The sulfur trioxide may be formed by

$$O + SO_2 \longrightarrow SO_3$$

The small amount of molecular oxygen probably arises by attack of O on SO_3[8] according to

$$O + SO_3 \longrightarrow O_2 + SO_2$$

The alternate explanation that the O_2 is formed by recombination of O atoms in the spurs (analogous to $H + H = H_2$ in irradiated water) can be ruled out by the following argument: for gas phase reactions, the specific rate constant for $O + O + M = O_2 + M$ is 9.8×10^8 l.^{2} mole^{-2} sec.$^{-1}$,[11] whereas for $O + SO_2 + M = SO_3 + M$ it amounts to 3×10^{10} l.^{2} mole^{-2} sec.$^{-1}$.[12] In the condensed phase, in analogy to the events in irradiated water, the O atoms are probably clustered a few ångstrom units apart within isolated volume elements,[13,14] each O atom being surrounded by SO_2 molecules. Therefore, no significant amounts of O_2 arising from recombination of O atoms can be expected.

The reaction $O + SO_3 \rightarrow SO_2 + O_2$ is competitive with $O + SO_2 \rightarrow SO_3$. Applying the usual equations for such cases,[15] the following relations are obtained among $G(SO_3)$, $G(O_2)$, G_O (the primary yield of oxygen atoms), the specific rate constants k of $O + SO_3 = SO_2 + O_2$ and $O + SO_2 = SO_3$, respectively, and the concentrations of SO_2 and SO_3

$$\frac{G_O}{1 + \frac{k_{O+SO_3}[SO_3]}{k_{O+SO_2}[SO_2]}} = G(SO_3) \qquad (1)$$

$$\frac{G_O}{1 + \frac{k_{O+SO_2}[SO_2]}{k_{O+SO_3}[SO_3]}} = G(O_2) \qquad (2)$$

Equations 1 and 2 show that at low doses ($[SO_3] \approx 0$) $G(SO_3) \approx G_O$ and $G(O_2) \approx 0$. With increasing accumulation of SO_3, the rate of oxygen production increases whereas the rate of sulfur trioxide formation decreases. Equation 2 is integrated to give the concentration of oxygen molecules as a function of the dose. Noting that $G(O_2) = d[O_2]/d$ dose, $[SO_2] \approx$ constant, $(k_{O+SO_2}/k_{O+SO_3})([SO_2]/[SO_3]) >> 1$, $G_O \approx G(SO_3)$, and $[SO_3] \approx G(SO_3) \times$ dose, one obtains

$$[O_2] \approx \frac{(G(SO_3) \times \text{dose})^2}{2[SO_2]\frac{k_{O+SO_2}}{k_{O+SO_3}}} \qquad (3)$$

where concentrations are in number of molecules per cm.^{3} and dose is in units of 100 e.v. cm.$^{-3}$. Figure 2 shows the curve according to eq. 3 with $k_{O + SO_2}/k_{O + SO_3} \approx 0.1$. From eq. 1 follows $G_O = 1.35$. According to the proposed mechanism, G_O equals G_{SO}; therefore, the net decomposition of pure liquid sulfur dioxide proceeds with a primary G-value of 1.35.

Acknowledgments.—The author wishes to thank Dr. W. J. Burlant for many fruitful discussions and his continued interest in this work, J. C. Neerman for the mass spectrometric analyses, and M. Valukonis for help with the experiments.

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(12) F. Kaufman, *Proc. Roy. Soc.* (London), **A247**, 123 (1958).
(13) A. O. Allen, "The Radiation Chemistry of Water and Aqueous Solutions," D. Van Nostrand Co., Inc., Princeton, N. J., 1961, p. 7.
(14) C. J. Hochanadel, "Comparative Effects of Radiation," John Wiley and Sons, Inc., New York, N. Y., 1960, Chapter VIII.
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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, POLYTECHNIC INSTITUTE OF BROOKLYN, BROOKLYN 1, N. Y.]

