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Plant & Cell Physiol., 6 (1965)

DEGRADATION AND FORMATION OF SULFOLIPID OCCURRING CONCURRENTLY WITH DE- AND RE-GENERATION OF CHLOROPLASTS IN THE CELLS OF *CHLORELLA PROTOTHECOIDES*[1]

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(Received December 24, 1964)

1. It has been demonstrated that when the cells of *Chlorella protothecoides* are grown mixotrophically under illumination in a medium rich in nitrogen source (urea) and poor in glucose, the normal green cells are obtained, while in a medium rich in glucose and poor in the nitrogen source, entirely chlorophyll-less cells with profoundly degenerated plastids ("glucose-bleached" cells) are produced, irrespective of whether in the light or in darkness. The "glucose-bleached" cells turn green with regeneration of fully organized chloroplasts when incubated in a nitrogen-enriched medium in the light ("light-greening"), while in the dark they become pale green with formation of only partially organized chloroplasts ("dark-greening"). When, on the other hand, the green cells are transferred into a medium enriched with glucose, they are bleached fairly rapidly with degeneration of chloroplasts in the light as well as in darkness ("bleaching"). Using ^{35}S as a tracer, investigations were made on the changes of contents of the algal cells in sulfolipid and other sulfur compounds during the processes of the greening and bleaching.

2. By determining the radioactivities of chromatographically separated sulfur-containing compounds of the uniformly ^{35}S-labeled green ("G") and "glucose-bleached" ("W") cells, it was found that the concentration of a species of sulfolipid (discovered by BENSON et al.) as well as those of glutathione, sulfotrioses and most of the other sulfur-containing compounds were at least 5 times higher in the "G" cells than in the "W" cells, while sulfoquinovosyl glycerol was present in approximately equal amounts in the two types of cells.

3. Phospholipid contents and compositions in the two types of algal cells were found to be practically identical.

4. The sulfolipid content of algal cells increased and decreased almost in parallel with the processes of greening and bleaching, respectively.

5. Studying the mode of incorporation of radiosulfate into various sulfur compounds of algal cells during the processes of "light- and

[1] This work was partly reported at the Symposium on Biochemistry of Lipids, sponsored by the Agricultural Chemical Society of Japan, Sapporo, July, 1964.

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dark-greening" and "bleaching" (lasting about 70 hr), it was found that active ^{35}S-incorporation into sulfolipid occurred throughout the process of "light-greening," while in the "dark-greening" and "bleaching" the active incorporation abruptly ceased after the initial 24 hr period of experiments. It was suggested that the biosynthesis of the sulfolipid is closely related to the formation of photosynthetic apparatus in chloroplast.

6. When the ^{35}S-labeled green cells were bleached in a medium containing no radiosulfate, the ^{35}S-sulfolipid and most of other ^{35}S-sulfur compounds decreased markedly but the ^{35}S-sulfoquinovosyl glycerol increased considerably. It was inferred that the deacylation of the sulfolipid, a surfactant lipid, with formation of water soluble sulfoquinovosyl glycerol may be a cardinal event of bleaching process, causing a disintegration of the intact architechture of photosynthetic apparatus.

7. Based on these observations it was concluded that the sulfolipid is an integral component of photosynthetic structure.

It has been known that a sulfolipid is contained in various photosynthetic organisms including algae, bacteria and green leaves (*1-4*), and it was revealed to be 6-sulfo-α-D-quinovopyranosyl-(1→1′)-2′, 3′-di-O-acyl-D-glycerol (*2-9*). In all photosynthetic plants thus far examined its content was found to equal or exceed those of any of the phospholipids (*2, 4, 10*). BENSON et al. (*4, 10*) assumed that this sulfolipid forms—by virtue of its surfactant nature—an important architectural component in the oriented matrix of pigments, enzymes, etc. in the photosynthetic apparatus.

SHIHIRA-ISHIKAWA and HASE (*11*) have recently demonstrated the reversible degeneration and regeneration of chloroplasts ("bleaching" and "greening") in the cells of *Chlorella protothecoides* by controlling light conditions as well as the concentration balance between glucose (or other organic carbon sources) and nitrogen source. Taking advantage of this characteristic of the alga, investigations were made of the problem as to whether and in what manner the contents of the solfolipid and other sulfur-containing compounds would change during the processes of the de- and re-generation of chloroplasts. The observations to be described in the present paper seem to provide strong experimental evidence in favor of the assumption made by BENSON et al.

MATERIAL AND METHODS

The strain of *Chlorella protothecoides* and the culture (shaking) apparatus used were the same as those reported by SHIHIRA-ISHIKAWA and HASE (*11*). The temperature and light intensity used in the culture were 23° and 2 klux throughout the present experiments.

Preparation of the "green" (G) and "glucose-bleached" (W) cells

The methods of preparation of these cells were the same as those de-

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scribed previously (*12*): the green cells were obtained by growing, in the light, the alga for 5 days in the medium containing 0.5% urea and 0.1% glucose in addition to the basal mineral nutrients of the following composition: per liter, KH_2PO_4, 0.7 g; K_2HPO_4, 0.3 g; $MgSO_4 \cdot 7H_2O$, 0.3 g; $FeSO_4 \cdot 7H_2O$, 3 mg; ARNON's "A_5" solution (*13*) 1 ml; thiamine hydrochloride, 10 μg. The entirely chlorophyll-less "glucose-bleached" cells were obtained by culturing the alga in the medium containing, besides the basal nutrients, 0.1% urea and 1.0% glucose, under otherwise the same conditions as those used for the green cells.

