

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 ( $1.1 \times 10^{10}$  l./sec.) leads to a quenching, then the lifetime of excited chlorophyllin *a* is  $2.8 \times 10^{-9}$  sec. The intrinsic lifetime obtained by integrating over the red absorption band and using the Ladenburg formula gives a lifetime of  $2.2 \times 10^{-8}$  sec., indicating that the fluorescence efficiency is about 10%. A more powerful fluorescence quencher for chlorophyllin (as well as for chlorophyll<sup>27</sup>), *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

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

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 m $\mu$ .

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.<sup>28</sup> Apparently this metastable species is identical with that observed in flash spectroscopy<sup>29-31</sup> and attributed to triplet-triplet absorption.

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## Photochemical Properties of Chlorophyllin *a*<sup>1</sup>

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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<sup>3</sup> 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.<sup>4-7</sup> 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.<sup>8</sup> This reaction (the Krasnovsky reaction) is also exhibited by chlorophyll analogs.<sup>6,9</sup> 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.<sup>5,10</sup> Since the precise conditions for the photoreduction of chlorophyll are difficult to reproduce and since the system is not completely reversible,<sup>11</sup> 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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(2) Public Health Service Research Career Awardee.

(3) G. Oster, S. B. Broyde, and J. S. Bellin, *J. Am. Chem. Soc.*, **86**, 1309 (1964).

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(10) J. W. Coleman and E. I. Rabinowitch, *J. Phys. Chem.*, **63**, 30 (1959).

(11) T. T. Bannister, *Plant Physiol.*, **34**, 246 (1959).

(12) G. K. Oster and G. Oster, *J. Am. Chem. Soc.*, **81**, 5543 (1959).

(13) G. Oster, J. S. Bellin, R. W. Kimball, and M. Schrader, *ibid.*, **81**, 5095 (1959).

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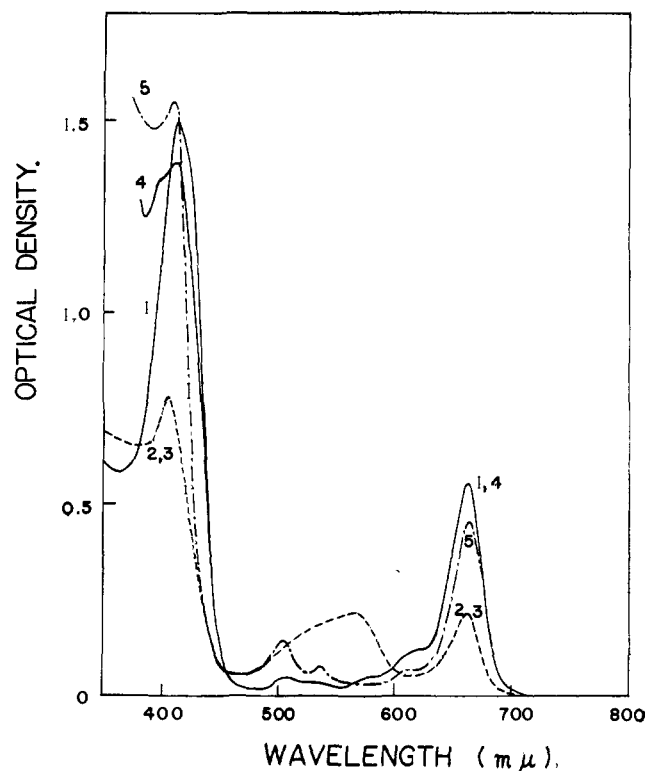


Fig. 1.—Spectral changes of chlorophyllin *a* on photoreduction. All solutions contain  $10^{-5}$  *M* chlorophyllin *a*, 0.20% PVP, and 6% pyridine in 0.1 *M* pH 7.0 phosphate buffer. With 0.01 *M* ascorbic acid, original spectrum (curve 1) is transformed on illumination for 6 min. with red light into curve 2, which on standing in the dark for 24 hr. reverts to curve 4. With 0.07 *M* ascorbic acid curve 1 is transformed on illumination into curve 3, which on standing reverts to curve 5.

proceed *via* a long-lived metastable state and that binding to a polymeric substrate can, in some cases, increase the transition to this state and hence enhance the quantum yield of the reaction.<sup>15-17</sup> The present paper consists of a study of the kinetics of some photochemical reactions of chlorophyllin *a*. These reactions include the photoreduction of chlorophyllin *a* in the presence of pyridine and ascorbic acid, the chlorophyllin *a*-sensitized photoreduction of the azo dye fast red S, and the chlorophyllin *a*-sensitized photooxidation of *p*-toluenediamine in the presence of oxygen. The effect on these reactions of binding chlorophyllin *a* to polyvinylpyrrolidone in solution is examined. This effect could be of importance because chlorophyll is in the bound state in the chloroplast.