Spectral Properties of Chlorophyllin *a*[1]

BY GERALD OSTER, SUSE B. BROYDE, AND JUDITH S. BELLIN[2]

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The infrared and visible absorption spectra and the fluorescence spectrum of chlorophyllin *a* were determined and compared with those of chlorophyll *a*. The infrared spectrum showed the absence of a carbonyl group except when chlorophyllin *b* was present as an impurity. This provides the basis of a convenient method of analysis of the chlorophyll *b* content of a given chlorophyll preparation. Since the cyclopentanone ring is absent in chlorophyllin, the observed infrared bands help to clarify those observed in the case of chlorophyll. The visible absorption spectrum of chlorophyllin *a* has features in common with that of chlorophyll *a*. In particular, the formation of colloidal aggregates is shown by the appearance of an absorption band at 735 mμ just as is observed for microcrystals of chlorophyll *a*. The appearance of a pronounced absorption maximum at 688 mμ attributed to dimer formation is analogous to that observed by difference spectra in the case of concentrated solutions of chlorophyll *a*. In stretched polymer films chlorophyllin *a* and chlorophyll *a* exhibit a positive dichroism which is greater for the blue absorption maximum than for the red. The fluorescence spectrum of chlorophyllin *a* is markedly dependent on the wave length of excitation.

Introduction

Because of the oleophilic character of chlorophyll, photochemical and spectral studies of this pigment have of necessity been carried out in organic solvents. In the chloroplast, however, some of the chlorophyll molecules are in contact with an aqueous medium. We have therefore undertaken a study of the water-soluble chlorophyll derivative chlorophyllin, in aqueous solution. Chlorophyllin, the product of saponification of chlorophyll in air, has been known for more than 50 years,[3] yet the photochemical and spectral properties of this interesting pigment have not been studied extensively. A preliminary account of our

(1) (a) Supported by the United States Air Force through the Air Force Cambridge Research Laboratories under Contract No. AF19(628)-475 and by the National Institutes of Health under Research Grant No. C6351. (b) Taken in part from the dissertation of Suse B. Broyde, submitted June, 1963, to the Faculty of the Polytechnic Institute of Brooklyn in partial fulfillment of the requirements for the degree of Doctor of Philosophy.
(2) Public Health Service Research Career Awardee.

(3) (a) R. Willstätter and A. Stoll, "Untersuchungen über Chlorophyll," Springer-Verlag, Berlin, 1913; (b) S. Aronoff in "Handbuch der Pflanzenphysiologie," Vol. 5, Part 1, W. Ruhland, Ed., Springer-Verlag, Berlin, 1960.

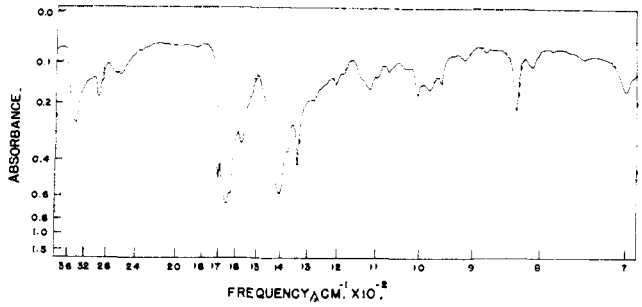

Fig. 1.—Infrared absorption spectrum of chlorophyllin *a* in KBr pellet.

work[4] and its relevance to photosynthesis[5] has been presented. A comparison of some spectral and photochemical properties of chlorophyllin *a* and *b* has been reported recently.[6]

One objective of the present study was to compare the spectral properties of chlorophyll and chlorophyllin since these two pigments differ in important structural respects, notably the absence of the cyclopentanone ring in this latter compound. As will be shown, a study of the spectral properties of chlorophyllin helps to elucidate the spectral characteristics of chlorophyll itself. A subsequent paper[7] will discuss the relevance of the spectral properties of chlorophyllin to its photochemical properties.

