absence of any $NADP^+$-photoreducing activity suggest that sodium dodecyl sulfate causes a further splitting of the particles into smaller but inactive subunits. It is remarkable that the percentage of non-pigment lipids is significantly higher in whole chloroplasts and quantasome preparations[8]. The relatively low concentration of these compounds in the blue-green particles raises the question whether digitonin can substitute in part for lipids in providing the photochemical apparatus with the appropriate medium and structure.

The yellowish-green band from the isodensity centrifugation, which does not have any $NADP^+$-photoreducing activity, exhibits a relatively strong fluorescence at about 680 mμ, at room temperature as well as at 77 °K. P700 was not found to be present in this fraction. The band could be separated into 2 components by either chromatography on a DEAE-cellulose column, or isodensity centrifugation using a higher ratio of digitonin:chlorophyll (10:1, w/w). The absorption and fluorescence maximum of the light fraction, which is not adsorbed on DEAE-cellulose, are located at 670.5 and 678.5 mμ, respectively. The pigment composition of this fraction (A) is given in Table II. The fraction has been enriched in chlorophyll *a* and xanthophylls. These results suggest that this component contains solubilized pigments which have been split from the native pigment–proteins by the digitonin.

The pigment composition of the heavier fraction, which is readily adsorbed on DEAE-cellulose, is also given in Table II. It is seen that this component (B) contains

TABLE II

PIGMENT COMPOSITION OF THE YELLOWISH-GREEN DIGITONIN PARTICLES AS COMPARED TO THAT OF HEAVY TRITON[3] AND DIGITONIN[11] PARTICLES AND COMPONENT II OF SODIUM DODECYL SULFATE-SOLUBILIZED CHLOROPLASTS[10]

A, light fraction, which is not adsorbed on DEAE-cellulose; B, heavy fraction, which is readily adsorbed on DEAE-cellulose. The same methods were used as described for Table I.

Pigment	*A*	*B*	*Heavy Triton particles*	*Heavy digitonin particles*	*Component II of sodium dodecyl sulfate-solubilized chloroplasts*
Chlorophyll *a*	86	64	67	67	64
Chlorophyll *b*	14	36	33	33	36
Chlorophyll *a*:chlorophyll *b*	6.1	1.8	2.0	2.0	1.8
β-Carotene	6	1	6	7	5
Lutein	58	14	16	15	16
Lutein-epoxide	8	—	—	—	—
Violaxanthin	60	8	3	6	8
Neoxanthin	11	3	2	5	5

a relatively high concentration of chlorophyll *b* and xanthophylls, but only a little β-carotene. The pigment composition resembles more or less that obtained by KE, SELISKAR AND BREEZE[11] and VERNON, SHAW AND KE[3] for their "heavy" particles, and by OGAWA, OBATA AND SHIBATA[10] for pigment protein II prepared from sodium dodecyl sulfate-solubilized chloroplasts. Both the absorption and the fluorescence band of this fraction are shifted about 3 mμ towards longer wavelengths as compared to the fraction containing solubilized pigments. These data suggest that these particles could be derived from pigment system II. The further properties of this fraction are under current investigation.

The availability of the blue-green fragments allows a close comparison to be made with the photoreactions catalyzed by solubilized chlorophyll *a* itself. The most conspicuous, perhaps essential differences between these two preparations are the presence of P700 and protein in the digitonin particles and the shift of the red absorption maximum of chlorophyll *a* towards 679 mμ, which presumably may be due to a characteristic pigment–(lipo)protein association. Characterization of this protein and study of its interaction with chlorophyll may provide information on the mechanism of the photosynthetic quantum conversion process.

The technical assistance of Miss M. VAN LEEUWEN is gratefully acknowledged.

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Received October 18th, 1967

Biochim. Biophys. Acta, 153 (1968) 497–500

BBA 43184

Continuous recording of pH and pCa during calcium binding by muscle microsomes

The methods which have been routinely used for studying calcium binding by muscle microsomes involve separating the particles from their suspension medium, either by centrifugation (*e.g.* ref. 1) or filtration (*e.g.* ref. 2). These procedures impose limits on the time resolution obtainable. In addition, the properties and contents of the microsomal membranes are subject to alteration by forces acting on the particles during filtration or centrifugation. The uptake of calcium by isolated sarcotubular vesicles has been detected in measurements of turbidity changes, which can be monitored continuously[3]. OHNISHI AND EBASHI[4] have utilized an indicator technique to obtain continuous measurements during calcium binding. HERNIO AND SARIS[5] have described experiments in which pH was recorded continuously. The changes in pH were found to be correlated both with H^+ production resulting from ATP hydrolysis and with ammonia evolution accompanying adenylate deamination[5]. The approach described in the present paper permits continuous and sensitive measurements of net calcium movements and associated changes in energy utilization.

In our experiments, pH and pCa were simultaneously recorded. The A. H. Thomas No. 4858-L15 combination pH electrode and the Orion[6] calcium electrode were used. The electrodes were connected, with the pH electrode reference in common, to Radiometer model 22 pH meters. The outputs of the meters were further amplified and then recorded by means of a dual channel recorder.

In the experiment shown in Fig. 1A, the endogenous creatine kinase of the microsomal preparation, together with added creatine phosphate, served as an ATP-regenerating system. The use of this regenerating system simplifies the interpretation of H^+ changes. When the added adenine nucleotide is maintained primarily in the form of ATP, ammonia production due to adenylate deamination is minimized and