### Experimental

**Materials.**—Chlorophyllin *a* was obtained as described previously.<sup>3</sup> Chlorin *a* acid (hereafter referred to as chlorin *a*) was prepared by dissolving chlorophyllin *a* in pH 5.0 acetate buffer under which conditions the magnesium is removed from the porphyrin ring.

Polyvinylpyrrolidone (PVP) was obtained from Antara Chemicals and was of Type NP-K90 (weight average molecular weight  $3.6 \times 10^5$ ). All other chemicals were obtained as reagent grade from Fisher Scientific Co. Traces of oxygen were removed from prepurified nitrogen (Airco) by bubbling through a solution of chromous chloride. Oxygen-nitrogen mixtures were obtained from Matheson Co.

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(16) G. Oster and J. S. Bellin, *ibid.*, **79**, 294 (1957).

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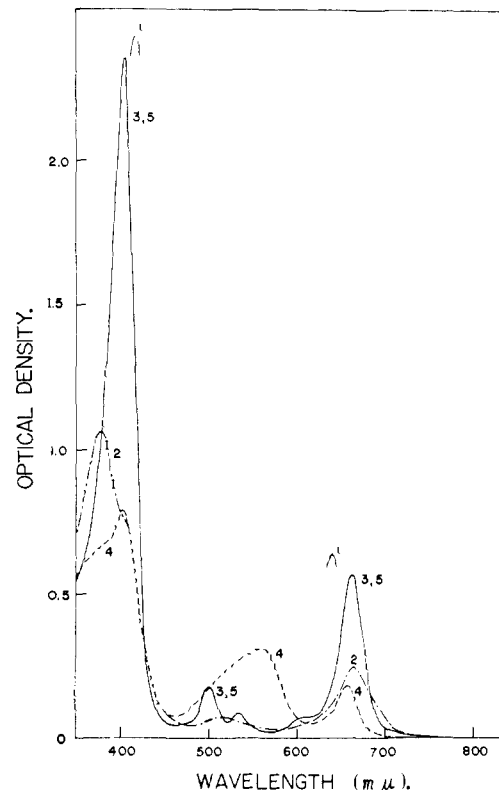


Fig. 2.—Spectral changes of chlorin *a* on photoreduction (1 indicates peaks of original chlorophyllin). Solutions contain  $10^{-5}$  *M* chlorin *a* and 0.05 *M* ascorbic acid in 0.1 *M* pH 5.0 acetate buffer. Chlorin *a* (curve 2) changes to curve 3 on addition of 6% pyridine. On illumination with red light for 4 min. curve 4 is obtained which on standing in the dark for 24 hr. gives curve 5.

**Procedures.**—Most photochemical rate studies were performed using a 500-watt tungsten lamp slide projector as the light source. For certain experiments a 1000-w. mercury-xenon lamp (Hanovia 528B9) was employed in conjunction with a heat filter and a bi-convex lens arranged so that the sample (in a  $5 \times 5 \times 1$  cm. cell) was uniformly illuminated. A red interference filter (Farrand 10- $m\mu$  band width) having a maximum in transmittance at the red absorption maximum (either 660 or 690  $m\mu$ ) of the solution in question was placed between the light source and the reaction cell. For studies using blue incident light from the stronger source a 420- $m\mu$  interference filter was employed. In order to monitor the concentration of photoreduced chlorophyllin *a* (absorbing maximally at 550  $m\mu$ ) the transmittance of the solution was determined at 30-sec. intervals by interposing a green transmitting filter combination (Corning numbers 2075-S, 8484, 4303, and 5120 in conjunction with a 550- $m\mu$  interference filter). For the chlorophyllin *a*-sensitized photoreduction of fast red S a 540- $m\mu$  interference filter was employed and for the sensitized photooxidation (in the presence of oxygen) a 525  $m\mu$  interference filter was used instead of the filter combination.<sup>18</sup> This arrangement<sup>18</sup> as well as a description of the detector (silicon cell), light intensity measurements, and the deaerating procedures employed are described in detail elsewhere.<sup>18,19</sup> Except where otherwise noted all solutions were freshly prepared in 0.1 *M* phosphate buffer at pH 7.0. Quantum yield measurements were obtained from initial rates only.