Experimental

A. Preparation of Chlorophyllin *a*.—Chlorophyll is extracted from frozen spinach and the chlorophyll *a* component is obtained by chromatographic purification on a powdered sucrose column.[8] The pigment is dissolved in benzene and diluted 1:14 with pentane. Saponification is carried out by the addition of 1 ml. of a filtered 7% solution of KOH in methanol to 50 ml. of the chlorophyll *a* solution. On vigorous shaking the solution turns brown momentarily (Molisch phase test[9,10]), and after a few minutes bluish crystals of chlorophyllin *a* settle out. The crystals are separated, washed with pentane, and dissolved in anhydrous methanol. Carbon dioxide is then bubbled through the solution (in the dark) for 1 hr. in order to remove any remaining KOH as the insoluble methyl carbonate. The solution is then filtered through a very fine sintered glass filter and evaporated to dryness. Partition chromatography of the pigment on paper using either pyridine–phosphate buffer or pyridine–ethyl acetate–phosphate buffer as the eluent reveals the presence of only one component.

B. Other Materials.—Polyvinylpyrrolidone (PVP) used for the binding studies was obtained from the Antara Chemical Division of the General Aniline and Film Corp. The particular sample employed (NP-K90) has a weight average molecular weight of 3.6×10^5. Polyvinyl alcohol used as a medium for dichroic studies was obtained from E. I. du Pont de Nemours and Co., Inc., as Elvanol 72–60. All other chemicals were reagent grade obtained from Fisher Scientific Co.

C. Procedures.—Infrared absorption spectra of the pigments dispersed in KBr pellets were determined on a Perkin–Elmer Model 21 spectrophotometer.

Visible absorption spectra were obtained on the Cary Model 11 recording spectrometer. In the case of solutions, absorption cells having 1-cm. path length were used and an appropriate sham was inserted for concentrated solutions. Dichroic spectra in the visible region were carried out on oriented films of polyvinyl alcohol containing chlorophyllin *a*. Polaroid dichroic filters (Type HNB) were inserted in both the analyzing and reference beams with their direction of vibration vertical. Since it was found that both beams are strongly polarized, the sample was interposed between the polarizer and the detector. Absorption spectra were taken with the direction of stretch of the sample at various angles with respect to the orientation of the polarizer.

The dichroic samples were prepared completely in the dark by adding a concentrated water solution of chlorophyllin *a* to a 10% water solution of polyvinyl alcohol and stirring for 2 hr. with a magnetic stirrer. The resulting homogeneous viscous solution was cast on a silicone-treated glass plate and allowed to dry. The stripped-off film was held firmly at both ends, then warmed to about 75° by holding it momentarily over a hot plate. Then the film was rapidly stretched about threefold and cooled to room temperature in this condition. Dichroic films of chlorophyll *a* can also be obtained by this technique, by adding a methanol solution of chlorophyll *a* to a methanol solution of polyvinylbutyral. The dichroism of these films, however, is less pronounced than those for chlorophyllin *a*–polyvinyl alcohol films due to poorer orientation possibilities with polyvinylbutyral.

Fluorescence spectra obtained by excitation with various wave lengths were determined in the laboratory of Dr. S. S. Brody at the IBM Watson Research Lab. This instrument is a modification of that described[11] and consists of a xenon source whose output passes through a monochromator and an auxiliary filter. The fluorescent light which is at right angles to the exciting beam is examined from the front surface of the sample by passing it through a second monochromator to a cooled end-on multiplier phototube (Dumont Type 6911). All data obtained are corrected for the small amount of scattered exciting light as determined in "blank" runs.

Quenching of the fluorescence was studied in an Aminco light scattering instrument[12] using the 436 mμ mercury line and measuring with a yellow filter the relative light intensity falling on an RCA Type 1P28 multiplier phototube.

Results and Discussion

Infrared Spectra.—The infrared spectrum of chlorophyllin *a* is illustrated in Fig. 1. In common with chlorophyll *a*, there are bands at 3425, 2950, and 1655 cm.$^{-1}$. The bands at 1740, 1700, and 1610 cm.$^{-1}$ which are present in the spectrum of chlorophyll *a* are absent in the case of chlorophyllin *a*. Two peaks at 1570 and 1400 cm.$^{-1}$ which appear in the chlorophyllin *a* spectrum are absent in that for chlorophyll *a*.

