THE ACTION OF SODIUM DODECYL SULFATE ON THE CHLOROPHYLL-PROTEIN COMPOUND OF THE SPINACH LEAF*

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I

INTRODUCTION

Evidence has been presented that in the green leaf chlorophyll is bound to protein by true chemical linkage (Smith, 1941). In order to elucidate additional properties of this compound, the effect of sodium dodecyl sulfate was studied. Sreenivasaya and Pirie (1938) demonstrated that the tobacco mosaic virus protein is split by sodium dodecyl sulfate into fragments of smaller size than the initial virus preparation, and at the same time, the nucleic acid is separated from the protein. Anson (1939) observed that various detergents including some which contain sodium dodecyl sulfate denature hemoglobin and egg albumin. Keilin and Hartree (1940) found that cytochrome *c* is reversibly changed by sodium dodecyl sulfate apparently affecting the linkage of the heme group to the protein since the absorption spectrum is modified.

II

Effect of Sodium Dodecyl Sulfate

When a solution of sodium dodecyl sulfate (SDS)[1] is added to an alkaline leaf extract, every trace of opalescence disappears and a brilliantly clear green solution is obtained. SDS thus has a similar action to digitonin or bile salts (Smith, 1941), but differs from these in its much greater effectiveness. The SDS clarified preparations show a clear red fluorescence which appears to be greater than that of the untreated leaf extract, but have a

* Part of this work has already been presented in a preliminary communication (Smith, 1940 *a*).

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[1] The SDS used in these experiments was part of a gift to Professor D. Keilin from Imperial Chemical Industries.

much smaller fluorescence than an equivalent concentration of free chlorophyll in acetone or ether.

The most striking action of the SDS is that in addition to clarifying the solution, magnesium is eliminated from the chlorophyll converting it to phaeophytin. The reaction is extremely rapid in weakly acid solutions

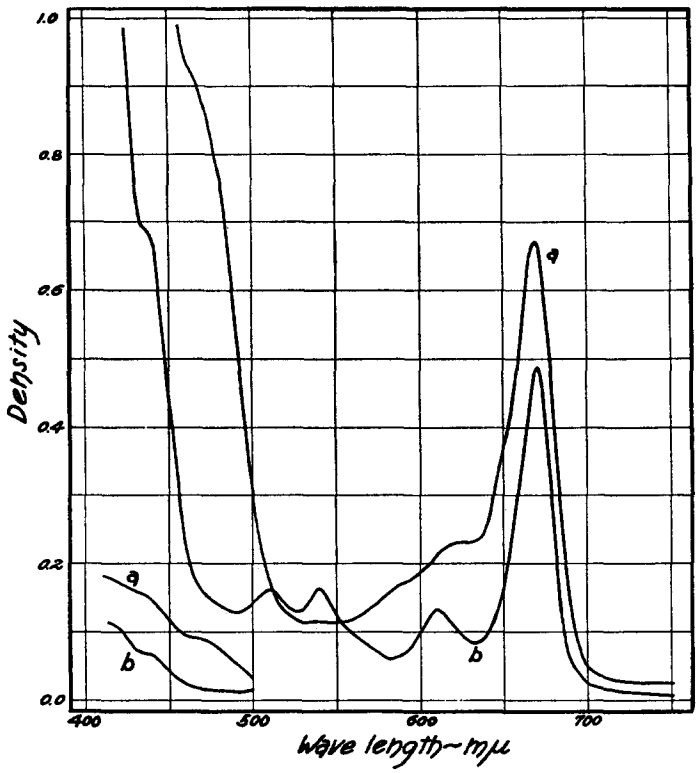

FIG. 1. The absorption spectra of the chloroplast pigment in SDS solutions at pH 8.90 (Curve *a*) and pH 5.30 (Curve *b*). In the short wave region of the spectrum, the curves have been plotted at a tenth of the measured density values. The data are given in Table I.

and takes place slowly in more alkaline solutions. This change is apparent by the striking color change from the original brilliant green first to an olive green and finally to a yellow or brown depending on the concentration of the pigment. In Fig. 1 are shown the absorption spectra of two solutions identical in all respects except that *a* was buffered at pH 8.9 and *b* at pH 5.3. The spectrum of the alkaline solution was measured immediately after addition of the SDS. No significant change occurred during the course of the measurements. After addition of the SDS solution to the acid buffered

solution (*b*), it was allowed to stand overnight to permit the reaction to go to completion.