### Results

**Photoreduction of Chlorophyllin *a*.**—Illumination of an anaerobic aqueous solution of chlorophyllin *a* containing pyridine and ascorbic acid (Fig. 1, curve 1) causes decrease in the absorption maxima at 660 and 418  $m\mu$ , and a new maximum at 550  $m\mu$  appears (Fig. 1, curve 2). At concentrations of ascorbic acid less than about  $4 \times 10^{-2}$  *M* the original chlorophyllin *a* is almost completely regenerated if allowed to stand in the dark for several hours (Fig. 1, curve 4). At higher reductant

(18) G. Oster, G. K. Oster, and G. Karg, *J. Phys. Chem.*, **66**, 2514 (1962)

concentrations reversibility is less complete (Fig. 1, curves 3 and 5). Figure 2 illustrates the results obtained by illuminating a solution of chlorin *a* in the presence of pyridine and ascorbic acid at pH 5.0. The pink species (maximum at 550  $m\mu$ ) produced (Fig. 2, curve 4) is identical with that illustrated in Fig. 1, curve 2, and is completely regenerated on standing in the dark (Fig. 2, curve 5). A solution of either chlorophyllin *a* or chlorin *a* containing ascorbic acid becomes turbid when illuminated with red light and reverts back to the original clear green solution on standing for some hours. Upon the subsequent addition of pyridine to the turbid solution, it becomes clear and pink in color. The pink species was found to be photochemically inactive and the dark recovery is unaffected by illumination with green light.

The quantum yield for the production of pink pigment in the presence of ascorbic acid and pyridine is independent of the intensity of red actinic light over a 100-fold range as demonstrated with neutral density filters. The quantum yield of the reaction is nearly five times greater for red light than for blue. Using the xenon-mercury lamp, it was found that with radiation of 660  $m\mu$  ( $7.85 \times 10^{-9}$  einstein/cm.<sup>2</sup>/sec.) the quantum yield for pink pigment production is  $3.3 \times 10^{-3}$ , the same value as was obtained with a tungsten lamp. The quantum yield for blue light from the xenon-mercury lamp ( $26.5 \times 10^{-9}$  einstein/cm.<sup>2</sup>/sec.), on the other hand, was  $0.71 \times 10^{-3}$ .

The Krasnovsky reaction for chlorophyllin *a* or chlorin *a* in aqueous media is readily observable even if only small amounts of pyridine are added. In a typical case, namely  $10^{-5}$  *M* chlorophyllin *a* and  $10^{-3}$  *M* ascorbic acid, the quantum yield increases approximately linearly with increasing pyridine concentration up to about 6%, whereupon it decreases until at 15% pyridine the rate is about half that of its maximum value after which it is independent of pyridine concentration.

For ascorbic acid concentrations less than about  $4 \times 10^{-2}$  *M* where reversibility occurs (Fig. 1) the quantum yield  $\Phi$  as a function of ascorbic acid concentration ( $A_R$ ) follows the empirical expression  $\Phi = (A_R)/\alpha + \beta(A_R)$  where the empirical constants  $\alpha$  and  $\beta$  are 3.76 moles/l. and 70, respectively (Fig. 3). At reductant concentrations greater than about  $4 \times 10^{-2}$  *M* there are deviations from linearity in the reciprocal plot (Fig. 3).

The quantum yield for the production of pink species is independent of chlorophyllin *a* concentration up to at least  $2 \times 10^{-4}$  *M* in chlorophyllin *a* and is  $3 \times 10^{-3}$  for red light. At low chlorophyllin *a* concentrations in the presence of 0.20% PVP, which is more than sufficient to bind all the chlorophyllin *a*, the quantum yield increases with increasing concentration of bound pigment and reaches the same level value found for free pigment, namely,  $3 \times 10^{-3}$  at chlorophyllin *a* concentrations greater than  $1.5 \times 10^{-5}$  *M*. Under these same conditions chlorin *a* bound to PVP also exhibits this behavior, reaching a level value for the quantum yield of  $5.5 \times 10^{-3}$ . For both cases the quantum yield as a function of bound pigment concentration (*D*) can be described by the empirical formula  $\Phi = (D)/[\gamma + \delta(D)]$  where  $\gamma$  and  $\delta$  are  $2.79 \times 10^{-4}$  mole/l. and 315, respectively, for chlorophyllin *a*,

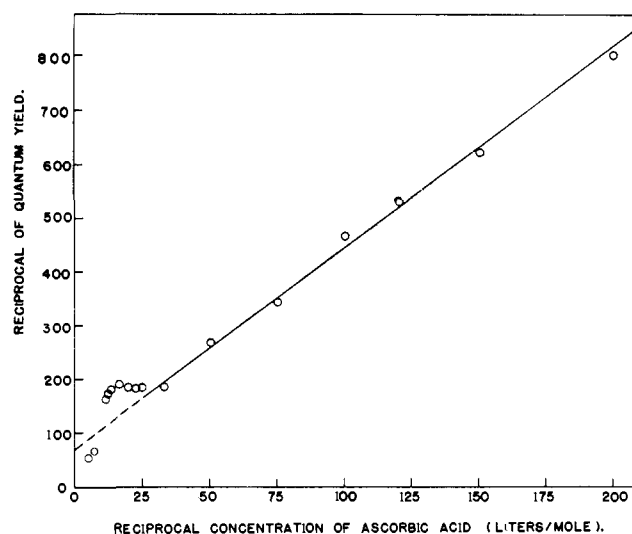


Fig. 3.—Quantum yield of photoreduction of chlorophyllin *a* as a function of ascorbic acid concentration; chlorophyllin *a* concentration  $10^{-5}$  *M*, 0.2% PVP, and 6% pyridine in 0.1 *M* pH 7.0 phosphate buffer.

and  $4.81 \times 10^{-4}$  mole/l. and 165, respectively, for chlorin *a*.