A spectrum we obtained for chlorophyll *a* in KBr pellets is identical with that obtained for chlorophyll *a* in organic solvents[13] except that the 3425-cm.$^{-1}$ band which we observed is associated with the moisture content of the sample. The absorption band at 2950 cm.$^{-1}$ is due to CH stretching found both with chlorophyll and with chlorophyllin. The absorption at 1655 cm.$^{-1}$ in chlorophyllin *a* which in chlorophyll *a* itself appears at 1660 cm.$^{-1}$ has, for the latter substance, been attributed to either C=C in phytol[14] or to a perturbed carbonyl[13,15] of the cyclopentanone ring. Since chlorophyllin *a* possesses neither of these structures, they cannot be the origin of this vibration. We therefore attribute the band at 1655 cm.$^{-1}$ for both chlorophyll *a* and chlorophyllin *a* to C=C bonds in the porphyrin structure. The absorption band at 1610 cm.$^{-1}$ present in chlorophyll *a* but absent in chlorophyllin *a* has been attributed to C=C bonds in the chlorophyll ring[13,14] but more likely it is associated with the enol form of the cyclopentanone ring[16] since

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(5) G. Oster and S. B. Broyde, Conference on Photosynthesis CNRS, Gif-sur-Yvette (France), July, 1962, to be published.

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(16) A. N. Sidorov and A. N. Terenin, *Opt. i Spektroskopiya*, **8**, 254 (1960); see, however, A. S. Holt, *Proc. Intern. Congr. Biochem.*, **6**, 59 (1963).

this band occurs only in those porphyrins containing a cyclopentanone ring.[17]

In agreement with Willstätter's contention (ref. 3, p. 303) that chlorophyllin is the salt of a tricarboxylic acid, we find that the band at 1740 cm.$^{-1}$ present in chlorophyll and ascribed to ester groups is absent in chlorophyllin and is replaced by bands at 1400 and 1570 cm.$^{-1}$ characteristic of carboxylate ion.[18]

The strong absorption band at 1700 cm.$^{-1}$ for chlorophyll *a* is also seen in the spectrum of chlorophyllin *b*. Its appearance as a shoulder in Fig. 1 is due to trace amounts of chlorophyllin *b* as an impurity since only the *b* component possesses the strongly absorbing carbonyl (aldehyde) group. This provides the basis of a useful technique for detecting trace amounts of chlorophyll *b* in samples of chlorophyll *a*. The infrared spectrum of the saponified sample establishes the proportion of the two components.

Visible Absorption Spectra.—The visible light absorption spectra of chlorophyllin *a* in water and in methanol are illustrated in Fig. 2. For comparison the spectrum of chlorophyll *a* (in methanol) is also given. The principal bands of chlorophyll *a* at 428 and 660 mμ are shifted to 418 and 640 mμ (or 655 mμ) for chlorophyllin *a*. The shoulder on the blue band of chlorophyll *a* is absent in the chlorophyllin *a* spectra, presumably due to loss of the cyclopentanone ring since it is also absent in the allomerized chlorophyll where the ring has been transformed.[13] Chlorophyllin *a* in water also lacks fine structure. The relative height of the blue to red peaks is about three times greater than for chlorophyll. At concentrations of chlorophyllin *a* greater than 8.3×10^{-6} *M* the absorption at 418 mμ (but not at 640 mμ) does not follow Beer's law unless the path length of the absorption cell is reduced so as to maintain the optical density below 1.5. The same behavior was observed for chlorophyll *a*. This phenomenon is also characteristic of dichroic systems[19] (*cf.* below).

Since crystals of chlorophyllin *a* are hygroscopic and may also contain traces of potassium methyl carbonate, it was found more practicable to determine its molar extinction coefficient by saponification of a known concentration of chlorophyll *a*. Typically a pellet of KOH is added to a methanolic solution of chlorophyll *a* of about 5×10^{-6} *M* (concentration obtained from its known molar extinction coefficient). The solution is then treated with CO_2 to remove excess alkali as the insoluble methyl carbonate. The spectrum in methanol is given in curve B in Fig. 2 where we have assumed complete conversion of chlorophyll *a* to chlorophyllin *a*. The methanolic solution is then evaporated to dryness and redissolved in buffer to give curve A of Fig. 2.