The spectrum of the chlorophyll-protein compound even in the alkaline SDS solution shows striking changes. The maximum of the main red band which is at 678 in the leaf extract is shifted to 670 mμ. The prominent bands in the blue at 470 and 437 mμ are reduced in the SDS treated solution to slight inflections, as the entire curve appears to rise towards a maximum in the ultraviolet.

The spectrum of the acid SDS solution is plotted on the same density scale as the alkaline one. It shows a decided decrease in density at the red maximum which remains at 670 mμ, a pronounced shift of the entire blue absorption region towards the ultraviolet, and the appearance of three new absorption bands at 610, 540, and 510 mμ. The absorption minima are at 632.5, 583, 527.5, and 490 mμ. The actual measurements of both spectra are given in Table I. Comparison of the acid spectrum with the absorption spectrum of phaeophytin *a* in dioxane (Stern and Wenderlein, 1936) shows that phaeophytin, the magnesium-free derivative of chlorophyll, has been formed. In dioxane, the band maxima are from 1 to 5 mμ further towards the blue end of the spectrum than for the pigment in aqueous solution.

Ultrafiltration of a clarified extract through a 3 per cent Bechold collodion membrane or dialysis does not result in any loss of chlorophyll or carotenoid pigment showing that these pigments remain attached to large molecules. After prolonged dialysis against alkaline buffer solutions, the SDS may be nearly completely removed. The solution remains clear. The pigment and protein are precipitated by acidifying with dilute acetic acid, and can be redissolved with alkali. The complex is precipitated from solution by a tenth saturation with ammonium sulfate. This precipitate cannot be redissolved in water or neutral buffer, but is readily dissolved in SDS solution. The low concentration of salt required and its subsequent insolubility indicate that the protein is denatured by SDS. This is true for the protein compound whether the prosthetic group is phaeophytin or chlorophyll. No separation of the pigment from the protein can be obtained by fractional precipitation.

SDS readily dissolves the protein denatured by boiling. However, in these preparations, phaeophytin is formed much more rapidly than in control preparations buffered at the same pH. In one experiment, two 3 ml. samples of a leaf extract were strongly buffered at pH 8. One was boiled for 5 minutes, cooled to room temperature, and 1 ml. of 5 per cent SDS added to both solutions. In the boiled solution, phaeophytin formation

was complete in less than 3 hours, while the control solution still showed some green color and incomplete phaeophytin formation after 20 hours.

TABLE I

The Effect of Sodium Dodecyl Sulfate on the Absorption Spectrum of the Chloroplast Pigment. Data of Fig. 1

Measurements were made with the spectrophotometer of Shlaer (1938).

λ	Density at pH 8.90	Density at pH 5.30	λ	Density at pH 8.90	Density at pH 5.30
mμ			mμ		
750	0.0244	0.0052	560	0.1168	0.0964
740	0.0260	0.0088	550	0.1127	0.1220
730	0.0244	0.0096	542	—	0.1612
720	0.0260	0.0116	541	—	0.1628
710	0.0319	0.0152	540	0.1151	0.1628
700	0.0462	0.0216	538	—	0.1600
690	0.1327	0.0576	530	0.1134	0.1304
685	0.2369	0.1156	527.5	—	0.1284
680	0.4116	0.2508	525	—	0.1288
675	0.5855	0.4172	520	0.1268	0.1372
672	0.6426	0.4744	511	—	0.1612
671	—	0.4848	510	0.1772	0.1616
670	0.6712	0.4872	509	—	0.1612
669	0.6712	0.4848	500	0.3066	0.1436
668	0.6695	—	495	—	0.1316
665	0.6375	0.4232	490	0.5359	0.1276
660	0.5158	0.3312	485	—	0.1320
650	0.3721	0.1636	480	0.7619	0.1404
640	0.2545	0.0944	475	—	0.1480
635	—	0.0840	470	0.8854	0.1592
632.5	—	0.0824	465	—	0.1808
630	0.2302	0.0840	460	0.9408	0.2324
625	0.2302	—	455	—	0.3188
620	0.2302	0.1108	450	1.1567	0.4304
612	—	0.1308	445	—	0.5684
610	0.2134	0.1320	440	1.4860	0.6768
608	—	0.1304	435	—	0.6904
600	0.1873	0.0984	430	1.5750	0.7612
590	0.1722	0.0664	425	—	0.9280
585	—	0.0616	420	1.7302	1.0836
583	—	0.0604	415	—	1.1252
580	0.1537	0.0624	410	1.8287	—
570	0.1336	0.0784			