The reaction is retarded by very small amounts of nitrobenzene. The plot of the reciprocal of the rate *vs.* nitrobenzene concentration is linear and the rate is reduced by a factor of two at a concentration of nitrobenzene of  $6.2 \times 10^{-6}$  *M*.

In the presence of pyridine the pink product can be produced by replacing ascorbic acid by reductants such as phenylhydrazine hydrochloride or cysteine. However, EDTA and other ethylenediamine derivatives which serve as electron donors for light-excited synthetic dyes<sup>12,18,19</sup> are ineffectual in this reaction. In the presence of ascorbic acid, pyridine can be replaced by 3,4-pyridinedicarboxylic acid. The rate of formation of the pink species is considerably less with 2-methylpyridine and does not proceed at all with 2,6-dimethylpyridine.

**Chlorophyllin *a*-Sensitized Photoreduction.**—Chlorophyllin *a* sensitizes the photoreduction of a variety of substrates just as do chlorophyll<sup>20-22</sup> and various photoreducible synthetic dyes.<sup>23</sup>

Chlorophyllin *a* is a sensitizer for the photoreduction of tetrazolium salts, indophenol dyes, and many azo dyes where the electron donor may be ascorbic acid, phenylhydrazine hydrochloride, cysteine, or methionine. For the quantitative studies the light-insensitive anionic azo dye fast red S was employed as the substrate since its absorption maximum (480  $m\mu$  when free and 504  $m\mu$  when bound to PVP) lies between the two absorption maxima of chlorophyllin *a*, and the reduced form is colorless. Ascorbic acid was used as the hydrogen donor and it, as well as the azo dye and chlorophyllin *a*, bind, as do most anions, to PVP.<sup>24</sup> Binding to PVP enhances the quantum yield of photoreduction considerably, and for complete binding (0.20% PVP) the quantum yield with red light is  $4.1 \times 10^{-3}$ , a factor

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(20) J. C. Ghosh and S. B. Sen-Gupta, *J. Indian Chem. Soc.*, **11**, 65 (1934).

(21) R. Livingston and R. Pariser, *J. Am. Chem. Soc.*, **78**, 2948 (1956).

(22) R. Livingston and R. Pariser, *ibid.*, **70**, 1510 (1948).

(23) G. Oster, *J. chim. phys.*, **55**, 899 (1958).

(24) G. Oster, *J. Polymer Sci.*, **16**, 235 (1955).

18 times that for unbound chlorophyllin *a*. For fully bound chlorophyllin *a* (the rates for unbound species were too low for accurate measurement) the quantum yield of photoreduction of fast red S is independent of chlorophyllin *a* concentration at least up to  $10^{-5} M$  and is independent of light intensity.

The dependence of quantum yield  $\Phi$  on ascorbic acid concentration ( $A_R$ ) can be represented by  $\Phi = (A_R)/[a + b(A_R)]$  where the empirical constants  $a$  and  $b$  are  $4.6 \times 10^{-2}$  mole/l. and 160, respectively, for optimal azo dye concentration (see below). The quantum yield as a function of fast red S concentration first increases then passes through a maximum (at  $2.2 \times 10^{-5} M$  azo dye, *i.e.*, at ten times the chlorophyllin concentration employed) and then decreases. The quantum yield as a function of azo dye concentration ( $S$ ) is given roughly by an equation of the form  $\Phi = (S)/[c + d(S) + e(S)^2]$ .

Nitrobenzene retards the reaction according to the Stern-Volmer expression, and half retardation occurs at a nitrobenzene concentration of  $5.2 \times 10^{-5} M$ . Pyridine also retards the reaction, but the concentrations required are large and the reaction is half retarded at 0.4 *M* pyridine.

**Chlorophyllin *a*-Sensitized Photooxidation.**—In the presence of oxygen, chlorophyllin *a* is an effective sensitizer for the photooxidation of many biological substrates and of easily autoxidizable substances, particularly *p*-toluenediamine (PTD) where the quantum yield with red light can be as high as 0.8. In the over-all reaction chlorophyllin *a* remains unchanged and PTD is oxidized. The fluorescence intensity and spectrum of chlorophyllin *a* are unchanged by the presence of PTD at the concentrations employed (up to  $2.5 \times 10^{-3} M$ ) in this quantitative study. Binding of chlorophyllin *a* to the polymer PVP increases the quantum yield only by a factor of 3.5 over that of the free pigment.