Chlorophyllin *a*, as does chlorophyll, readily loses magnesium under acid conditions to produce chlorin e_6 *a* acid, hereafter referred to as chlorin *a*. This is manifested by a shift in absorption spectrum to about 400 and 660 mμ for the two maxima.[7] Chlorin *a* is less soluble in aqueous media than is chlorophyllin

(17) J. E. Falk and J. B. Willis, *Australian J. Sci. Res.*, **4**, 579 (1951).

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(19) E. H. Land and C. D. West in "Colloid Chemistry," Vol. V1, J. Alexander, Ed., Reinhold Publishing Corp., New York, N. Y., 1946, p. 160. A mathematical formulation is given in Sect. 14.6 of M. Born and E. Wolf, "Principles of Optics," Pergamon Press, London, 1959.

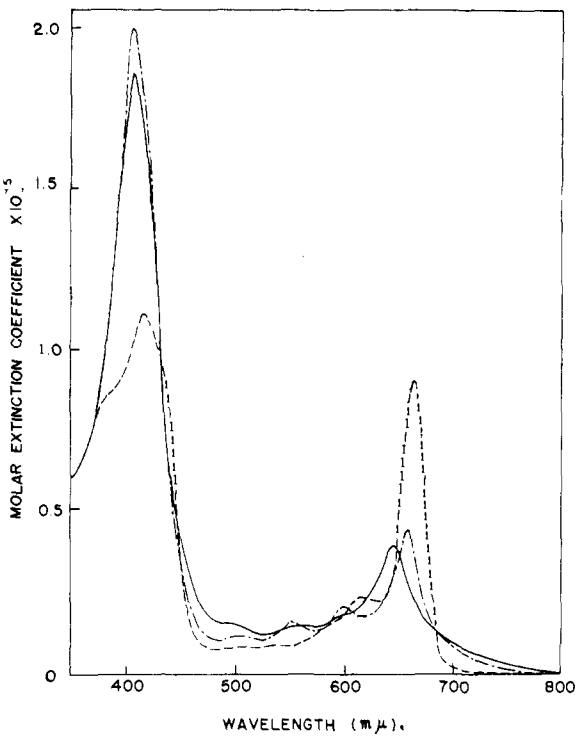

Fig. 2.—Visible absorption spectra of chlorophyllin *a*: A, ———, 0.1 *M* phosphate buffer pH 7.0; B, — · —, in methanol; C, - - - -, chlorophyll *a* in methanol.

a, and its strong red fluorescence is best manifested if methanol or pyridine is added to the aqueous system.

Addition of pyridine to an aqueous solution of chlorophyllin *a* produces a shift of the red peak to 660 mμ and the shoulder at 688 mμ now becomes pronounced. On the addition of trace amounts (*e.g.*, 0.01%) of the water-soluble polymer PVP, which is known to bind anionic dyes, there is an immediate shift of the 640-mμ peak to 660 mμ and on standing in the dark the peak at 688 mμ appears slowly; as it increases, the 660-mμ peak decreases. The formation of the 688-mμ species in the presence of PVP is enormously accelerated by red light. The 688-mμ absorbing species may be related to that obtained for chlorophyll *a* in dioxane–water mixtures[20] or possibly to the 682-mμ absorbing species observed by difference spectra for very concentrated alcoholic solutions of chlorophyll *a*,[21] which is believed to be a dimer.[21-23]

On standing for several hours in the dark chlorophyllin in aqueous buffer at pH 7 forms a nonfluorescent colloidal aggregate with an absorption maximum at 735 mμ. This reaction is accelerated by light. It is of interest that either chlorophyll *a* or ethyl chlorophyllide *a* microcrystals, produced by the addition of water to the organic solutions, also show an absorption peak at 735 mμ.[24] Since both chlorophyll *a* and chlorophyllin *a* give the characteristic peak at 735 mμ, it is evidently the state of aggregation rather than the exact nature of the pigment which is responsible for this effect. On dilution of this colloidal chlorophyllin *a* system with buffer there is a reversion to the 640-mμ absorbing species. Addition of alkali also

(20) B. B. Love and T. T. Bannister, *Biophys. J.*, **3**, 99 (1963).

