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REVERSIBLE PHOTOCHEMICAL OXIDATION OF CHLOROPHYLL BY NITRO COMPOUNDS

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In describing the photochemical phase of photosynthesis it is often assumed (^{1}) that chlorophyll, under the action of a quantum of visible light, acts as an electron donor. In this connection, studies of the photochemical oxidation of the pigment acquire great importance. Of special interest here are processes that are accompanied by the "storage" of light energy in the reaction products, i.e., processes reversible in the dark.

Meanwhile, our knowledge of the reversible photochemical oxidation of chlorophyll is far from complete. Apparently this is connected with the extremely low stability of the active oxidation product and the resulting difficulties in its identification. The most fruitful method for studying reversible photooxidation has proved to be low-temperature quenching of the products of the elementary photoprocess. The point is that in frozen solutions, where the kinetic motions of molecules are strongly inhibited, the probability of recombination of active products is reduced, thereby creating conditions for their accumulation.

Using this technique, Kachan and Dain (^{2}) succeeded in carrying out reversible photooxidation of chlorophyll under the action of ultraviolet light, and Linschitz and Renner (^{3}), upon illumination with red light in the presence of quinone. Recently, the photochemical oxidation of chlorophyll by quinones under low-temperature conditions has been studied in detail in the works of Krasnovsky and Drozdova (^{4}), in which the method of differential spectroscopy made it possible to establish the nature of the absorption of the product of reversible oxidation of chlorophyll. In the work of Tollin and Green (^{5}), assumptions were put forward concerning the mechanism of the photochemical interaction of chlorophyll with quinones.

Although the product of reversible photooxidation of chlorophyll appears only under special conditions, its role in the occurrence of a number of photoreactions of the pigment is apparently very great. This is indicated, in particular, by interesting results on the photooxidation of chlorophyll with methyl red in acidic media, obtained by Evstigneeva, Gavrilova, and Savkina (^{6}).

According to all the data, quenching of chlorophyll fluorescence by a number of oxidizers can also be regarded as reversible oxidation of the excited pigment molecule by quencher molecules. Indeed, as our studies ([7]) have shown, quenching of chlorophyll fluorescence depends on the oxidation–reduction properties both of the fluorescing molecules and of the quencher molecules. A systematic study of this phenomenon using quenchers—aromatic nitro compounds—gave grounds for concluding that the quenching is based on an act of reversible electron transfer from the chlorophyll molecule to the nitro-compound molecule. However, there were no direct data confirming the presence of separate stages of this process. In the present work an attempt was made to identify the products of the reversible photochemical interaction of chlorophyll with these oxidizers. For this purpose, the method of low-tem···

temperature quenching. The investigation carried out showed that irradiation with red light of frozen solutions leads to bleaching of chlorophyll and the formation of a new yellow product. Thawing of the solution is accompanied by almost complete regeneration of the initial pigment. In the course of the photochemical reaction, free radicals accumulate, detected by the EPR method.

The procedure for studying frozen solutions was borrowed from work (4). The solvent was a mixture of ethyl alcohol with glycerol (1 : 2), which below −60° gives a transparent glassy mass suitable for spectrophotometry. Irradiation and recording of the absorption spectra of the solutions were carried out in plane-parallel cuvettes, with a layer thickness of 3–4 mm. A 1000-W cinema lamp served as the irradiation source. A KS-12 light filter was used. During irradiation the cuvette was in a transparent Dewar vessel filled with a cooling mixture (methanol + carbon dioxide). The temperature during irradiation was $-75 \pm 2°$.

Table 1

Quantum yields of the photochemical oxidation of chlorophyll a by various nitro compounds in toluene solution

Nitro compound	Quantum yield of the photoreaction
Nitrobenzene	$3.5 \cdot 10^{-6}$
m-Dinitrobenzene	$1.9 \cdot 10^{-5}$
m-Nitrophenol	$3.9 \cdot 10^{-6}$
2,4-Dinitrophenol	$1.2 \cdot 10^{-5}$

The absorption spectra of the solutions were recorded on an SF-10 spectrophotometer at the maximum recording speed (90 sec.). No measures were taken to thermostat the frozen solutions during recording. Control experiments showed that over the entire time required for this purpose the solution retained a solid consistency. To avoid fogging of the cuvette, its walls were coated with an alcohol-glycerol mixture.

Measurements of the EPR spectra were carried out on an RE-1301 radiospectrometer. All experiments were performed in the absence of oxygen. The latter was removed from the solution by prolonged pumping on a high-vacuum apparatus according to the method described in (8).

The quantum yield of the photochemical reaction was determined actinometrically, by the chlorophyll-a-sensitized photoreduction reaction of methyl red with phenylhydrazine (9). The solutions contained chlorophyll a at a concentration of the order of 10^{-5} mol/l and the nitro compound at a concentration of 10^{-2} mol/l.

Nitrobenzene, *m*-dinitrobenzene, *m*-nitrophenol, and 2,4-dinitrophenol of chemically pure grade were used in the work after additional purification (distillation or recrystallization).

Below are the results obtained in studying the photochemical reaction of chlorophyll a with a series of nitro compounds at room temperature and under low-temperature conditions.