"Greening" and "bleaching" experiments

In the "greening" experiment the glucose-bleached cells separated from the preculture medium were incubated in the medium—referred to G-medium—containing 0.5% urea and the basal mineral nutrients but without addition of glucose, in the light ("light-greening") or in darkness ("dark-greening").

In the "bleaching" experiment the green cells were incubated, in darkness, in the medium containing 1.0% glucose and the basal mineral nutrients in the absence of urea. This medium shall be called B-medium in the following. In the control expriment the green cells were incubated in the basal mineral medium. During the 72 hr period of these incubations, aliquots of the cell suspensions were withdrawn at intervals from the cultures and subjected to the determinations of packed volume and chlorophyll content of cells (*14*), as well as to chromatographic analyses of the sulfolipid and related compounds.

Labeling of the cells with radioisotopes

Algal cells uniformly labeled with ^{35}S were prepared by growing the cells in the above media containing radiosulfate (1 mc/mM). Starting with these ^{35}S-labeled cells or with the non-labeled cells, the following eight sets of experiments were carried out.

(A) Greening experiments
  (I) Labeled W-cells were incubated in labeled G-medium under illumination.
  (II) Non-labeled W-cells were incubated, under illumination, in labeled G-medium, to investigate the ^{35}S-incorporation during the process of "light-greening."
  (III) The same as above, but without illumination, to investigate the ^{35}S-incorporation during the process of "dark-greening."

(B) Bleaching experiments
  (IV) Labeled G-cells were incubated in labeled B-medium.
  (V) Non-labeled G-cells were incubated in labeled B-medium.
  (VI) Labeled G-cells were incubated in non-labeled B-medium.

(C) Control experiments
  (VII) Non-labeled G-cells were incubated in labeled basal medium.

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(VIII) Labeled G-cells were incubated in non-labeled basal medium.

Cells uniformly labeled with ^{32}P were also prepared for phospholipid analyses using the media containing inorganic phosphate-^{32}P.

Determination of algal components

For the determination of sulfur-containing or phosphor-containing compounds on a microscale, radiochemical procedures were extensively employed. Radioactivity of a compound from the uniformly labeled cells represented its relative molar concentration.

Algal suspensions, well washed with water, were directly applied to the origin of chromatogram and subjected immediately to chromatography. Quantitative extraction of all compounds were effected with the chromatographic solvent as development proceeded. The omission of previous extraction of the compounds enabled to avoid their possible fractionation and oxidation. Radioactivities of the spots separated on paper chromatograms were directly measured with a wide area, end-window G. M. tube (Nihon Musen Co., Tokyo, Type 5006), and corrected for disintegration per minute with the use of average counting efficiencies obtained with a liquid scintillation spectrometer.

Two types of chromatography were routinely employed: two-dimensional chromatography on Toyo No. 51 paper (Toyo Roshi Co., Tokyo) first with phenol-water (500:130 w/w), second with butanol-propionic acid-water (142 : 71 : 100 v/v/v) (*15*), and a descending ion-exchange resin-paper chromatography of lipid mixtures on Amberlite WB-2 (Rohm and Haas Co., Philadelphia) using diisobutylketone-acetic acid-water (8 : 5 : 1 v/v/v). The former chromatography separated most of the water-soluble components, and the latter complex lipid mixtures into more than 12 individual lipids.

Identification of the algal compounds was carried out either by co-chromatography and co-electrophoresis on paper with authentic compounds or by chromatographic and electrophoretic investigation of their deacylation, hydrolysis or chemical modification products (*10, 16*).

RESULTS AND DISCUSSION

1. Changes in chlorophyll content and volume of the algal cells during the processes of greening and bleaching

Characteristic changes in chlorophyll content and volume of the algal cells during the processes of greening and bleaching are shown in Fig. 1. When the "glucose-bleached" cells were incubated, under illumination, in the nitrogen-enriched mineral medium without added glucose, active synthesis of chlorophyll started after the lag period of about 15 hr, and continued for the following period of experiment ("light-greening"). In the dark, on the other hand, there occurred much less active formation of chlorophyll which soon came to a standstill ("dark-greening"). During these greening processes the cell volume increased slightly. When the