(21) S. S. Brody and M. Brody, *Nature*, **189**, 547 (1961).

(22) S. S. Brody and M. Brody, *Trans. Faraday Soc.*, **58**, 416 (1962).

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(24) E. Rabinowitch, E. F. Jacobs, A. S. Holt, and R. A. Kromhout, *Z. Physik*, **133**, 261 (1952).

TABLE I

DICHROISM OF CHLOROPHYLLIN *a*

	λ, mμ									
	418		525		575		645		688	
θ, degrees	$D\theta - D_{90}$	$D\theta/D_{90}$	$D\theta - D_{90}$	$D\theta/D_{90}$	$D\theta - D_{90}$	$D\theta/D_{90}$	$D\theta - D_{90}$	$D\theta/D_{90}$	$D\theta - D_{90}$	$D\theta/D_{90}$
0	1.15	1.77	0.07	3.34	0.07	2.67	0.52	2.80	0.17	2.00
15	1.06	1.71	.07	3.34	.07	2.67	.48	2.65	.17	2.00
30	0.74	1.50	.05	2.66	.05	2.20	.35	2.20	.14	1.82
45	.53	1.35	.04	2.27	.04	1.95	.24	1.83	.11	1.65
60	.24	1.16	.01	1.27	.01	1.33	.10	1.34	.05	1.30
75	.13	1.09	.01	1.16	.01	1.23	.05	1.18	.02	1.12
90	..	1.00	..	1.00	..	1.00	..	1.00	..	1.00

causes reversion to the original chlorophyllin *a*. If the colloidal system is extracted with chloroform and the dried extract is suspended in KBr, the infrared spectrum is identical with that of ordinary chlorophyllin *a* described above except for the presence of bands at 1250 and 1739 cm.$^{-1}$ which are characteristic of undissociated carboxylic acids.

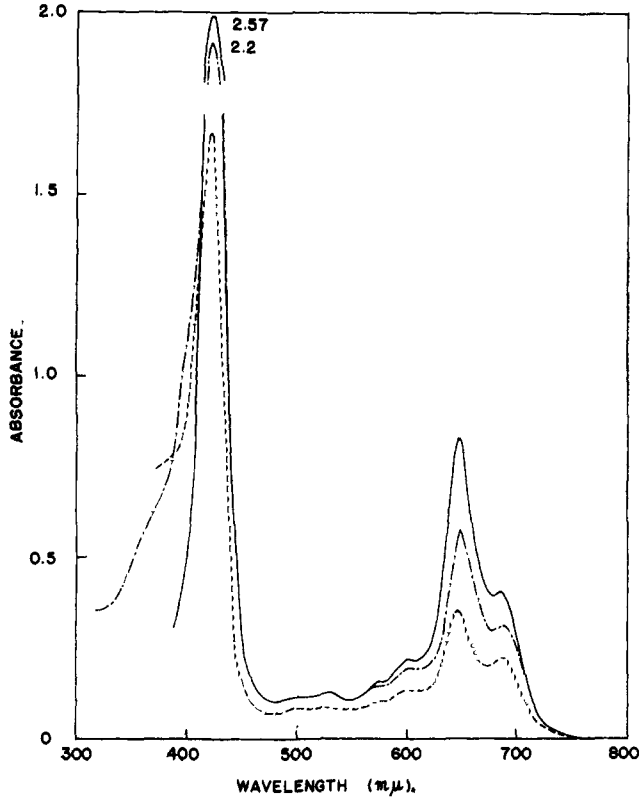

Fig. 3.—Dichroic spectra of chlorophyllin *a* in stretched film of polyvinyl alcohol: ———, parallel ($\theta = 0°$); - - - -, perpendicular ($\theta = 90°$); — · —, unstretched film.