The quantum yield for the photooxidation decreases markedly with increasing chlorophyllin *a* concentration ( $D$ ). According to the Stern-Volmer expression  $\Phi = p/[1 + q(D)]$  where, for bound pigment, the empirical constants are 0.8 and  $5.6 \times 10^5$  l./mole, respectively, at PTD concentration of  $2.0 \times 10^{-3} M$ , and a bound chlorophyllin concentration of  $10^{-5} M$ .

The quantum yield of photooxidation is sensitive to variations in oxygen concentration below  $4 \times 10^{-4} M$ , above which it is independent of the oxygen concentration. Empirically the quantum yield as a function of oxygen concentration ( $O_2$ ) (solubility in water based on Henry's law) is given by the expression  $\Phi = (O_2)/[m + n(O_2)]$  where  $m$  and  $n$  are  $1.39 \times 10^{-4}$  mole/l. and 3, respectively, when the bound chlorophyllin concentration is  $1.0 \times 10^{-5} M$  and the PTD concentration is  $2.0 \times 10^{-3} M$ . In a similar way the quantum yield depends on PTD concentration ( $P$ ) following the empirical expression  $\Phi = (P)/[r + s(P)]$  where  $r$  and  $s$  are  $2.26 \times 10^{-3}$  mole/l. and 1.8, respectively, when the bound chlorophyllin concentration is  $1.0 \times 10^{-5} M$  and the system is in equilibrium with pure oxygen ( $(O_2) = 1.28 \times 10^{-3} M$ ).

### Discussion

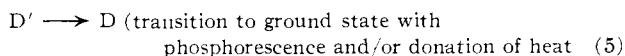
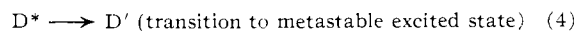
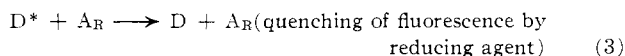
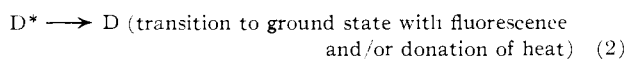
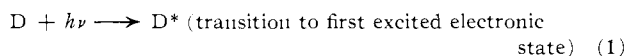
The pink product produced in the Krasnovsky reaction is a manifestation of the complexation by pyridine of a photoreduced chlorophyll *a* species where

ascorbic acid is the hydrogen donor. The fact that the pink species can be obtained by adding pyridine *subsequent* to the photoreduction of either chlorophyllin *a* or chlorin *a* clearly demonstrates this contention. A pink product may also be produced from chlorin *a* by using phenylhydrazine as both the reducing agent and complexing agent (compare ref. 25). The poorly soluble reduction product obtained on the illumination of chlorophyllin *a* (or chlorin *a*) in the presence of ascorbic acid may be analogous to the transient electrode-active intermediates found on photoreduction of chlorophyll in pyridine which can be stabilized at low temperatures (compare ref. 26 with ref. 27 and 28). The complexation apparently occurs *via* the nitrogen of the pyridine ring since substitution at the 2- and 6-positions blocks the formation of the pink species. Addition of excess PVP also inhibits the formation of the pink species, perhaps because it acts to prevent complexation by pyridine (see below). Thus the role of pyridine is not solely one of providing a polar environment as has been suggested<sup>11</sup> for the case of chlorophyll *a* and ascorbic acid.

In agreement with other workers<sup>6</sup> we find that neither the cyclopentanone ring nor magnesium is required for the production of the pink species. Indeed, stable photoreduced intermediates are produced with cyanine<sup>29</sup> and with xanthene dyes<sup>30,31</sup> illuminated in the presence of pyridine and a reducing agent. It would appear, therefore, that the *in vivo* formation of a transient pink species<sup>6,10</sup> may not be due to the Krasnovsky intermediate but rather to a reduced plant enzyme (*e.g.*, cytochrome *f*<sup>6,10,32</sup>).

The dependence of the quantum yield of photoreduction and of fluorescence on the wave length of exciting light agrees with our contention<sup>3</sup> that conversion between the two electronic states associated with the two main absorption bands in chlorophyllin *a* (and indeed in chlorophyll *a*) does not readily take place. Excitation with blue light may lead to a different metastable state than does excitation with red light. This phenomenon has been clearly demonstrated in the case of bound malachite green.<sup>17</sup>

A scheme which is consistent with the data on the kinetics of both the Krasnovsky reaction and the sensitized photoreduction of fast red S is



(25) V. B. Evstigneev and V. A. Gavrilova, *Dokl. Akad. Nauk SSSR*, **91**, 899 (1953).