This suggests that the action of the SDS takes place at several different linkages in the chlorophyll-protein compound. Anson has shown that Duponol WA, which consists mostly of SDS, readily denatures hemoglobin with liberation of sulfhydryl groups. Similarly it appears that SDS de-

natures the chlorophyll-protein compound splitting linkages which facilitates the removal of magnesium from the molecule. When denaturation is first accomplished by heating, the magnesium is rapidly removed.

It should be emphasized that SDS removes magnesium from the chlorophyll-protein compound in neutral and in slightly alkaline solutions. Heretofore, acid has been used to remove magnesium from chlorophyll dissolved in organic solvents. SDS is without effect on chlorophyll dissolved in acetone. Magnesium is removed by SDS from colloidal chlorophyll suspended in neutral solutions but at a slower rate than from the chlorophyll-protein compound.

III

Kinetics

1. Methods and Procedure.—All of the measurements were made with unpurified extracts of spinach leaves prepared as already described (Smith, 1941). The conversion of the pigment from chlorophyll to phaeophytin is readily observed spectrophotometrically because of the pronounced differences in the absorption spectra of the two pigments. The changes were followed at three separate wave lengths: at 670 mμ, the principal maximum in the red where a large decrease in density takes place (Fig. 1), similarly at 610 mμ, the maximum of a phaeophytin absorption band; and at 540 mμ where an increase in density takes place and a new absorption band appears. The measurements were made using the sensitive photoelectric spectrophotometer of Shlaer (1938).

After several preliminary experiments, all of the experiments were carried out over a period of 6 days on a single extraction which was kept in the refrigerator; no sensible change occurred in the extract during this period. Measurements were made at five pH values and at six different concentrations of sodium dodecyl sulfate, making ten runs in all since one run served for both series. The experiments of both series were made in random order.

The procedure was as follows: A sample of the extract was removed from the refrigerator and allowed to come to room temperature. To 0.5 ml. of extract were added 1.0 ml. of the appropriate sodium phosphate buffer (in one experiment sodium acetate buffer was used), a quantity of water where it was necessary to bring the solution to the final volume of 3 ml., and finally the sodium dodecyl sulfate solution. The final buffer concentrations were always tenth molar. The solution was mixed rapidly and pipetted into an absorption cell of 5 mm. optical thickness. Measurements were then carried out as rapidly as possible at the three wave lengths. In all cases, the pH of a sample of the reaction mixture was measured with the glass electrode. The measurements were made at room temperature (22–24°C.).

2. Effect of pH.—The change in density at 670 mμ at different pH's is shown in Fig. 2 and the data are given in Table II. For the data of pH 7.96, the correct time values are twice those in the figure. It is apparent that there is a large change in the rate of the reaction depending on the pH of the solution. At pH 7.96 nearly 5 hours (294 min.) are required for half

