PROPERTIES OF CHLOROPLAST DISPERSIONS IN THE PRESENCE OF DETERGENTS

by

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Clear aqueous chloroplast solutions were first prepared by EMIL SMITH with the aid of detergents[1,2]. When 0.25% sodium dodecyl sulfate was employed as the dispersing agent, the chlorophylls and carotenoids remained bound to the lipoprotein, which was split into small units (apparent mol. wt. 20–25,000[3]). The molecular size of the dispersed lipoprotein was twelve times greater in dilute digitonin, desoxycholate and bile salt solutions, but these detergents removed the pigments from the lipoprotein[3]. More recently TAKASHIMA[4] prepared clear chloroplast solutions in 50% aqueous α-picoline, from which chlorophyll-lipoprotein and carotenoid crystallized separately. The molecular size of the chlorophyll-lipoprotein in aqueous α-picoline[4] was about the same as in chloroplast solutions prepared with sodium dodecyl sulfate[3].

This paper reports studies of several properties of chloroplast and grana dispersions in the presence of synthetic and natural detergents.

MATERIALS AND METHODS

Nostoc muscorum provided by Dr. G. C. GERLOFF, University of Wisconsin, was cultured in Chu No. 10 medium[5] plus Eyster's micronutrients (0.01 p.p.m. cobalt, 0.1 p.p.m. boron and manganese, 0.4 p.p.m. molybdenum and 1.2 p.p.m. iron). *Chlorella pyrenoidosa* provided by Dr. R. EMERSON, University of Illinois, was cultured in Warburg's medium[6]. *Oscillatoria* sp. and *Chondrus crispus* were collected from their natural habitats. Chromoproteins were rapidly extracted from the blue-green algae by adding a few drops of toluol and of *n*-octanol to washed algal suspensions, which were subjected to five-minute blendor treatments in a cold room (toluol released the chromoproteins, and *n*-octanol prevented foaming). The crude extract following centrifugation and washing contained the phycobilins and most of the chlorophyll-lipoprotein. Crystalline C-phycocyanin was obtained by repeated fractional precipitation with 17–22% $(NH_4)_2SO_4$. Chloroplast fragments were isolated from market spinach at 0° C, washed twice with distilled water or $M/20$ phosphate, pH 7.0, and recovered in a refrigerated Servall centrifuge.

The following detergents were employed in this study:

Anionic — sodium dodecyl sulfate (SDS)
Cationic — zephiran chloride (high molecular alkyl benzyl ammonium chlorides)
Non-ionic — "Tween-20" (polyoxyethylene sorbitan monolaurate)
"Triton X-100" (alkylated aryl polyetheralcohol)
Steroidal — digitonin, bile salts
Triterpenoid — purified saponin from soapbark.
purified saponin from soybean and alfalfa tops[7].

Dodecyl sulfate contents of chloroplast suspensions (free and total) were determined by the rosaniline method of KARUSH AND SONENBERG[8], which was recalibrated with a Beckman DU spectrophotometer. To measure free SDS, the chloroplast substance was precipitated with half-saturated ammonium sulfate, and analyses were made on greatly diluted aliquots of the clear supernate. Total SDS was measured in greatly diluted suspensions in which the chlorophyll content amounted to less than 0.5 μg per extraction tube.

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Chloroplast dispersion by dodecyl sulfate was assessed quantitatively by 30 min centrifugation at 25,000 *g*, and filtration with Schleicher and Schuell "Ultra Filters" (Type 1 dense, pore size 0.1–0.3 micron) and Seitz E.K. filters (pore size *ca.* 0.1 micron). Chlorophyll contents of the original suspension, supernate and filtrates were measured spectrophotometrically[9]. In the absence of detergents, all chlorophyll in the greenish supernate obtained by centrifuging crude spinach chloroplast suspensions at 25,000 *g* was retained on the Seitz filters, and over 95% was retained on the ultra filters. Pure C-phycocyanin in water passed the ultra filter but was retained on the Seitz filter, presumably by adsorption.

Bonding of the pigments in aqueous chloroplast suspensions was assessed by liquid-liquid extraction with benzene[10]. The liquid phases were mixed thoroughly in 10 ml tubes with a plunger having 1 mm clearance (100 strokes), followed by centrifugation and spectrophotometric analyses of the benzene extract.