(26) V. B. Evstigneev and V. A. Gavrilova, *ibid.*, **103**, 97 (1955).

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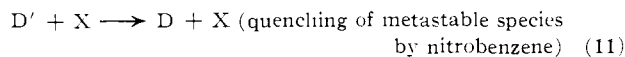
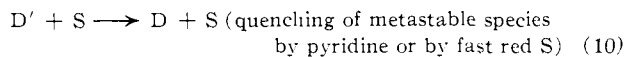
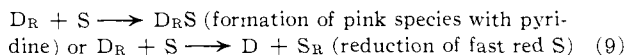
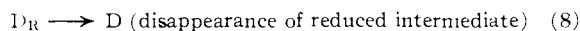
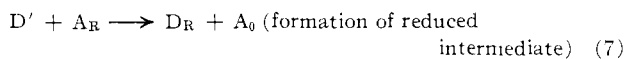
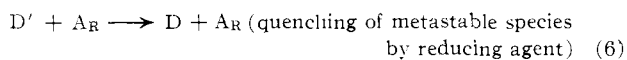
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(31) N. N. Bubnov, V. F. Tsepalov, and V. Ya. Shlyapintokh, *Izv. Akad. Nauk SSSR, Ser. Fiz.*, **23**, 1265 (1959).

(32) See articles by L. N. M. Duysens and by B. Chance in "Research in Photosynthesis," H. Gaffron, *et al.*, Ed., Interscience Publishers, Inc., New York, N. Y., 1957.



Using the usual steady-state argument for the transient species  $D^*$ ,  $D'$ , and  $D_R$ , one obtains the following expression for the quantum yield

$$\Phi = \frac{\left[ \frac{k_4}{k_2 + k_4 + k_3(A_R)} \right]}{\left[ \frac{k_7(A_R)}{k_5 + (k_6 + k_7)(A_R) + k_{10}(S) + k_{11}(X)} \right] \left[ \frac{k_9(S)}{k_8 + k_9(S)} \right]}$$

The proposed scheme is compatible with the fact that the quantum yield of photoreduction of chlorophyllin *a*, in the absence of PVP, and of sensitized photoreduction of fast red S is independent of chlorophyllin *a* concentration. This implies that self-quenching of the metastable state, *i.e.*,  $D' + D \rightarrow 2D$ , is unimportant. Flash spectroscopic studies of chlorophyll in benzene at room temperature (for review, see ref. 33) demonstrate that the rate constant for this step is only one-hundredth that of encounter frequencies. The apparent unimolecular disappearance of the reduced intermediate (step 8) could be a bimolecular reaction with an excess of some electron acceptor. If the acceptor were ground-state chlorophyllin *a* molecules (*i.e.*, *via* dismutation) then, in order to have a constancy of quantum yield, one would have to postulate a compensating step such as  $D^* + D \rightarrow D' + D$ . Although there is evidence for this latter process in the case of some synthetic dyes in the bound state,<sup>15,16</sup> our data on chlorophyllin-*a* do not require such a step. Our postulated intermediate  $D_R$  appears to be analogous to that found by flash spectroscopy of chlorophyll in pyridine containing ascorbic acid.<sup>34</sup> This chlorophyll intermediate decays unimolecularly in part to give, in the presence of pyridine, the Krasnovsky pink species.<sup>34</sup>

The observed increase in quantum yield with increasing low pyridine or fast S concentrations is consistent with step 8. At higher substrate concentrations the quantum yield decreases, presumably due to step 10. Both pyridine and PVP have affinity for anions and hence would be expected to be competitive. This may explain the observed inhibition by PVP of the Krasnovsky reaction.

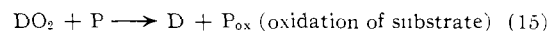
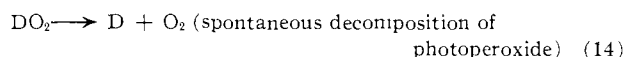
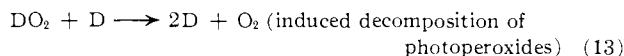
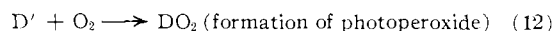
The observed linearity of the reciprocal quantum yield *vs.* reciprocal ascorbic acid concentration (Fig. 3) shows that step 3 is not important at reductant concentrations less than  $4 \times 10^{-2} M$ . At higher concentrations fluorescence quenching (step 3) decreases the quantum yield, and further increase in ascorbic acid induces formation of chlorin *a* (compare, in Fig. 1,

curves 4 and 5) which has a higher quantum yield of photoreduction than does chlorophyllin *a*.