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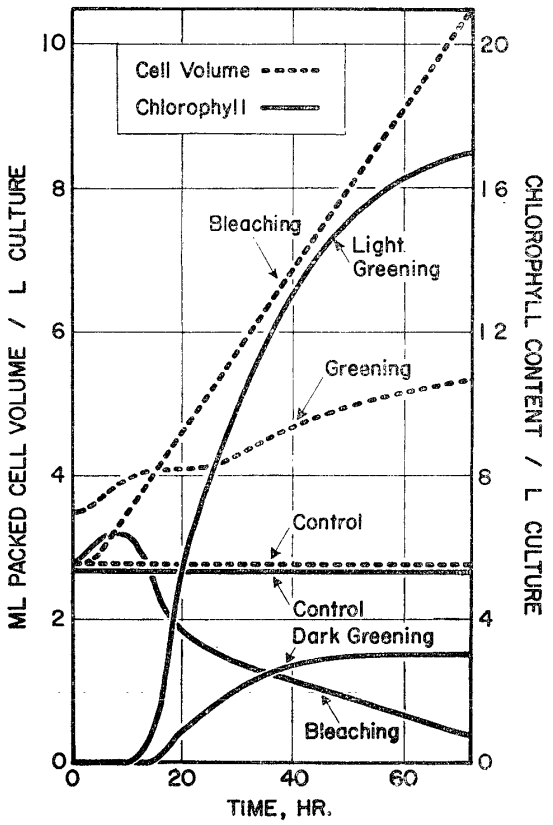

Fig. 1. Changes in volume and chlorophyll content of algal cells during the processes of greening and bleaching in the light and in darkness. For explanation, see text.

green cells were incubated in the glucose-enriched medium in the absence of the nitrogen source (in darkness), a marked degradation of chlorophyll took place after a slight increase during the initial short period, being accompanied by a considerable increase in the cell mass ("bleaching"). In the control culture, in which the green cells were incubated in the basal mineral medium without addition of glucose, no significant changes in chlorophyll and volume of the cells were observed.

Electronmicroscopic studies have revealed that during the processes of bleaching and greening a marked degeneration and regeneration, respectively, of chloroplast structures were occurring (*11* and unpublished data). The cells obtained in the "light-greening" contain fully organized chloroplasts, while those in the "dark-greening" show only partially organized chloroplasts without lamellar structures. It has also been shown in separate studies that the formation and degradation of chlorophyll are, on the whole, paralleled by the appearance and disappearance, respectively, of photosynthetic activities of the algal cells, except that in the "dark-greening" no CO_2-fixing capacity of the cells is produced although their photo-phos-

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phorylating power is developed (OH-HAMA, SHIHIRA-ISHIKAWA and HASE, to be published).

2. Differences in concentrations of sulfur-containing compounds between the green and "glucose-bleached" cells

As shown in Table I, the concentrations of the sulfolipid, an unidentified lipid[2] and glutathione were found to be markedly higher in the green cells than in the glucose-bleached cells. On the other hand, the deacylated sulfolipid, 6-sulfo-α-D-quinovopyranosyl-(1→1′)-glycerol, which was found in

TABLE I

Concentrations of major sulfur-containing compounds in the green and glucose-bleached cells of Chlorella protothecoides[a]

	^{35}S cpm/ml cells		"G"/"W" ratio
	"G" cells	"W" cells	
Sulfolipid	35,700	7,820	5
Sulfoquinovosyl glycerol	33,700	36,600	1
Glutathione	40,800	3,490	11
Unidentified lipid	32,100	425	76
Total soluble S-compounds	191,000	85,800	2

[a] Two types of algal cells uniformly labeled with ^{35}S (1 mc/mM) were analyzed by means of two-dimensional paper chromatography. The observed ^{35}S activities represent relative molar concentrations of individual components. Four compounds listed here constituted more than 90% of the algal sulfur content, excluding inorganic sulfate and insoluble macromolecules.

TABLE II

Phospholipid composition of the green (G) and glucose-bleached (W) cells of Chlorella protothecoides (*^{32}P-labeled*)[a]

	"G" cells		"W" cells		"G"/"W"
	cpm[a]	%	cpm[a]	%	
Phosphatidyl choline	4,440	54.5	4,020	42.9	1.1
Phosphatidyl ethanolamine	2,130	26.1	3,140	33.3	0.7
Phosphatidyl glycerol	605	7.4	920	9.8	0.7
Phosphatidyl serine	431	5.4	862	9.1	0.5
Phosphatidyl inositol	540	6.6	484	5.1	1.4
Total lipid-P	8,140	100	9,430	100	0.9

[a] ^{32}P-Counts per minute per 0.1 ml of packed algal cells. Specific activity of radiophosphor was same in both types of cells, and the cpm represents relative molar concentration of each lipid.

[2] Like in some other strains of green algae (*16*), a considerable ^{35}S-activity was found in an unknown spot obtained from "G"-cells on two-dimensional paper chromatograms. The spot moved as far as green pigments did, suggesting that the compound may be a lipid. This compound, whose structure analysis is in progress, is contained in extremely high concentrations in some plants such as *Lemna perpusilla* (SHIBUYA, MARUO and BENSON, in preparation).

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the water-soluble fraction (*16*), was present in nearly the same concentrations in these two types of cells. In W-cells it formed the major sulfur-containing compound. Experiments also revealed that the concentrations of most of the minor sulfur-containing components such as lysosulfolipid (*17*), methionine, taurine, cystine and sulfotrioses (*16*) were several times higher in "G"-cells than in "W"-cells.