Electrophoretic migration was measured in the Spinco Model R Paper Electrophoresis apparatus under conditions outlined by BLOCK, DURRUM AND ZWEIG[11]. Chromatographic separations were effected by the column method described by HAXO, O'HEOCHA AND NORRIS[12] with the following modifications: the chromoproteins were adsorbed on a minimum of gel particles which was placed on the prepared column as a slurry. The pigmented gel particles settled to a shallow "starting zone" from which the chromoproteins subsequently migrated as sharply defined bands. The gel column was previously extracted as well as developed with dilute detergent solutions in applications to chloroplastin. Tricalcium phosphate and c γ-alumina were prepared as directed by SWINGLE AND TISELIUS[13]. Silica gel (Mallinckrodt) and other detergents were obtained commercially.

Hill reaction measurements on washed chloroplast fragments were made manometrically in orange-red light at 10° C, using quinone as oxidant in *M*/20 phosphate, pH 7.0, which contained *M*/100 KCl[14]. Photosynthesis of dilute *Chlorella* suspensions was measured in saturating white light at 25° C in *M*/10 No. 9 bicarbonate buffer.

RESULTS

Adsorption of dodecyl sulfate by chloroplast substance

Fig. 1. provides comparative data on dodecyl sulfate sorption by spinach chloroplast fragments and by living *Chlorella* cells having the same content of chlorophyll and presumably of chloroplast substance. The pH was maintained at 8.9 with *M*/10 borate as in the studies of chloroplast dispersion, and the total SDS concentration was varied from $5 \cdot 10^{-3}$ to $4.6 \cdot 10^{-2}$ *M*. In the presence of small amounts of SDS, there was vastly greater sorption by the chloroplast fragments than by the *Chlorella* cells. At the highest of the tested SDS concentrations, sorption of SDS still remained eight times greater in the chloroplast suspensions. The markedly different inhibiting effects of synthetic detergents on the Hill reaction of isolated chloroplasts and on *Chlorella* photosynthesis (Table III) correspond closely with the sorption data of Fig. 1.

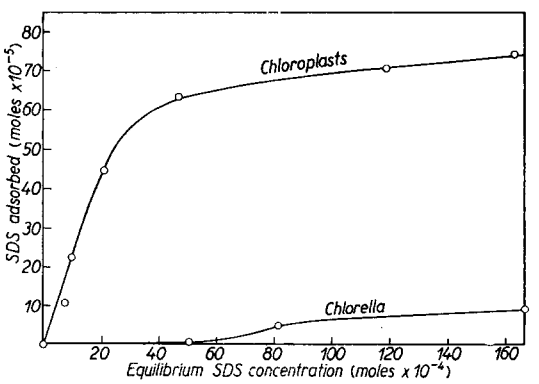

Fig. 1. Sorption of dodecyl sulfate by isolated spinach chloroplasts and by living *Chlorella* cells (0.7 mg chlorophyll, 0.125–1.15 mmoles dodecyl sulfate in 25 ml 0.1 *M* borate, pH 8.9).

Chloroplast dispersion by SDS

Fig. 2 illustrates the effect of SDS concentration on chloroplast dispersion. Sedimentation at 25,000 *g* was reduced appreciably by 10^{-3} *M* SDS, and was reduced

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90% by 10^{-2} *M* SDS. Most of the supernatant chlorophyll was still in particles too large to pass the 0.1–0.3 micron ultra filter at the latter SDS concentration. A similar concentration was required to eliminate sedimentation at 25,000 *g* and to effect maximum passage of the chloroplast substance through the ultra and Seitz filters. A distinct threshold was apparent in the SDS concentration required to produce particles which pass either of these filters. The range of SDS concentrations over which chloroplast dispersion rose steeply, as assessed by the ultra filters (Fig. 2), coincided with the critical region for SDS micelle formation, interfacial tension lowering and detergency established by PRESTON[15]. The chlorophyll content of the ultra filtrates attained a maximum at 0.02 *M* SDS, and decreased significantly with further increases in SDS concentration. Even with 0.02 *M* SDS, there was 25% retention of the chloroplast substance on the ultra filter. The Seitz filtrates attained maximum chlorophyll contents in the range 0.02–0.045 *M* SDS, but there was 35% retention of chloroplast substance on the Seitz filters under these conditions even after thorough washing. Chloroplast dispersion by SDS was relatively insensitive to temperature in the range 0–25° C.