The observed retardation of the reactions with nitrobenzene makes it possible to calculate the lifetime of the metastable excited state. The Krasnovsky reaction is retarded to half its maximum value by  $6.2 \times 10^{-6} M$  nitrobenzene. If every encounter ( $6.6 \times 10^9$  collisions/sec./1. mole in water at room temperature) is effective in step 11, then the lifetime  $1/k_5 = 2.4 \times 10^{-5}$  sec. This is about 10,000 times greater than the lifetime of the first excited singlet state as determined by fluorescence quenching.<sup>3</sup> The retardation by nitrobenzene was less effective for the sensitized photoreduction than for the Krasnovsky reaction presumably because quenching by substrate (step 10) is more competitive with step 11 in the case of fast red S than in the case of pyridine. Indeed the rate constant for step 10 is nearly one thousand times greater for the sensitized photoreduction than for the Krasnovsky reaction. This also explains the observed decrease of quantum yield at fast red S concentrations greater than  $2 \times 10^{-5} M$  or at pyridine concentrations greater than 6% (0.76 *M*). A further reflection of this is the fact that high concentrations of pyridine retard the photosensitized reduction of fast red S.

The highest observed quantum yield for the Krasnovsky and the sensitized photoreduction reactions are  $3 \times 10^{-3}$  and  $4 \times 10^{-3}$ , respectively, for chlorophyllin (80% higher for chlorin). According to the scheme, the limiting steps would be numbers 4 or 6. In the chlorophyllin-sensitized photooxidation quantum yields a 1000-fold higher than this were obtained. Since this reaction also takes place through the participation of a long-lived species (see below) we conclude that step 6, namely, quenching of the metastable species by ascorbic acid, is the limiting factor in photoreduction of chlorophyllin, rather than the radiationless conversion to the metastable state, which probably takes place with high efficiency.

The chlorophyllin-sensitized photooxidation of PTD has some of the characteristics of those described for proflavin as the sensitizer.<sup>13</sup> It also resembles the earlier studies on photooxidation of allylthiourea sensitized by ethyl chlorophyllide<sup>35</sup> or by chlorophyll.<sup>36</sup> A scheme which describes the results on photosensitized oxidation of PTD would contain steps 1, 2, 4, and 5 listed above plus the steps



Assuming that the photoperoxide  $DO_2$  is a labile species and applying the steady-state assumption for concentration of  $DO_2$  as well as for  $D^*$  and  $D'$  we obtain for the quantum yield

$$\Phi = \left[ \frac{k_4}{k_2 + k_4} \right] \left[ \frac{k_{12}(O_2)}{k_5 + k_{12}(O_2)} \right] \left[ \frac{k_{15}(P)}{k_{13}(D) + k_{14} + k_{15}(P)} \right]$$

In the case of the proflavin-sensitized photooxidation,<sup>13</sup>

(33) R. Livingston, *Quart. Rev. (London)*, **14**, 174 (1961).

(34) R. Livingston and P. J. McCartin, *J. Am. Chem. Soc.*, **85**, 1571 (1963).

(35) H. Gaffron, *Biochem. Z.*, **264**, 251 (1933).

(36) R. Livingston and K. E. Owens, *J. Am. Chem. Soc.*, **78**, 3301 (1956).

self-quenching of metastable dye molecules, namely,  $D' + D \rightarrow 2D$ , was comparable with induced decomposition of photoperoxide, step 13. In the case of chlorophyllin, however, concentration quenching of the metastable state is apparently unimportant (see above). Since about four molecules of oxidized PTD are involved in the formation of the observed product,<sup>13</sup> the highest observed over-all quantum yield, namely, 0.8, should be reduced by a factor of four. Hence  $k_3/k_4 = 4$  or one out of every four molecules in the first excited singlet state is converted to the long-lived metastable species. From the observed oxygen dependence  $k_3 \cdot k_{12} = 4.15 \times 10^{11}$  mole/l. If every encounter between  $D'$  and  $O_2$  leads to a reaction, then  $k_{12} = 6.6 \times 10^9$  sec./l. mole, so that the lifetime of the metastable species is  $1/k_3 = 1.3 \times 10^{-8}$  sec., a value one-tenth that obtained with nitrobenzene quenching of the Krasnovsky reaction. Hence we

conclude that only one out of ten encounters between  $D'$  and  $O_2$  leads to reaction. These calculated lifetimes demonstrate that oxygen reacts with the long-lived metastable species  $D'$  rather than with the first excited singlet species  $D^*$ . Although oxygen quenched the fluorescence of chlorophyll,<sup>33</sup> this step does not appear to be of importance for chlorophyllin-sensitized photooxidation. The data for the chlorophyllin dependence shows that the induced decomposition, step 13, is 275 times more rapid than the oxidation of the substrate, step 15.