3. *Phospholipids*

Table II shows the results of analysis (Amberlite WB-2 chromatography) of phospholipids in ^{32}P-labeled "G" and "W" cells. In sharp contrast to

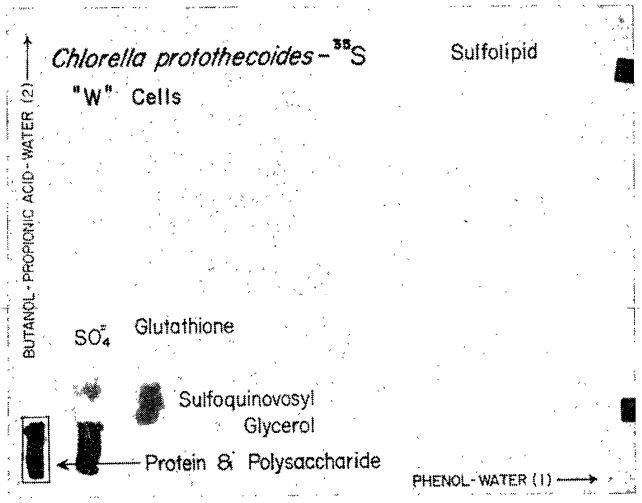

Fig. 2. Autoradiogram of the major sulfur-compounds of "W"-cells uniformly labeled with ^{35}S.

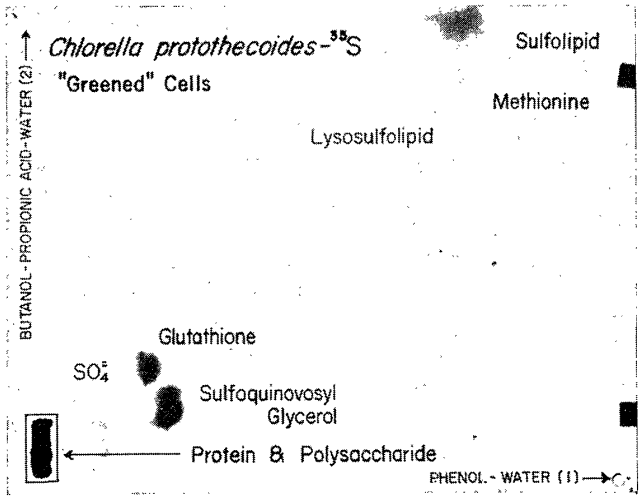

Fig. 3. Autoradiogram of the major sulfur-compounds of the 72 hours-greened cells uniformly labeled with ^{35}S.

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the results obtained with the sulfur-containing compounds, essentially the same composition of the major phospholipids was found with these two types of algal cells. A repeated analysis gave practically the same result.

4. Changes in quantities of sulfur compounds of algal cells during the processes of greening and bleaching

(*i*) *Greening* (*Expt. A-I*)—The glucose-bleached cells uniformly labeled with ^{35}S were incubated, in the light, in the greening medium containing radiosulfate of the same specific activity (1 mc/mM) as that in the preculture, and the process of cell greening was followed for 72 hr. The specific activity of ^{35}S was, therefore, constant throughout the experiment, and the determination of the radioactivities of the sulfur-containing compounds separated from the algal cells served as a measure of their relative molar concentra-

TABLE III

Change in relative quantities of the major sulfur-containing compounds of ^{35}S-labeled algal cells during the process of light-greening[a]

		Time (hr)						
		0	3	9	15	24	48	72
Ptotein and polysaccharide[b]	1	4,010	5,710	7,120	9,570	19,500	22,000	21,400
	2	7,110	9,280	13,000	19,600	38,500	67,800	80,500
$SO_4^=$	1	7,610	12,800	13,000	13,900	25,700	25,100	16,000
	2	13,500	20,800	23,700	28,500	50,800	77,300	60,200
Sulfoquinovosyl glycerol	1	3,810	5,080	5,140	4,440	5,070	4,710	4,950
	2	6,760	8,260	9,380	9,110	10,000	14,500	18,600
Glutathione	1	292	335	472	677	2,710	2,490	3,610
	2	518	545	862	1,390	5,350	7,670	13,600
Sulfolipid	1	640	802	1,100	1,190	1,640	3,790	4,870
	2	1,130	1,300	2,010	2,440	3,240	11,700	18,300
Lysosulfolipid	1	57	85	147	197	475	354	571
	2	101	138	318	404	938	1,090	2,150
Methionine	1	89	172	338	387	283	406	1,250
	2	158	280	617	793	558	1,250	4,700
Total[c]	3	16,500	24,700	27,300	30,400	55,400	58,900	52,700
	4	29,300	40,600	49,900	67,200	109,000	181,000	198,000

[a] The algal cell suspensions were withdrawn at intervals as indicated, and analyzed by two-dimentional paper chromatography. The radioactivity of ^{35}S in this table represents relative molar concentration of each compound since the specific activity was constant throughout the 72 hours' period of experiment.

1 : ^{35}S dpm per μ liter of packed algal cells.

2 : ^{35}S dpm per ml of culture.

3 : ^{35}S cpm per μ liter of packed algal cells.

4 : ^{35}S cpm per ml of culture.

[b] Radioactivity remained at the origin of the chromatogram. Repeated extraction of this area did not cause loss of the activity.

[c] Radioactivity of the cell sample was measured on planchet without subjecting to chromatography.