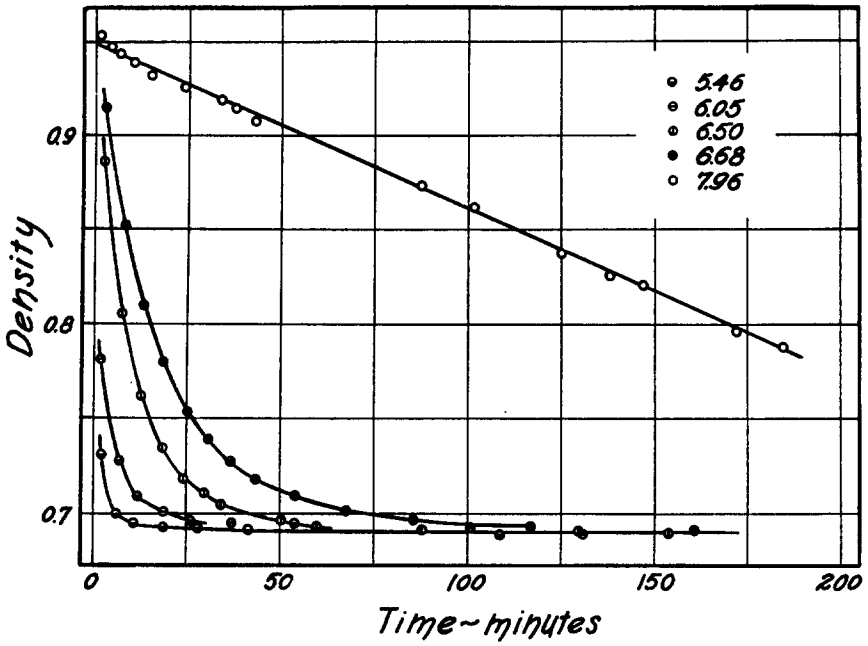

FIG. 2. Density changes at 670 mμ in solutions of different pH. For the data of pH 7.96 the plotted time values should be doubled. The numerical values are in Table II.

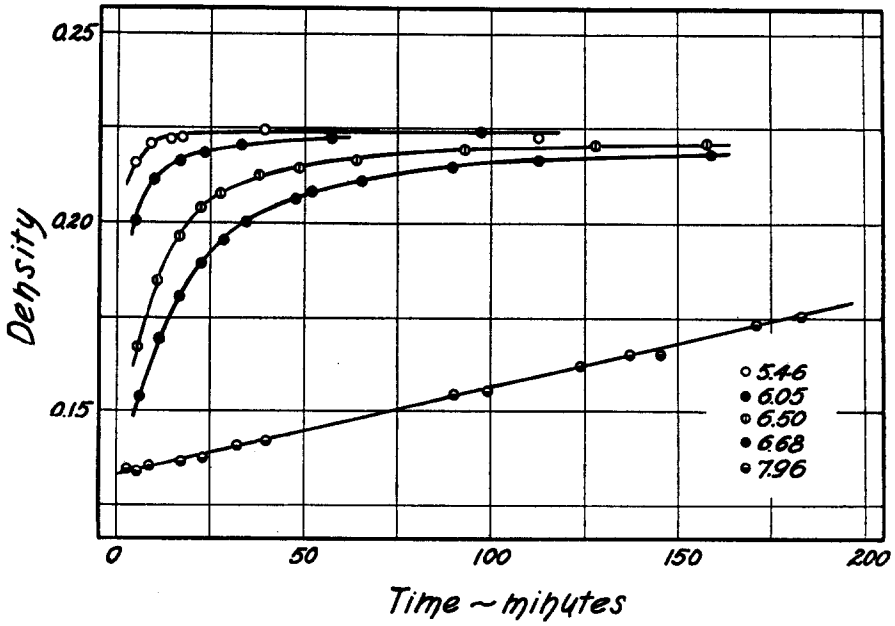

FIG. 3. Density changes at 540 mμ in solutions of different pH. For the data of pH 7.96 double the plotted time values. The data are given in Table III.

TABLE II

Change in Density at 670 mμ at Different pH's

Data of Fig. 2. SDS concentration constant at 1.67 per cent. Solution at pH 5.46 in 0.1 M sodium acetate buffers; others in 0.1 M sodium phosphate buffers.

pH = 5.46		pH = 6.05		pH = 6.50		pH = 6.68		pH = 7.96	
Time	Density	Time	Density	Time	Density	Time	Density	Time	Density
min.		*min.*		*min.*		*min.*		*min.*	
2.2	0.7308	1.7	0.7812	2.2	0.8860	2.5	0.9144	2.0	0.9536
6.3	0.7000	7.0	0.7280	7.5	0.8056	8.0	0.8520	7.5	0.9472
10.8	0.6952	11.8	0.7088	12.7	0.7624	13.3	0.8096	13.0	0.9436
18.9	0.6932	19.0	0.7008	18.5	0.7344	18.7	0.7800	20.0	0.9392
28.0	0.6928	26.0	0.6960	24.3	0.7184	25.2	0.7536	29.7	0.9320
41.5	0.6916	37.0	0.6948	29.7	0.7108	30.8	0.7392	47.5	0.9248
109.	0.6888	54.0	0.6948	34.3	0.7044	36.7	0.7276	67.0	0.9184
		101.	0.6924	50.2	0.6964	43.7	0.7180	75.0	0.9140
		131.	0.6892	60.0	0.6936	54.0	0.7088	85.5	0.9072
				88.0	0.6912	67.7	0.7016	175.	0.8728
				130.	0.6908	85.7	0.6964	203.	0.8616
				154.	0.6900	117.	0.6932	250.	0.8372
						161.	0.6916	276.	0.8248
						255.	0.6900	294.	0.8200
								344.	0.7960
								369.	0.7880