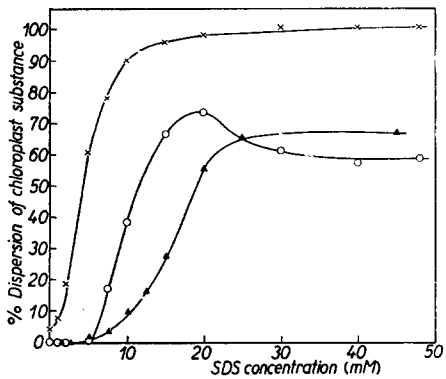

Fig. 2. Chloroplast dispersion by dodecyl sulfate in *M*/10 borate, pH 8.9. ×——× supernate at 25,000 *g*; O——O ultra filtrate; △——△ Seitz filtrate.

Benzene extraction of aqueous chloroplast dispersions

When washed chloroplast fragments suspended in water or dilute buffers are subjected to cold liquid-liquid extraction with benzene, the bulk of the chloroplast pigments remain in the aqueous phase. Their solubility in benzene is increased only to a small extent by flocculating the chloroplast protein with heat or half-saturated ammonium sulfate, but is strongly increased by *ca.* 10% sodium chloride.

The effects of detergents upon the benzene-solubility of chlorophyll in aqueous chloroplast suspensions vary with the type of detergent and its concentration, as well as with the pH of the aqueous phase. The chlorophyll is completely extractable with benzene in the presence of 0.05%–1% Zephiran chloride at pH 7.0–9.0. Unlike the remaining classes, cationic detergents cause flocculation of the chloroplast substance at the concentrations which render the pigments completely extractable with benzene. In the presence of increasing amounts of the anionic detergent SDS at pH 7.0 and 9.0, the benzene-solubility of chlorophyll rises to about 20% at 10^{-2} *M* SDS, beyond which

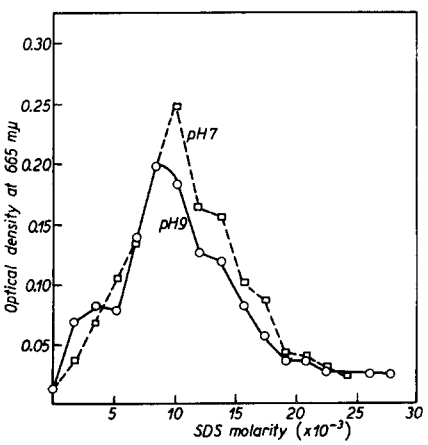

Fig. 3. Benzene-extractibility of chlorophyll in aqueous chloroplast dispersions containing dodecyl sulfate.

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it falls to the control level (Fig. 3). The benzene-extractibility of the chlorophyll is significantly lower in the presence of 0.05 *M* SDS than in the absence of detergent, which indicates affinity of the detergent for the pigment molecules. It is noteworthy that increased solubility of chlorophyll in benzene becomes apparent at lower SDS concentrations than are required for maximum dispersion of the chloroplast substance.

Further experiments established that the chlorophylls and carotenoids in aqueous chloroplast suspensions are both extracted more readily with benzene in the presence of 10^{-2} *M* SDS. Benzene extracts were evaporated under nitrogen, dissolved in petroleum ether and chromatographed on MgO-Celite with petroleum ether-acetone[16]. The resulting chromatograms exhibited α-carotene, β-carotene and xanthophyll bands in addition to chlorophyll, as with total pigment extracts of chloroplasts. The increase in benzene-extractibility was assessed spectrophotometrically (Table I). Although 10^{-2} *M* SDS caused similar increases in the extraction of chlorophyll and carotenoid with benzene, it had only a slight effect upon their solubility in petroleum ether (Table I).