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tions in the cells. Figures 2 and 3 are the typical autoradiograms of the ^{35}S-compounds in the starting "W"-cells and the 72 hours-greened cells, respectively.

Table III shows the result of the determination of the radioactivities of sulfur-containing compounds in the cells at various stages of the light-greening. Figure 4 illustrates the changes in concentration of the major sulfur-containing compounds in the cells during the process of light-greening. The sulfolipid increased gradually and its intracellular concentration became approximately 5 times higher after 72 hours' incubation.

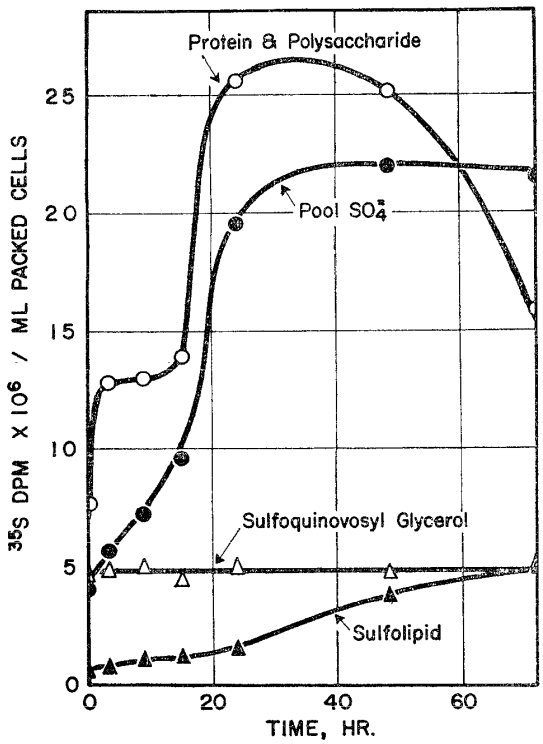

Fig. 4. Changes in content of major sulfur-containing compounds of algal cells during the process of light-greening. Specific activity of ^{35}S was constant throughout the 72 hours' experiment.

Other minor compounds, such as sulfotrioses, behaved in almost the same manners as the sulfolipid. Radioactivities of the protein and polysaccharide fraction as well as of inorganic sulfate pool increased most rapidly after 15 hours and soon reached the maximum. Sulfoquinovosyl glycerol was the only compound whose content in cells remained almost constant throughout the experiment. It should be noted that the greening medium contained only urea besides the basal mineral nutrients, and the growth of the algal cells did not take place markedly because of the lack of exogenous carbon and energy sources. The cells at the end of the

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greening (designated as "greened" cells in Fig. 3), therefore, may be different from the "G"-cells, and the disagreement between the data obtained with the "greened" cells (Fig. 4 and Table III) and those with the "G"-cells (Table II) is understandable.

(*ii*) *Bleaching* (*Expt. B-IV*)—A similar experiment at a constant ^{35}S-specific activity was conducted for the process of bleaching. Table IV shows the radioactivities of the major sulfur compounds separated on two-dimensional chromatograms of the uniformly ^{35}S-labeled cells withdrawn at intervals during the process of bleaching. It is seen from the table that the amount of sulfolipid (expressed per unit volume of culture) increased during the initial 24 hr period and decreased in the later period of experiment, while its content in cells (expressed per unit volume of packed cells) was almost constant during the initial 24 hr period but afterwards dropped sharply. As will be seen from the results of later experiments, these changes in

TABLE IV

Changes in relative quantities of the major sulfur-containing compounds of ^{35}S-labeled algal cells during the process of bleaching[a]

		Time (hr)						
		0	3	9	15	24	48	72
Protein and polysaccharide[b]	1	19,800	19,700	20,200	16,700	14,600	7,700	6,560
	2	35,600	36,100	40,400	40,600	44,500	38,200	37,700
$SO_4^=$	1	1,140	3,500	4,150	2,940	3,920	7,700	9,250
	2	2,050	6,400	8,300	7,140	12,000	38,200	53,200
Sulfoquinovosyl glycerol	1	2,900	1,860	2,880	3,180	4,650	5,610	6,790
	2	5,220	3,400	5,760	7,730	14,200	27,800	39,100
Glutathione	1	2,270	1,690	1,140	790	152	95	78
	2	4,090	3,090	2,280	1,920	1,200	472	449
Sulfolipid	1	2,230	2,160	1,910	2,350	2,460	1,080	945
	2	4,010	3,950	3,820	5,710	7,510	5,400	5,440
Lysosulfolipid	1	397	594	426	351	339	199	192
	2	715	1,090	852	853	1,040	986	1,110
Total[c]	3	1,530	1,490	1,460	1,210	1,090	727	763
	4	2,760	2,730	2,920	2,940	3,320	3,600	4,390

[a] The algal cell suspensions were withdrawn at intervals as indicated, and analyzed by two-dimensional paper chromatography. The specific activity of ^{35}S was constant throughout the 72 hours' transition, and the radioactivity in this table represents relative molar concentration of each compound.

1 : ^{35}S dpm per μ liter of packed algal cells.

2 : ^{35}S dpm per ml of culture.

3 : ^{35}S cpm per μ liter of packed cells.

4 : ^{35}S cpm der ml of culture.