TABLE III

Change in Density at 540 mμ at Different pH's

Data of Fig. 3. Measurements made alternately with those given in Table II using the same solutions.

pH = 5.46		pH = 6.05		pH = 6.50		pH = 6.68		pH = 7.96	
Time	Density	Time	Density	Time	Density	Time	Density	Time	Density
min.		*min.*		*min.*		*min.*		*min.*	
5.0	0.2160	5.0	0.2008	5.7	0.1672	6.2	0.1542	5.5	0 1344
9.0	0.2208	10.0	0.2116	10.8	0.1848	11.5	0.1694	10.9	0.1340
14.3	0.2220	17.0	0.2168	16.6	0.1964	16.8	0.1802	17.3	0.1352
17.2	0.2224	23.7	0.2184	22.5	0.2040	22.7	0.1894	34.7	0.1364
39.5	0.2244	33.3	0.2204	27.7	0.2076	28.8	0.1954	46.0	0.1376
113.	0.2224	57.5	0.2224	38.0	0.2124	34.5	0.2002	64.5	0.1408
		97.5	0.2240	48.5	0.2144	47.7	0.2062	80.0	0.1420
				64.0	0.2168	52.0	0.2082	180.	0.1544
				93.0	0.2192	65.5	0.2110	198.	0.1556
				128.	0.2208	89.7	0.2150	248.	0.1624
				158.	0.2212	113.	0.2170	274.	0.1656
						159.	0.2182	291.	0.1656
						251.	0.2210	342.	0.1736
								366.	0.1756

of the total density change. On the other hand, at pH 5.46 the reaction is practically complete in 10 minutes. It has not been feasible to measure the rate in more acid solutions because of the time required for mixing and transferring the solutions to the spectrophotometer.

In Fig. 3 and Table III are presented the data which were obtained on the same solutions at 540 mμ. The character of the data is exactly the same as those at 670 mμ except that they show an increase in density instead of a decrease. The precision of the measurements with the spectrophotometer used is well illustrated by these data since the density changes at this

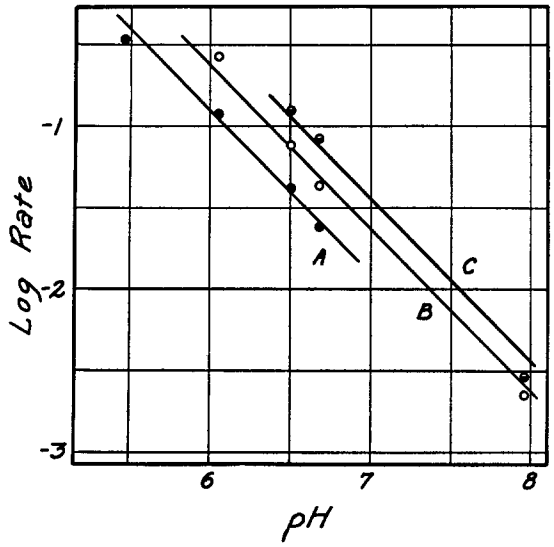

FIG. 4. Rate of phaeophytin formation as a function of pH at three density values: *A*, 0.72; *B*, 0.76; *C*, 0.80. These are the data for 670 mμ and are given in Table IV.

wave length are very small. The data obtained at 610 mμ have been omitted since they are so similar in character to those at 670 mμ. It is evident that the pH of the solution has little or no effect on the final phaeophytin spectrum since the final density is the same at all pH's both at 670 and 540 mμ; this is likewise true for the data at 610 mμ.

The influence of pH on the rate of the reaction can be estimated from the time required to reach a given density value at any one pH. In Fig. 4 are plotted the log rates (-log times) *versus* the pH, for three different density values. The lines drawn through the data have a slope of minus one indicating that the log rate is inversely proportional to the pH, or that the rate of the reaction is directly proportional to the hydrogen ion concentration. In other words, in the presence of sodium dodecyl sulfate the removal

of magnesium of the chloroplast pigment appears to be influenced by a simple hydrogen ion catalysis. The data in Fig. 4 are given in Table IV together with the data obtained from the measurements at 540 mμ. The effect of hydrogen ion concentration is identical at the three measured wave lengths.