TABLE I

RELATIVE SOLUBILITY IN ORGANIC SOLVENTS OF CHLOROPLAST PIGMENTS IN AQUEOUS CHLOROPLAST MEDIA

Chloroplast medium	*Organic solvent*	*Relative solubility of pigments in organic solvent*	
		Chlorophyll (E at 665 mμ)	*Carotenoid (E at 430 mμ)*
Water	Benzene	0.009	0.029
0.01 *M* SDS in water	Benzene	0.089	0.170
Water	Petroleum ether	0.004	0.022
0.01 *M* SDS in water	Petroleum ether	0.008	0.027

The effects of the non-ionic detergent "Tween 20" upon the benzene-solubility of chlorophyll in aqueous chloroplast dispersions resembled those of SDS at neutrality (Fig. 4). In alkaline media, the benzene-solubility of chlorophyll remained negligible over a wide range of "Tween 20" concentrations. Increasing benzene-solubility of chlorophyll was observed with increasing concentrations of bile salts, which remained high at high concentrations of this natural detergent (Fig. 5). Although digitonin removes the chloroplast pigments from the lipoprotein in aqueous dispersions[3], the solubility of the chlorophyll remains low in the presence of this natural detergent. Digitonin apparently has sufficient affinity for the freed pigments to prevent their removal from the aqueous phase by benzene, in marked contrast to cationic detergents such as Zephiran chloride. Tests with triterpenoid saponins gave results similar to those obtained with digitonin.

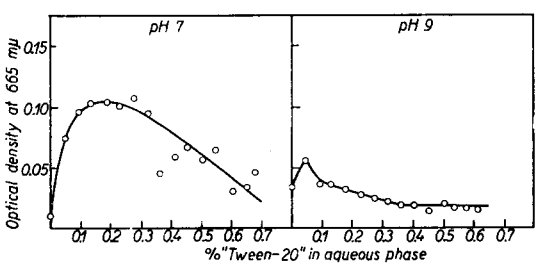

Fig. 4. Benzene-extractibility of chlorophyll in aqueous chloroplast dispersions containing "Tween 20".

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Absorption spectra of chloroplast-detergent solutions

The comparative effects of SDS and "Tween 20" on the visible absorption spectrum of chloroplast suspensions are of particular interest since SDS leaves the pigments attached whereas "Tween 20" frees them so that they may be separated chromatographically in a protein-free state. As noted above, the effects of these detergents on the benzene-extractibility of chlorophyll in neutral aqueous media are quite similar. They also induce identical shifts in the red absorption band; upon dispersing spinach chloroplast substance in 0.25% SDS, 1% "Tween 20" or 50% α-picoline, the red absorption peak is shifted in each case from 677 to 670 mμ.

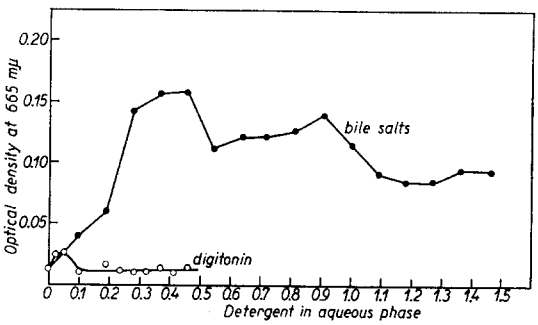

Fig. 5. Benzene-extractibility of chlorophyll in aqueous chloroplast dispersions containing digitonin and bile salts.

Column chromatography

When phycoerythrins and phycocyanins are separated on tricalcium phosphate or aluminum hydroxide columns, the chloroplastin of aqueous algal extracts is strongly adsorbed at the top of the column[12]. Chloroplastin from *Nostoc* migrated slowly as a single sharply defined band when dispersed with 0.25% SDS in *M*/10 borate, pH 8.9, and chromatographed on gel columns which had been previously extracted with the same solvent. Spinach chloroplast substance also migrated under these conditions as a single narrow band, which contained the carotenoids and chlorophyll as well as the chloroplast lipoprotein. Chloroplastin from *Nostoc* and from spinach chloroplasts upon admixture migrated slowly as a single band. The latter result was also obtained during paper electrophoresis, in which the solubilized chloroplastin migrated more rapidly than either phycoerythrin or phycocyanin (*vide infra*).