[b] Radioactivity remained at the origin of the chromatogram. Repeated extraction of this area did not cause any loss of the activity.

[c] Radioactivity of the cell suspension was directly measured on planchet without chromatography. Correction for counting efficiency was not made.

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TABLE V

Incorporation of radiosulfate into various algal sulfur compounds during the process of light-greening[a]

		Time (hr)						
		0[b]	3	9	15	24	48	72
Protein and polysaccharide	1	25	143	1,370	7,250	12,600	13,100	14,600
	2	108	677	6,970	31,000	65,900	80,200	92,700
$SO_4^=$	1	80	779	2,520	6,880	4,520	7,570	9,290
	2	352	3,710	12,900	35,200	23,600	46,800	59,000
Glutathione	1	0	7	73	409	749	1,420	1,160
	2	0	31	371	2,100	4,920	8,750	7,350
Sulfoquinovosyl glycerol	1	0	61	204	1,700	1,720	5,030	6,160
	2	0	288	1,080	8,650	9,030	31,000	38,800
Sulfolipid	1	0	10	82	293	861	2,450	3,150
	2	0	47	417	1,490	4,500	15,100	19,800
Lysosulfolipid	1	0	19	111	303	427	828	753
	2	0	89	568	1,540	2,230	5,080	4,760

[a] Non-labeled algal cells were incubated in an ^{35}S-radioactive medium and the aliquotes were withdrawn at intervals as indicated. The values in this table show the radioactivity incorporated into the chromatographically separated sulfur compounds. For further explanation see the legend of Table III and text.

[b] Intracellular radioactivities found at zero time are due to the incorporation during a very short period of time elapsed between the inoculation and withdrawal of the aliquot.

TABLE VI

Incorporation of radiosulfate into various algal sulfur compounds during the process of dark-greening[a]

		Time (hr)						
		0	3	9	15	24	48	72
Protein and polysaccharide	1	14	144	1,540	7,200	15,700	17,200	15,500
	2	55	604	7,700	37,400	83,200	103,000	93,000
$SO_4^=$	1	49	498	2,210	6,580	5,830	7,110	5,520
	2	192	2,080	11,100	34,200	30,900	42,400	33,100
Glutathione	1	0	18	105	380	588	960	917
	2	0	75	525	1,980	3,120	5,720	5,500
Sulfoquinovosyl glycerol	1	0	0	41	406	1,220	2,710	2,700
	2	0	0	204	2,110	6,460	16,200	16,200
Sulfolipid	1	0	0	75	297	800	728	657
	2	0	0	376	1,540	4,240	4,340	3,940
Lysosulfolipid	1	0	0	72	52	297	561	138
	2	0	0	358	269	1,570	3,340	828

[a] For explanation, see the legends of Table V.

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the total amount of sulfolipid are the resultant of its synthesis and breakdown. Marked net increase in the amounts of sulfate and sulfoquinovosyl glycerol were observed in the cells during the process of bleaching. The total ^{35}S-activity in the protein and polysaccharide fraction remained almost constant throughout the experiment, its content in cells (per packed cell volume) decreasing considerably with the increase in the cell mass. The cellular concentration of glutathione markedly decreased as the bleaching proceeded, while those of several other water-soluble sulfur compounds decreased only gradually.

5. Incorporation of ^{35}S into the sulfolipid and other sulfur compounds by algal cells during the processes of greening and bleaching

The modes of incorporation of radiosulfate into the sulfolipid and other sulfur-containing compounds by algal cells were studied during the processes of their greening (in the light (Expt. A-II) and in darkness (Expt. A-III)) and bleaching (Expt. B-V). Tables V, VI and VII show the results

TABLE VII

Incorporation of radiosulfate into various algal sulfur compounds during the process of bleaching[a]

		Time (hr)						
		0	3	9	15	24	48	72
Protein and polysaccharide	1	19	56	260	402	913	1,500	2,620
	2	53	163	905	1,930	4,660	12,700	33,200
$SO_4^=$	1	234	1,700	3,990	3,100	4,510	5,680	6,810
	2	655	4,890	13,900	14,900	23,000	47,800	86,200
Glutathione	1	0	0	36	104	151	144	189
	2	0	0	126	499	770	1,220	2,390
Sulfoquinovosyl glycerol	1	0	0	91	266	1,170	2,120	2,610
	2	0	0	317	1,280	5,970	17,900	33,000
Sulfolipid	1	0	28	205	392	769	481	496
	2	0	82	713	1,880	3,920	4,060	6,280
Lysosulfolipid	1	0	0	13	43	77	22	50
	2	0	0	46	205	392	181	628

[a] For explanation, see the legends of Table V.

of the experiments. During the greening and bleaching processes investigated the most striking mode of radiosulfate incorporation was observed for the sulfolipid. Figure 5 reproduces the time courses of ^{35}S-incorporation into the sulfolipid in the three different transition processes. In the control experiment shown in the figure, the non-^{35}S-labeled green cells were incubated, in the dark, in the basal mineral medium containing radiosulfate (Expt. C-VII). The time courses of the ^{35}S-incorporation under these different conditions were found to be identical during the initial 24 hr period of