3. Effect of Sodium Dodecyl Sulfate Concentration.—In Fig. 5 and Table V are presented the data obtained for the change in density at 670 mμ. The

TABLE IV

Rate of Phaeophytin Formation As a Function of pH

The data of 670 mμ are shown graphically in Fig. 4. These values were obtained by interpolation from the measurements given in Tables II and III. The value in brackets was obtained by extrapolation.

Wave length	pH	Density = 0.7200		Density = 0.7600		Density = 0.800	
		Time	−log time	Time	−log time	Time	−log time
mμ		*min.*		*min.*		*min.*	
670	5.46	2.7	−0.431	—	—	—	—
	6.05	8.4	−0.924	3.7	−0.568	—	—
	6.50	24.0	−1.380	13.1	−1.117	8.0	−0.903
	6.68	41.4	−1.617	23.3	−1.367	15.0	−1.176
	7.96	—	—	[440.]	[−2.644]	340.	−2.532
		Density = 0.2200		Density = 0.1700			
		Time	−log time	Time	−log time		
		min.		*min.*			
540	5.46	8.2	−0.914	—	—		
	6.05	30.8	−1.489	—	—		
	6.50	105.	−2.021	6.3	−0.799		
	6.68	217.	−2.337	11.7	−1.068		
	7.96	—	—	314.	−2.497		

plotted time values should be doubled for the data at the lowest SDS concentration (0.0209 per cent). The solutions were all buffered at pH 6.50. The curves show a marked effect of SDS concentration when the concentration is low. With these solutions there was no noticeable difference in character or rate of clarification of the solutions. When SDS was added to make the final concentration 0.01 per cent, or half that of the lowest concentration given in Fig. 5, no clarification of the solution was obtained. It was not possible to measure this solution spectrophotometrically. There seems to be a fairly abrupt transition below the least effective concentration of SDS indicating a threshold effect.

Relative rates of phaeophytin formation as a function of SDS concentra-

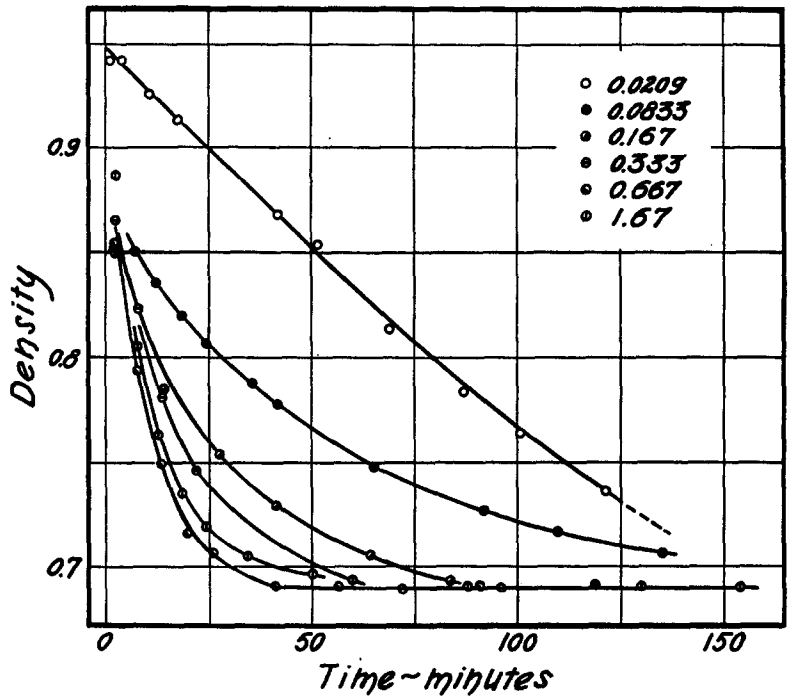

FIG. 5. Density changes at 670 mμ at different SDS concentrations, and at a constant pH of 6.50. For the data of 0.0209 per cent SDS, the correct time values are twice those plotted. The data are given in Table V.