The chromatographic behaviour of chloroplastin-SDS was tested on several adsorbents, using different aqueous developing solutions. (Adsorbents—Celite, paper, starch, tricalcium phosphate, aluminum hydroxide, silica gel; developing solvents—SDS up to 0.4%, $(NH_4)_2SO_4$ up to 10%, NaCl up to 0.1 *M*, *M*/10 phosphate and borate buffers). The chloroplastin band was more diffuse on Celite, paper and starch than on the remaining adsorbents, and its migration rate was always much slower than that of phycocyanin or phycoerythrin under the same conditions. No evidence of separable components in the chloroplastin-SDS complex was obtained with these adsorbents and developing solutions.

Since SMITH AND PICKELS[3] had demonstrated that the chloroplast pigments are removed from the lipoprotein when digitonin and bile salts are used as dispersing agents, use of these and other detergents offered a means of separating the pigments in a protein-free condition on aqueous gel columns. In our exploratory tests, 1–5% "Tween 20" gave the best aqueous separations of protein-free chlorophyll and carotenoid fractions on tricalcium phosphate and silica gel columns. The eluted chlorophyll and carotenoid fractions obtained with "Tween 20" were shown to be free of proteins by ring tests with saturated ammonium sulfate solution. When whole

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chloroplast solutions prepared with "Tween 20" were floated over ammonium sulfate solution, a white precipitate formed at the interphase which gradually increased in depth; this protein precipitate remained white at the interphase, but acquired greenish color on its upper edge. When chloroplast solutions prepared with SDS were subjected to this test, a dense green precipitate formed at the interphase. When the tube contents were mixed and centrifuged a dark green sediment was deposited from the chloroplast-SDS solutions, whereas a light green floc collected at the surface of chloroplast-"Tween 20" solutions.

TAKASHIMA[4] used 50% aqueous α-picoline to prepare clear chloroplast solutions from which chlorophyll-lipoprotein and carotenoid were crystallized separately. Upon chromatographing chloroplast-α-picoline solutions on tricalcium phosphate gel, a protein-free carotenoid band rapidly separated from the almost immobile chlorophyll-lipoprotein. Chloroplastin thus migrates chromatographically as a unit with SDS, with the chlorophyll bound and the carotenoid free in aqueous α-picoline, and with both the chlorophyll and carotenoid in a protein-free state in "Tween 20" solutions.

Filter paper electrophoresis

The "chloroplastin-SDS" complex migrated electrophoretically toward the anode at a uniform rate for a period of about two hours. As in column chromatography, the chlorophylls, carotenoids and lipoprotein migrated as a unit, when the chloroplast substance was dispersed with SDS (Table II). Tests with bromophenol blue revealed the absence of unpigmented protein bands (*i.e.* there was no evidence of colorless "stroma" protein outside the chromoprotein zone). The "chloroplastin-SDS" complex migrated much more rapidly in the electrical field than either R-phycoerythrin or C-phycocyanin under the same conditions. Chloroplastin from *Nostoc* grana and from spinach chloroplasts migrated at identical rates during filter paper electrophoresis, and did not separate upon admixture.

TABLE II

ELECTROPHORETIC MIGRATION OF PLANT CHROMOPROTEINS

(dissolved in 0.025 *M* SDS made up in 0.1 *M* borate, pH 8.9, and applied to filter paper previously impregnated with this solvent; subjected to 150 volts across 29 cm for 70 min).

Chromoprotein	*Source*	*Movement, mm*
Chloroplastin	Spinach chloroplasts, *Nostoc muscorum*	28
R-phycoerythrin	*Chondrus crispus*	19
C-phycocyanin	*Nostoc muscorum*	18

Inhibition of photochemical activity by detergents

THOMAS, BLAAUW AND DUYSENS[17] discovered that when spinach chloroplast fragments are reduced ultrasonically below a critical volume of $10^6 A^3$ in the absence of oxygen, their capacity for the Hill reaction drops to zero. Maximum photochemical activity was observed in particles having a volume of $> 10^9 A^3$ [17]. Assuming either spherical or cubical shapes for the chloroplast particles, those possessing full activity ($> 10^9 A^3$) should be retained on filters with pore sizes in the range 0.1–0.2 microns. Barring adsorption, chloroplast particles of the critical size ($10^6 A^3$) should pass the ultra filters used in the present study. In addition to reducing the particle size, direct effects of detergents on the Hill reaction are to be expected because of their high surface activity, which is also a property of the urethanes[18].