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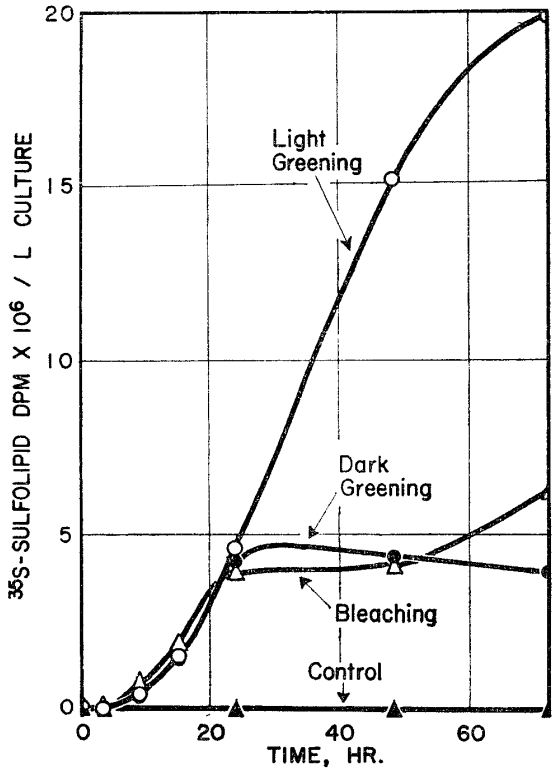

Fig. 5. Incorporation of ^{35}S into the sulfolipid in the four different transition processes of algal cells.

the experiments. During the following period of experiments, however, sharply different courses of the incorporation were observed: active incorporation continued throughout in the light-greening, while the incorporation abruptly ceased in the dark-greening and bleaching.

It has been revealed in the previous study (*12*) that the process of greening of the glucose-bleached cells consists of two consecutive phases: an early light-independent phase in which nucleic acids play an important role and the following light-dependent one in which full organization of chloroplasts takes place. It is during the latter light-requring phase that the formation of lamellar structures as well as active syntheses of chlorophyll and protein with the appearance of full photosynthetic activities takes place. In the dark, on the other hand, these light-requiring processes remain arrested. The results obtained here strongly suggest that the sulfolipid is an integral part of the photosynthetic structures, and that the biosynthesis of this lipid is closely related to the formation of the photosynthetic apparatus in chloroplasts. The observation that ^{35}S-incorporation into the sulfolipid came to a standstill at about the 24th hr in the bleaching experiment, may be taken as an indication that a repressive action of glucose on the sulfolipid synthesis started around that stage.

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TABLE VIII

Changes in radioactivity of the ^{35}S-labeled sulfur compounds of the green cells during the process of bleaching in a medium containing no radiosulfate ("chasing" experiment)[a]

		Time (hr)						
		0	3	9	15	24	48	72
Protein and polysaccharide	1	14,800	14,000	12,600	11,100	9,740	6,170	4,830
	2	41,900	39,400	45,900	46,600	49,700	48,800	48,100
$SO_4^=$	1	1,880	2,180	1,130	1,160	1,040	898	1,140
	2	5,280	6,120	4,110	4,900	5,310	7,100	11,000
Glutathione	1	2,830	1,570	1,090	1,060	682	239	138
	2	7,950	4,520	3,990	4,480	3,500	1,890	1,330
Sulfoquinovosyl glycerol	1	3,030	3,780	2,740	2,940	3,500	2,900	2,440
	2	8,500	11,000	9,940	12,400	17,900	22,900	23,500
Sulfolipid	1	1,380	1,500	1,110	664	638	139	96
	2	3,880	4,210	2,970	2,790	3,260	932	923
Lysosulfolipid	1	56	72	33	26	29	8	7
	2	156	200	120	111	147	64	71

[a] The green cells uniformly labeled with ^{35}S were incubated in a non-radioactive bleaching medium. For further explanation, see the legends of Tables III and V.

6. Fates of the ^{35}S-labeled compounds of the green cells during the process of bleaching in a medium containing no radiosulfate ("chasing" experiment)

The "G"-cells uniformly labeled with ^{35}S were transferred to the "bleaching medium," on the one hand (Expt. B-VI), and to the basal medium containing no radiosulfate on the other (Expt. C-VIII). Table VIII shows the changes in radioactivity of the major sulfur-containing compounds of the cells in the bleaching process. In the control culture incubated in the basal medium, the radioactivities of all compounds studied did not change significantly. During the process of bleaching, however, the concentrations of various labeled compounds changed in different ways suggesting different metabolic turnover of the compounds. Most striking were the behaviors of sulfolipid and 6-sulfo-α-D-quinovopyranosyl-(1→1′)-D-glycerol. Figure 6 reproduces the characteristic changes in radioactivity of the sulfolipid and glycoside. The ^{35}S activity of the sulfolipid showed a slight decrease at an early stage but remained constant during the following period up to the 24th hr, after which time a marked decrease took place. This characteristic change may be interpreted as follews: (i) the degradation of the ^{35}S-sulfolipid proceeds continuously from the beginning of bleaching process except during the initial very short period, (ii) an active incorporation of ^{35}S from intracellular sources such as $^{35}SO_4^=$ into the sulfolipid occurs during the initial period of about 24 hr, and (iii) the resultant of the degradation (loss of ^{35}S) of, and ^{35}S-incorporation into, the sulfolipid gives rise to the characteristic change in the ^{35}S-activity of the

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sulfolipid as observed above. The inference mentioned under (ii) is based on the observation described in the preceding section that an active incorporation of radiosulfate into the sulfolipid occurred during the initial 24 hr period of bleaching, becoming repressed afterwards. It seems highly likely that the degradation of the sulfolipid is a cardinal event in the bleaching process, proceeding in close association with the disappearance of chlorophyll and chloroplast structures.