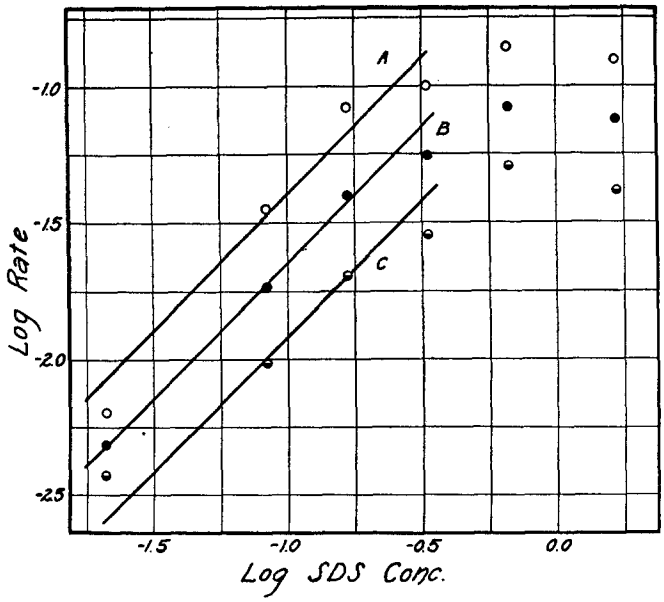

FIG. 6. Rate of phaeophytin formation as a function of SDS concentration at three different density values: *A*, 0.80; *B*, 0.76; *C*, 0.72. The data are given in Table VI.

tion were obtained from the data shown in Fig. 5 by the same procedure used for the pH data. The data obtained are given in Table VI. In Fig. 6 these data are plotted as log rate *versus* log SDS concentration. The lines

TABLE V

Change in Density at 670 mμ at Different Concentrations of Sodium Dodecyl Sulfate

Data of Fig. 5. pH was constant at 6.50 in 0.1 M sodium phosphate buffer. SDS concentrations are given in per cent.

[SDS] = 0.0209		[SDS] = 0.0833		[SDS] = 0.167		[SDS] = 0.333		[SDS] = 0.667		[SDS] = 1.67	
Time	Density	Time	Density	Time	Density	Time	Density	Time	Density	Time	Density
min.		*min.*		*min.*		*min.*		*min.*		*min.*	
1.8	0.9418	2.0	0.8540	2.1	0.8500	2.3	0.8642	2.0	0.8529	2.2	0.8860
7.0	0.9413	7.0	0.8496	7.9	0.8224	8.0	0.8222	7.5	0.7936	7.5	0.8056
21.0	0.9250	12.0	0.8348	14.0	0.7844	13.5	0.7802	13.3	0.7488	12.7	0.7624
34.7	0.9127	18.3	0.8188	27.7	0.7540	22.0	0.7460	20.0	0.7157	18.5	0.7344
83.7	0.8679	24.3	0.8064	41.1	0.7292	72.0	0.6889	26.0	0.7068	24.3	0.7184
103.	0.8534	35.8	0.7868	64.3	0.7056	96.0	0.6900	41.3	0.6911	29.7	0.7108
138.	0.8130	41.8	0.7772	83.7	0.6936	119.	0.6917	56.8	0.6900	34.3	0.7044
174.	0.7834	65.3	0.7480					91.0	0.6905	50.2	0.6964
202.	0.7638	91.8	0.7264							60.0	0.6936
243.	0.7363	110.	0.7164							88.0	0.6912
		135.	0.7064							130.	0.6908
										154.	0.6900

TABLE VI

Influence of Sodium Dodecyl Sulfate Concentration on Rate of Phaeophytin Formation

Data of Fig. 6. These values were obtained by interpolation from the data of Table V. The value in brackets is an extrapolated one.

Sodium dodecyl sulfate concentration	Log SDS concentration	Density = 0.7200		Density = 0.7600		Density = 0.8000	
		Time	−log time	Time	−log time	Time	−log time
per cent		*min.*		*min.*		*min.*	
0.0209	−1.780	[268.]	[−2.428]	208.	−2.318	156.	−2.193
0.0833	−1.079	103.	−2.013	54.5	−1.736	28.	−1.447
0.167	−0.777	49.	−1.690	25.	−1.398	12.	−1.079
0.333	−0.478	35.	−1.544	18.	−1.255	10.	−1.000
0.667	−0.176	19.5	−1.290	11.9	−1.076	7.2	−0.857
1.67	0.223	25.	−1.380	13.1	−1.117	8.0	−0.903

drawn have a slope of unity, and show that the rate is directly proportional to the SDS concentration within the precision of the data. The levelling of the data at the higher SDS values indicates that under the conditions of these experiments 0.4 per cent SDS produces the maximum rate.