When dodecyl sulfate was added in the amount required for chloroplast solu-

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bilization (0.02 M) during a Hill reaction measurement, photochemical activity was instantly abolished. Tests at successively lower concentrations showed that the Hill reaction is abolished by SDS well below its threshold concentration ($5 \cdot 10^{-3}$ M) for chloroplast solubilization as assessed by ultrafiltration (Fig. 6). In common with the urethanes, the degree of inhibition was similar at limiting and saturating light intensities. Inhibition increased with the time that the chloroplast fragments were exposed to low ($<10^{-3}$ M) SDS concentrations, and was greater in dilute than in concentrated chloroplast suspensions. Since adsorbed SDS is removed with difficulty from chloroplasts, its inhibition of the Hill reaction is essentially irreversible, whereas urethane inhibition is reversible. Table III shows that the Hill reaction is inhibited by low concentrations of anionic, cationic and neutral synthetic detergents as well as by triterpenoid and steroidal detergents of plant origin. The inhibiting action of leaf saponins on the Hill reaction is of interest because of the known presence of heat-stable inhibitors in the cell sap of many leaves[19].

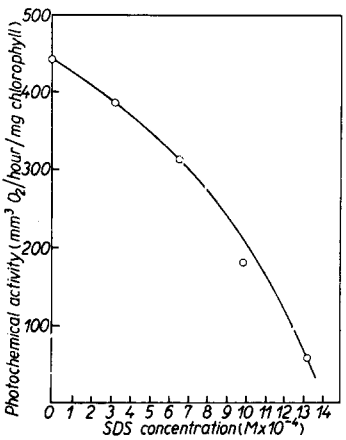

Fig. 6. Inhibition of the Hill reaction in isolated chloroplasts by dodecyl sulfate.

Photosynthesis in *Chlorella* was abolished by very low concentrations of the cationic detergent zephiran chloride, which also caused flocculation of the cells. (Table III). Photosynthesis in *Chlorella* otherwise was quite resistant to synthetic as well as natural detergents. The weaker inhibition of photosynthesis than of the Hill reaction presumably was caused by low penetration of the algal cells, as shown in the case of SDS.

TABLE III

INHIBITION OF THE HILL REACTION IN ISOLATED CHLOROPLASTS (10° C) AND OF PHOTOSYNTHESIS IN *Chlorella* (25° C) BY SYNTHETIC AND NATURAL DETERGENTS

Detergent	*Concentration*		*Hill reaction* 0–20 *min*	20–40 *min*	*Photosynthesis (steady state)*
	%	M	% inhibition		% inhibition
SDS	0.0095	$3.3 \cdot 10^{-4}$	15	25	nil
	0.0190	$6.6 \cdot 10^{-4}$	30	55	nil
	0.0285	$9.9 \cdot 10^{-4}$	60	80	10–20
	0.038	$1.32 \cdot 10^{-3}$	85	100	10–20
	0.24	$8.4 \cdot 10^{-3}$	100	100	37
Tween 20	0.12	$1 \cdot 10^{-3}$	65	100	nil
	0.41	$3.3 \cdot 10^{-3}$	100	100	nil
Zephiran chloride	0.05	—	100	100	100
Digitonin	0.07	—	55	90	nil
	0.20	—	100	100	nil
Soapbark saponin	0.42	—	15	20	—
	0.83	—	20	100	38
	1.25	—	50	100	—
	1.67	—	100	100	60
Alfalfa saponin	0.33	—	23	35	—
	0.83	—	37	45	nil

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DISCUSSION

It has been known for some time that brilliantly clear aqueous chloroplast solutions may be prepared with detergents, which may either free the pigments or leave them attached to the lipoprotein[1-3]. Since the lipoprotein, chlorophylls and carotenoids are all insoluble in water, preparation of stable aqueous chloroplast solutions must always involve linkage of one or more of these water-insoluble constituents with the hydrophobic part of the detergent molecules. The non-ionic detergent "Tween 20" renders these constituents separately water-soluble, and therefore must associate with each of them without linking them together. The anionic detergent SDS disperses and solubilizes the chloroplast substance with the chlorophylls and carotenoids bound to the lipoprotein. Concentrated α-picoline solutions remove the carotenoids but leave the chlorophyll attached to the protein. The lipid remains bound to the protein in the presence of these dispersing agents, all of which shift the red absorption peak for the chloroplast suspensions from 677 to 670 mμ.