Solutions of chlorophyll a containing nitro compounds are fairly stable at room temperature under irradiation with red light. Only upon very prolonged exposure is a decrease in the intensities of the main pigment absorption bands observed. According to all the data, the bleaching of chlorophyll observed in this case is associated with its destructive photooxidation. This is indicated primarily by the fact that the photoreaction considered is not accompanied by the formation of any colored products and, secondly, that its rate is directly dependent on the electron-acceptor ability of the nitro compound. The latter is clearly illustrated by the data in Table 1, where the quantum yields of the photochemical oxidation of chlorophyll by various nitro compounds are given. Both in the case of nitrobenzenes and in the case of nitrophenols, the quantum yield of the photoreaction is almost an order of magnitude higher in the presence of dinitro compounds.*

* The oxidizing potential of aromatic nitro compounds, as is known (10), increases as the number of nitro groups in the benzene ring increases.

A very interesting picture is presented by the photochemical interaction of chlorophyll *a* with nitro compounds in frozen solutions. Under these conditions one can establish a stage of reversible oxidation of the pigment. Upon irradiation of frozen solutions, a decrease in the principal absorption bands of the pigment is observed, accompanied by a simultaneous increase in the light absorption of the solution in the spectral region 470–570 mμ. This is illustrated by curves 1 and 2 in Fig. 1. The state of the solution corresponding to curve 2 is quite stable at low temperature. However, warming the solution to room temperature leads to a rather rapid regeneration of the initial state (Fig. 1, 3). The photochemical reactions of chlorophyll described are based on the process of its photochemical oxidation by the nitro compound. Indeed, even very prolonged irradiation of frozen solutions of chlorophyll that do not contain nitro compounds does not lead to any noticeable photochemical reaction. In addi-

Fig. 1

Figure 1: Fig. 1

tion, the rate of the photoreaction in a frozen solution depends on the nature of the nitro compound. If, under completely identical conditions, two solutions of chlorophyll *a* are irradiated—one containing nitrobenzene and the other the same amount of dinitrobenzene—then in 5 hours of irradiation, 12% of the chlorophyll is bleached in the former case, and 46% in the latter. This means that the rate of the photoreaction increases with increasing electron-acceptor activity of the nitro compound present in the system. It is necessary to add to this that pheophytin *a*, for which photochemical oxidation reactions are not at all characteristic, undergoes no changes whatever at low temperatures upon irradiation in the presence of nitro compounds.

Fig. 1. Reversible photoreaction in a frozen solution of chlorophyll *a* in the presence of *m*-dinitrobenzene. 1 —absorption spectrum before irradiation; 2 —the same after 5 hours of irradiation with red light; 3 —after thawing of the irradiated solution.

A characteristic feature of the reactions under consideration is that they take place in the presence of a considerable excess of nitro compounds, exceeding the pigment concentration by 2–3 orders of magnitude. It may be expected that under these conditions each pigment molecule is surrounded by oxidizer molecules.

In light of the data presented, the elementary processes underlying the photooxidation of chlorophyll may be represented by the following Scheme 1:

Scheme 1

$$\begin{tikzcd}
 & \text{final oxidation products} & \\
\mathrm{Chl} \arrow[r, shift left, "h\nu"] & {[\mathrm{Chl},\ \mathrm{RNO_2}]^*} \arrow[l, shift left, "\mathrm{RNO_2}"] \arrow[u, "()"'] \arrow[d, "()"] \arrow[r, "(a)"] & \mathrm{Chl}^+ + \mathrm{RON_2}^- \\
 & \mathrm{Chl} + \mathrm{RNO_2} &
\end{tikzcd}$$

Absorption of a light quantum leads to electron transfer from chlorophyll to the nearby nitro compound and to the formation of the corresponding

ion-radicals. Apparently, this process, as has already been noted in (^{7}), underlies the quenching of fluorescence, as well as bleaching of the pigment under low-temperature conditions. At room temperature there is a high probability of recombination of the ion-radicals (6), which accounts for the reversibility observed during quenching. Only a small fraction of them undergoes further

Fig. 2

Figure 2: Fig. 2

transformations leading to destruction of the pigment (8), proceeding with a very low quantum yield.

In frozen solutions there are favorable conditions for the stabilization of ion-radicals and their accumulation. Thawing the solutions, however,

Fig. 2. E.p.r. spectra of a frozen irradiated solution of chlorophyll *a* in the presence of *m*-dinitrobenzene. **1** –immediately after irradiation, **2** –after 6 min, and **3** –after 9 min of standing at room temperature

creates preconditions for recombination of the radicals and reversal of the process. This can explain the regeneration of the pigment observed experimentally.

Adopting scheme 1, one may expect that paramagnetic particles will be contained in the irradiated solutions. Indeed, study of the e.p.r. spectrum of an irradiated frozen solution showed the presence of a complex signal containing a number of lines. It is very interesting in this connection that, as the solution thaws, the magnitude of the signal gradually decreases. The latter is clearly seen in Fig. 2. This undoubtedly indicates the disappearance in the system, during thawing, of particles possessing an unpaired electron.

The obtained pattern of the e.p.r. spectrum does not provide a basis for determining its genesis. The concentration of the ion-radicals formed is apparently too low to yield lines sufficiently intense for interpretation.

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