4. *Effect of Temperature.*—The effect of temperature was observed only in a qualitative way. Aliquot portions of the same reaction mixture were

taken, exposed to different temperatures, and the color changes of the solutions observed visually. Temperature has an extremely large effect on the rate of the reaction. For the region between 20 and 30°C., the Q_{10} may be as high as 4 or 5. The high temperature coefficient may be only the usual high Q_{10} for protein denaturation since prior denaturation by boiling increases the rate of SDS action as described in Part II of this paper.

IV

DISCUSSION

The experiments of ultrafiltration, dialysis, and fractional precipitation show that the prosthetic group remains attached to the protein regardless of the presence or absence of magnesium in the molecule. This has been confirmed by an ultracentrifugal study of the solutions which showed in addition that the protein is split into particles of low molecular weight (Smith, 1940 *b*; Smith and Pickels, 1941). The action of SDS on the chlorophyll-protein compound differs from its action on the virus of tobacco mosaic disease; in the latter case, Sreenivasaya and Pirie showed not only a splitting of the protein, but also a separation of the prosthetic group (nucleic acid) from the protein.

The effect of SDS on cytochrome *c* (Keilin and Hartree) shows some similarity to the present experiments. Here also the prosthetic group remains attached to the protein, and it is the linkage of the metal, iron, which is apparently modified. However, with cytochrome *c* the effect was found to be reversible on removal of the SDS, while with the chlorophyll-protein compound, no reversal could be obtained.

The effect of pH shows that the lability of the magnesium atom is increased by the splitting and denaturing action of the SDS, and that it may not be the SDS itself which causes the removal of the metal. This is similar to the effect found by Inman and Crowell (1939) who observed that when trypsin is allowed to act on a leaf extract, the formation of phaeophytin by acid is enhanced. Nevertheless, when the chlorophyll-protein compound is split by digitonin or bile salts, no phaeophytin formation takes place even at pH 4.5. It appears that the SDS attacks different linkages in the molecule than those affected by digitonin or bile salts.

Since phaeophytin remains attached to the protein, it seems as though magnesium can play little part in binding the prosthetic group to the smaller protein units. On the other hand, the change in the spectrum produced by SDS at pH 9.0 indicates some modification, unless this effect can be ascribed wholly or in part to the solvent action of the SDS. The chlorophyll groups are probably oriented to the hydrophobic part of the SDS molecule

dissolving the chlorophyll in what may be considered an organic solvent. This may explain the shift of the red band from 678 mμ to 670 mμ, and also the modification of the absorption spectrum in the blue where the carotenoids as well as the chlorophyll would tend to disperse in the paraffin groups of the SDS.

It is too early to speculate much concerning the exact linkage of the chlorophyll to the protein. Linkages appear to be possible through the formyl group of chlorophyll *b*, the vinyl group, the labile hydrogen atoms of Stoll, and the magnesium. From the SDS action, it seems likely that the magnesium plays no rôle in binding chlorophyll to the smaller protein fragments, although it may be concerned in binding the intact molecule. This is indicated by the extreme lability of the magnesium in the presence of SDS and its stability in the presence of other detergents which also split the protein.

SUMMARY

1. Sodium dodecyl sulfate (SDS) attacks the chlorophyll-protein compound modifying its protein properties and absorption spectrum.

2. In the presence of SDS, chlorophyll is quantitatively converted to phaeophytin; *i.e.*, magnesium is removed from the molecule. This reaction, measured spectrophotometrically, proceeds at a rate directly proportional to the hydrogen ion concentration. At constant pH, the rate is proportional to the SDS concentration until a maximum rate is achieved.

3. The chlorophyll or phaeophytin (depending on the pH) remains attached to the protein, since the prosthetic group cannot be separated by ultrafiltration, dialysis, or fractional precipitation.

4. This suggests that the magnesium plays no part in binding chlorophyll to the split protein fragments, but may be concerned in binding the larger units, since the metal becomes extremely labile when the protein is split.

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