Spinach chloroplasts consist of laminated grana embedded in a colorless stroma, the pigmented grana accounting for less than half of the total lipoprotein[18,20,21]. Chloroplast solutions prepared with detergents as well as α-picoline appear to contain but a single lipoprotein—whatever differences there are in the lipoproteins of the grana and stroma become obliterated in the solubilization process. The dissolved lipoprotein molecules either are uniformly pigmented by redistribution, or their properties are little affected by the presence or absence of adsorbed pigments. Two chlorophyll molecules per 19–20,000 unit of solubilized lipoprotein[3,4], represents the average ratio for the chloroplast substance as a whole, including the colorless stroma.

The changes in benzene-solubility of the pigments in aqueous chloroplast suspensions induced by detergents are believed to result from the opposing effects of weakened pigment-lipoprotein bonding and affinity of the detergent for the pigments; whether benzene solubility of the pigments is increased or decreased depends upon which of these effects is predominant. The chloroplast pigments are rendered completely extractable with benzene by very low concentrations of quaternary ammonium salts, which apparently displace the pigments while possessing insufficient affinity for them to prevent their removal with benzene. Digitonin also removes the pigments from the lipoprotein, but its affinity for the freed pigments prevents their removal from the aqueous phase by benzene. Extraction of the pigments with benzene is increased by intermediate concentrations and depressed by high concentrations of "Tween 20" and SDS, yet the pigments are separated from the lipoprotein by "Tween 20" and not by SDS. The latter observation demonstrates the affinity of the latter detergents for the pigments in a protein-free state and in association with the lipoprotein respectively. The affinity of SDS for the pigments while adsorbed on the lipoprotein probably contributes to the maintenance of the chloroplastin as a water-soluble complex.

The preparation of clear chloroplast solutions with detergents and with α-picoline always involves an irreversible loss of photochemical activity. The Hill reaction of isolated chloroplasts is inhibited by much lower detergent concentrations than are required for particle disintegration. The inhibiting action of detergents resembles that of the urethanes with respect to the response at saturating and limiting light intensities, as well as with increasing time of exposure. The most important difference is

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the negligible effect of several detergents on photosynthesis in *Chlorella* cells, which are relatively impermeable to these detergents, but permeable to urethanes. The similar type of inhibition shown by detergents and urethanes on the Hill reaction of isolated chloroplasts suggests a common mode of action for these surface-active compounds. The previous interpretation of urethane inhibition[22] was that the chloroplast surface becomes coated to a variable extent by these compounds. Recently acquired information indicates that the physical site of urethane and detergent inhibition is not the external surface of chloroplasts or grana, but the protein-lipid interphases within grana. Photosynthesis by *Chlorella* and *Nostoc* is inhibited to the same extent by phenylurethane[23] although the surface/volume ratios of their chloroplasts and grana, respectively, are widely different. The minimum particle size for the Hill reaction is smaller than individual grana[17]. The interphase which is of decisive importance for photochemical activity therefore cannot be the external surface of either intact chloroplasts or intact grana. Detergent and urethane molecules all possess hydrophobic and hydrophilic groupings, in common with the chlorophylls and phospholipids located at the lipid-protein boundaries within grana. Surface-active compounds undoubtedly penetrate the grana at the protein-lipid interphase, where they become oriented in the same manner as the chlorophylls and phospholipids[24,25]. Transport of absorbed light energy is then blocked to a variable extent, depending upon the size and number of detergent or urethane molecules which become incorporated in the pigment-phospholipid films.