The radioactivity of sulfoquinovosyl glycerol, unlike those of other sulfur-containing compounds, increased markedly during the bleaching process. It was surmised that the ^{35}S of the glycoside came from the ^{35}S-sulfolipid. Although the pathways of biosynthesis and metabolism of the sulfolipid have not yet been elucidated, the unique structure of this glycoside, the deacylated form of the sulfolipid, suggests its close metabolic relation to the sulfolipid[3]. Taking into consideration the fairly well established biosynthetic pathways of galactolipids (*18*), we are led to a plausible assumption that sulfoquinovosyl glycerol is not a biosynthetic precursor but

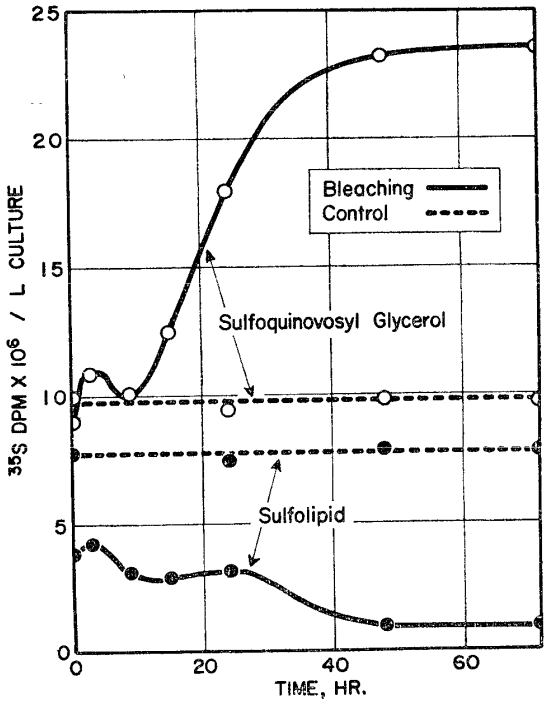

Fig. 6. Changes in radioactivity of the ^{35}S-labeled sulfolipid and sulfoquinovosyl glycerol during the process of cell bleaching in a medium containing no radiosulfate ("chasing" experiment).

[3] The glycoside has never been found in organisms without photosynthetic activity so far investigated. Recently YAGI and BENSON demonstrated an enzymatic hydrolysis of the sulfolipid with formation of lysosulfolipid and sulfoquinovosyl glycerol (*17*).

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the direct degradation product of the sulfolipid[3]. It is seen from the data in Fig. 6 or in Table VIII that during the process of bleaching the ^{35}S increment in the glycoside was much higher than the ^{35}S decrement in the sulfolipid. This result may appear to contradict with the above surmise. As mentioned earlier, however, the sulfolipid seems to be rapidly turning over during the initial 24 hr period of bleaching, incorporating ^{35}S from intracellular sources on the one hand, and being degraded, on the other, a situation that makes the apparently contradictory observation mentioned above understandable. In any event, it may be quite plausible that the ^{35}S-glycoside was, at least partly, a product of degradation of the ^{35}S-sulfolipid.

The deacylation of the sulfolipid, with formation of the water soluble glycoside, will naturally lead to a degradation of photosynthetic apparatus, if the sulfolipid is—by virtue of its surfactant nature—an architectural component in maintaining the functional structure of the apparatus, as has been suggested by BENSON (*19*). It may be added that the behavior of glutathione, which also showed a marked decrease in its ^{35}S-activity during the process of bleaching, is worthy of notice in connection with the processes of de- and re-generation of chloroplasts.

CONCLUDING REMARKS

The present study has demonstrated that in *Chlorella protothecoides* the de- and re-generation of photosynthetic apparatus are causally related, respectively, to the degradation and synthesis of the sulfolipid discovered by BENSON et al. in various photosynthetic organisms. The important role of the sulfolipid in the maintenance of photosynthetic micro-structures, as has been suggested in the present study, seems to be also evidenced by our recent finding (SHIBUYA and MARUO, submitted to publication) that the sulfolipid and the lysosulfolipid are specifically concentrated in the quantasome, a particulate fraction obtained from chloroplast (*20*).

During the course of this study, ROSENBERG and PECKER (*21*) reported similar results with *Euglena* cells in their process of light-induced greening.

The authors are indebted to Mr. K. TAKASHIMA and Miss S. MIHARA of this Institute for the preparation of algal cells. They are grateful to Professor B. MARUO for his critical discussion and to Professor H. TAMIYA for his valuable advice in preparation of the manuscript. This work was supported by the grants from the Ministry of Education and Charles F. KETTERING Foundation.

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