Addition of water-miscible organic solvents such as methanol or pyridine dissolves the colloid to give a red fluorescent solution absorbing maximally at 688 and at 640 mμ. The same effect can be obtained by adding trace amounts of PVP. It would appear that the 688-mμ absorbing species is a dimer formed *via* hydrogen bonding of the carboxylic acid groups and that the 735-mμ absorbing species is a colloidal aggregate of these dimeric species and is only poorly molecularly dispersed in purely aqueous media.

The dichroic absorption spectrum of chlorophyllin *a* in a stretched film of polyvinyl alcohol is illustrated in Fig. 3. The dichroism is always positive regardless of the relative orientations of the sample axis with respect to the direction of vibration of the polarizer, and the absorption is greatest when both axes coincide. A similar result, but less pronounced, was obtained with chlorophyll *a* in stretched films of polyvinylbutyral. In oriented micelles of ammonium oleate chlorophyll exhibits dichroism.[25] Some representative data for chlorophyllin *a* are given in Table I where the angle θ is that between the vibrational axis of the polarizer and the direction of stretch of the sample. It should be noted that the dichroism is greater for the blue peak than for the red region.

The fluorescence spectrum of chlorophyllin *a* varies markedly with the wave length of excitation and to some extent with the nature of the solvent. The value for the maxima in the fluorescence spectra are given in Table II. In pyridine or in methanol excitation with

TABLE II

FLUORESCENCE SPECTRA OF CHLOROPHYLLIN *a*

Solvent	Exciting light, mμ	Fluorescence maxima, mμ (rel. intensities in parentheses)	
Methanol	436	660 (1.6)	705 (1.9)
	640	647 (14.8)	705 (4.5)
Phosphate buffer (pH 7)	436	656 (0.6)	705 (0.4)
	640	645 (27.8)	705 (0)
Above with 10% pyridine	436	655 (5.1)	709 (5.0)
	640	645 (26.3)	715 (8.1)
	678	680 (100)	709 (21.8)

blue light produces less of the deep red (705–715 mμ) component than does excitation with red light. It is possible that this deep red fluorescence arises from the 688 mμ absorbing species which also absorbs light at 640 and 436 mμ. This species does not exist in aqueous buffer alone but does contribute to the fluorescence at *ca.* 710 mμ when pyridine is added. Here its contribution to the fluorescence is particularly evident when 678-mμ light is used for excitation. The data further illustrate that the shorter wave length red fluorescence is produced with a greater fluorescence efficiency when excited with red light than with blue light. In addition one also observes that excitation with blue light produces a fluorescence band at slightly longer wave lengths. These observations indicate that internal conversion between the two electronic states associated with the two principal absorption bands does not readily take place (compare ref. 26). The photochemical manifestations of these phenomena are considered elsewhere.[7]

The over-all red fluorescence of chlorophyllin *a* is quenched by a number of substances. In methanol

(25) *Cf.* H. Zocher, *Trans. Faraday Soc.*, **35**, 34 (1939); J. C. Goedheer, *Biochim. Biophys. Acta*, **16**, 471 (1955); compare W. D. Bellamy, G. L. Gaines, and A. G. Tweet, *J. Chem. Phys.*, **39**, 2528 (1963).

(26) G. Oster and G. K. Oster in "Luminescence of Organic and Inorganic Materials," H. Kallman and G. M. Spruch, Ed., John Wiley and Sons, Inc., New York, N. Y., 1962.

the fluorescence of chlorophyllin *a* is quenched by nitrobenzene following the Stern–Volmer relation with a quenching constant of 29 l./mole. If every encounter (1.1×10^{10} l./sec.) leads to a quenching, then the lifetime of excited chlorophyllin *a* is 2.8×10^{-9} sec. The intrinsic lifetime obtained by integrating over the red absorption band and using the Ladenburg formula gives a lifetime of 2.2×10^{-8} sec., indicating that the fluorescence efficiency is about 10%. A more powerful fluorescence quencher for chlorophyllin (as well as for chlorophyll[27]), *p*-quinone, gives a Stern–Volmer constant of 440 l./mole. Here, since the quenching constant is so high, the quenching can obviously not be attributed to diffusional encounters and must be due to complex formation. Direct evidence of this is seen by the fact that *p*-quinone, in amounts sufficient for appreciable quenching, causes a shift in the red absorption band of chlorophyllin *a* to shorter wave lengths by 15 mμ.