Binding of chlorophyllin *a* to PVP considerably enhances its photoreduction as shown in the photosensitized reduction of fast red S just as is the case for synthetic dyes.<sup>15-17</sup> For sensitized photooxidation the effect of binding is less pronounced because diffusion of oxygen to the excited molecule is retarded by the enveloping residues of the high polymer.<sup>17</sup>

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, STANFORD UNIVERSITY, STANFORD, CALIF., AND DIVISION OF MATERIALS SCIENCES, STANFORD RESEARCH INSTITUTE, MENLO PARK, CALIF.]

## Electron Transfer through Organic Structural Units. Aromatic and Heterocyclic Carboxylates as Bridging Groups in Oxidation-Reduction Reactions

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The specific rates of reduction of 49 carboxylatopentamminecobalt(III) complexes (containing benzene, thiophene, furan, pyridine, and pyrrole rings) with  $Cr^{2+}$  have been measured and their dependence on  $(H^+)$  noted. The majority of these lie between 0.08 and 0.30 l. mole<sup>-1</sup> sec.<sup>-1</sup>, indicating the usual electron-transfer path through the coordinated carboxylato group with the formation of a carboxylatochromium(III) complex. A number of much higher values point to the operation of several special effects. The reduction of a complex having a carbonyl *para* to the carboxylato proceeds through remote attack on the carbonyl group, forming the parent carboxylic acid and  $Cr(H_2O)_6^{3+}$ ; the rates of such reductions exhibit a term first order in  $(H^+)$ . If the carbonyl group lies *ortho* to the carboxylato, rates are again greater than normal, but the carboxylato group is bound to Cr(III) in the product, and the first-order acid term does not appear; acceleration here may be due to chelation and electron transfer through the carbonyl group, or, alternatively, may be attributed to conjugative stabilization of a radical-ion intermediate. Reduction of the complexes of dicarboxylic acids may proceed through the complex itself,  $(Ro-A-H)^{2+}$ , and through its conjugate base  $(Ro-A)^{-}$ . The conjugate bases are reduced with specific rates between 9 and 30. The basic path is observed only in those cases in which a strain-free model of a chelated transition state for reduction may be constructed, suggesting a chelated activated complex (although chelation does not appear to persist in the products). There is no evidence that a reducing electron may be transmitted through an *-S-* bridge in cases where such a bridge links conjugated carbon systems bearing carboxy groups. Rate laws for reduction of certain of the hydroxybenzoato complexes contain a term representing reduction of the conjugate base. This term appears only if the coordinated carboxyl is situated *ortho* to a hydroxy group. The specific rates for this path are very high ( $10^8$ - $10^9$ ) and suggest a transition state strongly stabilized by chelation; there is, however, no evidence for electron transfer through phenolic oxygen. Data on the pyridinecarboxylato complexes suggest that electron transfer through nitrogen occurs in reduction of the 4- and 2- (but not the 3-) carboxylato derivatives. The reductions of the 2-carboxy, the 2,6-dicarboxy, and the 2,5-dicarboxypyridine derivatives are fast, and deeply colored Cr(III) products, presumed to be chelates, are formed; but the reduction of the pyrrole-2-carboxylato derivative is much slower, and the predominant product appears to be nonchelated. In contrast to uncoordinated carbonyl-substituted benzoic acids, a number of the pyridinecarboxylato acids and their *N*-methyl derivatives are readily reduced by  $Cr^{2+}$  in the absence of Co(III). The general correlation between reducibility of the acid and reducibility of the Co(III) complex in this series suggests that, in some cases at least, reduction of the pyridinecarboxylato complexes may proceed through a radical-ion intermediate stabilized by conjugation. This description best applies to the surprisingly fast reduction of the protonated and *N*-methylated complexes of 4-pyridinecarboxylic acid.

Although the effects of ring substitution on the reductions of benzoatopentamminecobalt(III) complexes have been investigated in some detail,<sup>2</sup> a number of potentially instructive substituents have not yet been

examined. Moreover, scant attention has been paid to heterocyclic carboxylato groups as "electron-transfer bridges" in such reactions. In the oxidation-reduction reactions comprising the present study, only one reducing agent,  $Cr^{2+}$ , has been used, but we have introduced considerable variation in the carboxylato group bound to tripotivite cobalt. We have included, in addition to a number of new benzoato complexes, com-

<sup>1</sup> National Science Foundation Senior Postdoctoral Fellow, Stanford University, 1962-1963.

(2) (a) R. T. M. Fraser and H. Taube, *J. Am. Chem. Soc.*, **83**, 2239, 2242 (1961); (b) R. T. M. Fraser, *ibid.*, **83**, 565 (1961); **84**, 3436 (1962); (c) D. K. Selera and H. Taube, *ibid.*, **83**, 1785 (1961).