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SUMMARY

Chloroplastin solubilized with dodecyl sulfate migrates more slowly chromatographically and more rapidly electrophoretically than phycoerythrin and phycocyanin. Chloroplastin from *Nostoc* and from spinach chloroplasts is indistinguishable by these methods. The chlorophylls and carotenoids migrate with the solubilized lipoprotein in dodecyl sulfate solutions, and migrate separately in Tween 20 solutions; protein-free carotenoid rapidly separates from the chlorophyll-lipoprotein when aqueous α-picoline is employed as solvent and developing solution.

The chlorophylls and carotenoids in *Chlorella* and chloroplast suspensions are rendered completely extractable with benzene by very low concentrations of the cationic detergent zephiran chloride, which simultaneously inactivates photosynthesis and the Hill reaction. Anionic and neutral synthetic detergents as well as saponins had little effect on photosynthesis in *Chlorella* due to poor penetration, but inhibited the Hill reaction at lower concentrations than are required for chloroplast dispersion. Their inhibiting action resembles that of the surface active urethanes. It is concluded that the physical site of their inhibiting action is the pigment films within the laminated grana.

RÉSUMÉ

La chloroplastine solubilisée par le dodécylsulfate migre plus lentement par chromatographie et plus rapidement par électrophorèse que la phycoérythrine et la phycocyanine. Les chloroplastines

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isolées de *Nostoc* et des chloroplastes de l'épinard ne peuvent être distinguées par ces méthodes. Les chlorophylles et les caroténoïdes migrent en même temps que la lipoprotéine solubilisée dans des solutions de dodécylsulfate et migrent séparément dans des solutions de Tween 20; le caroténoïde débarassé de protéines se sépare rapidement de la chlorophylle-lipoprotéine quand on emploie l'alpha-picoline comme solvant et comme solution de développement.

Les chlorophylles et les caroténoïdes dans des suspensions de *Chlorella* et de chloroplastes deviennent totalement extractibles par le benzène en présence de très faibles concentrations d'un détergent cationique, le chlorure de zéphiran, qui inactive simultanément la photosynthèse et la réaction de Hill. Des détergents synthétiques anioniques et neutres, de même que les saponines ont peu d'effet sur la photosynthèse chez *Chlorella* en raison de leur pénétration faible, mais inhibent la réaction de Hill à des concentrations plus petites que celles qui sont nécessaires pour produire la dispersion des chloroplastes. Leur action inhibitrice ressemble à celle des uréthanes tensioactifs. Les auteurs concluent que le lieu physique de leur action inhibitrice est le film de pigment à l'intérieur du grana stratifié.

ZUSAMMENFASSUNG

In Dodekylsulfat gelöstes Chloroplastin weist eine geringere chromatographische und eine höhere elektrophoretische Beweglichkeit auf als Phycoerythrin und Phycocyanin. Aus *Nostoc*, bezw. aus Spinatchloroplasten gewonnenes Chloroplastin sind durch diese Methoden nicht zu unterscheiden. In Dodekylsulfatlösungen wandern Chlorophylle und Karotenoide mit dem löslich gemachten Lipoprotein, während sich dieselben Substanzen in Tween 20-Lösungen getrennt bewegen; falls wässeriges α-Pikolin als Lösungsmittel und Entwicklungslösung benützt wird, trennt sich proteinfreies Karotenoid schnell vom Chlorophyll-Lipoprotein.

Durch sehr geringe Konzentrationen des kationischen Detergents Zephiranchlorid, wird, bei gleichzeitiger Inaktivierung der Photosynthese und der Hill-Reaktion, die vollkommene Extrahierbarkeit von Chlorophyllen und Karotenoiden in *Chlorella*- und Chloroplastenaufschlemmungen durch Benzol ermöglicht: Anionische und neutrale synthetische Detergenten, sowie Saponine hatten wegen ihres geringen Penetrationsvermögens nur wenig Einfluss auf die Photosynthese, hemmten jedoch die Hill-Reaktion in geringeren Konzentrationen als diejenige, die für die Dispersion der Chloroplasten benötigt wird. Ihre Hemmungswirkung ähnelt derjenigen vor oberflächenaktiven Urethanen. Es wird daraus gefolgert, dass sich der physikalische Wirkungsort der Hemmung im Farbstoff-Film im Inneren der lamellaren Grana befindet.

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