Upon continuous strong illumination (from a high pressure mercury lamp) and in the absence of oxygen, both chlorophyll *a* and chlorophyllin *a* in plastics at room temperature exhibit a reversible brown coloration with a lifetime of about 1 sec.[28] Apparently this metastable species is identical with that observed in flash spectroscopy[29–31] and attributed to triplet–triplet absorption.

(27) R. Livingston and C. Ke, *J. Am. Chem. Soc.*, **72**, 909 (1950).

(28) G. Oster, to be published.

(29) R. Livingston, *J. Am. Chem. Soc.*, **77**, 2179 (1955).

(30) H. Linschitz and K. Sarkanen, *ibid.*, **80**, 4826 (1958).

(31) S. Claesson, L. Lindqvist, and B. Holmström, *Nature*, **183**, 661 (1959).

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Photochemical Properties of Chlorophyllin *a*[1]

BY GERALD OSTER, JUDITH S. BELLIN,[2] AND SUSE B. BROYDE

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Chlorophyllin *a* can be photoreduced and can sensitize photoreductions and photooxidations in a manner similar to that of chlorophyll *a*. Neither the cyclopentanone ring nor magnesium is essential for photochemical activity. Chlorophyllin *a* (or chlorin *a*) undergoes a reversible photoreduction in the presence of ascorbic acid to give a product which can in a subsequent reaction either complex with pyridine to give a stable pink substance (the Krasnovsky intermediate) or reduce an azo dye. The kinetics of these reactions are presented and require the participation of a long-lived metastable state of chlorophyllin. The quantum yield of the Krasnovsky reaction is greater for red light excitation than for blue light. In sensitized photooxidation the metastable species react with oxygen to give an unstable intermediate which oxidizes the substrate. Binding of chlorophyllin to a high polymer enhances its photochemical activity.

Introduction

In the previous paper[3] we demonstrated that many of the spectral properties of chlorophyll *a* are shown by its water-soluble derivative chlorophyllin *a* despite the absence of the cyclopentanone ring in this latter compound. In fact, a comparison of the spectral properties of the two pigments helped to explain those previously reported for chlorophyll *a*. The present paper will demonstrate that the photochemical properties of the two substances likewise resemble each other. It is particularly convenient to study the photochemistry of chlorophyll-like pigments in aqueous solution, since many physiological substances involved in photosynthesis are water soluble. The photochemical properties of chlorophyll, notably its photoreduction and its sensitization of photoreduction and photooxidation, in organic solvents have been extensively investigated.[4–7] The addition of water to organic solutions of chlorophyll yields colloidal systems whose photometry is difficult to define.

Chlorophyll dissolved in pyridine containing a trace of water and in the presence of a reductant such as ascorbic acid undergoes a reversible photoreduction yielding a pink intermediate.[8] This reaction (the Krasnovsky reaction) is also exhibited by chlorophyll analogs.[6,9] A photoreduced form of chlorophyll may be produced in photosynthesis since differential spectrophotometric studies of bacteria and algae show that under certain limiting conditions an intermediate is found which resembles Krasnovsky's pink substance.[5,10] Since the precise conditions for the photoreduction of chlorophyll are difficult to reproduce and since the system is not completely reversible,[11] a kinetic study of the Krasnovsky reaction has not been conducted. In the case of chlorophyllin, however, and especially for its magnesium-free analog chlorin, the reaction is easily reproduced and completely reversible.

Sensitized photoreductions and photooxidations, as well as reduction of the pigment itself, are also exhibited by synthetic dyes (see, for example, ref. 12–14). It has been shown that these reactions

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