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Abstract

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SPECTRAL STUDY OF THE PHOTOREDUCED FORM OF PHEOPHYTIN *a*

The reaction of reversible photochemical reduction of chlorophyll by ascorbic acid in solution was discovered by Krasnovsky in 1948 ([1]). Subsequently, this reaction was obtained for a large number of pigments of the porphyrin class, and the range of reducing agents was thereby substantially expanded ([2], [3]). The photoreduction reaction of the pigment proceeds in two stages. It is assumed that the primary photoreduced form, unstable under ordinary conditions, is the product of addition of an electron to the 18-membered cyclic system of conjugated bonds of the pigment molecule ([4]). The more stable secondary form, observed in Krasnovsky' s work ([1]), is presumably the product of addition of two H atoms to this molecule ([2], [5]). The question of the localization of the added H atoms in the molecule of the secondary photoreduced form of the pigment remained open. In this connection we carried out a series of experiments in order to obtain IR spectra of the secondary photoreduced form of the metal-free analogue of chlorophyll—pheophytin, since these spectra can provide direct information about the structure of the molecules of the compound under investigation. The choice of pheophytin as the first object of study was due to the fact that chlorophyll is subject to pheophytinization, which complicates the reaction of its photoreduction ([6], [7]).

The photoreduction reaction of pheophytin was carried out in our experiments in a pyridine solution containing 1-2% water, in a vacuum cuvette described earlier ([8]), at room temperature. H_2S was used as the reducing agent; its equilibrium pressure above the solution was 300–500 mm Hg. Before the photoreaction was carried out, the solutions were thoroughly degassed, and all subsequent operations with them were performed under vacuum conditions. The solutions were illuminated with condensed light from a 150 W incandescent lamp through a water filter. Spectral measurements in the visible region were made on an SF-2M instrument, and in the IR region on a UR-10 instrument. Pheophytin *a* was prepared by I. A. Popova at the Komarov Botanical Institute of the Academy of Sciences of the USSR.

After illumination of a solution of pheophytin *a* (concentration $5 \cdot 10^{-5}$ mol/l) in the presence of H_2S, its spectrum assumes the outlines characteristic of the secondary photoreduced form of the pigment ([2], [5]). If the H_2S and solvent are then frozen out of the cuvette, a layer of pigment can be obtained on its windows, convenient for studying its IR spectrum and apparently representing pheophytin

Fig. 1. IR absorption spectra of solid films: 1 —pheophytin a; 2 —photoreduced form of pheophytin a; 3 —product of oxidation of the photoreduced form of pheophytin a by atmospheric oxygen

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in its photoreduced form in the condensed state. Evidence for this assertion is provided by the reversibility of the spectral changes of the precipitated product: if it is again dissolved in the same solvent and H_2S is admitted, we obtain almost the same spectral picture (in the visible region), characteristic of the secondary photoreduced form, as was observed before this form was precipitated from solution. If, however, the precipitated substance is treated with pyridine not containing H_2S and air is admitted into the cuvette, then from this substance, according to spectral data (in the visible and IR regions) and chromatographic analysis, the initial pheophytin *a* is regenerated.

Figure 1 presents the IR spectra of solid layers of the initial pheophytin *a*, its photoreduced form obtained by precipitation from solution, and the product of oxidation of the photoreduced form of pheophytin by atmospheric oxygen (oxidation was carried out in pyridine, followed by its evaporation). Comparison of these spectra shows that the following spectral changes occur upon photoreduction of pheophytin. The absorption band corresponding to the stretching vibrations of the imino groups NH shifts from 3390 to 3410 cm^{-1}. The absorption bands at 685 and 618 cm^{-1}, corresponding to deformation vibrations of the imino groups NH (^{9}), almost completely disappear. The $C = O$ band of the cyclopentanone ring shifts from 1703 to 1693 cm^{-1}, and the intensity of this band decreases somewhat. New absorption bands appear at 1665 and 1584 cm^{-1}.

Fig. 1. IR absorption spectra of solid films: 1 —pheophytin *a*; 2 —photoreduced form of pheophytin *a*; 3 —product of oxidation of the photoreduced form of pheophytin *a* by atmospheric oxygen

The changes observed upon photoreduction of pheophytin in the visible and IR spectra may be attributed to the following structural transformations of the pigment molecule. The disappearance of the principal absorption bands of pheophytin in its electronic spectrum upon formation of the photoreduced form of the pigment (^{2,5}) shows that the conjugation in the 18-membered cyclic system is thereby disturbed to some extent. In the formation of the primary photoreduced form, which is assumed to be characterized by attachment of an electron to the pigment molecule excited by light, this disturbance is not local in character, since the attached electron is delocalized (^{2}). In the formation of the secondary photoreduced form, two whole H atoms are already added to the pigment molecule, and the disturbances of conjugation must have a local character. Significant changes in the absorption bands corresponding to vibra-

Fig. 2. Scheme of photoreduction of pheophytin

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tions of the imino groups NH suggest that the H atoms added to the pheophytin molecules are localized in the immediate vicinity of the imino groups NH. From the standpoint of general chemical considerations, the most suitable sites for addition of H atoms are the two N atoms of the tetrapyrrole ring that do not form imino groups. Indirect confirmation of the assumption that the H atoms add to N atoms is provided by the possibility of isotopic exchange H → D in the imino groups of pheophytin in the presence of heavy water ([9]) and by the phenomenon of pheophytinization of chlorophyll during its photoreduction ([6,7]). The changes occurring in the structure of the pheophytin molecule upon its photoreduction may, on the basis of the above assumption, be represented by the scheme shown in Fig. 2.

The spectral changes observed upon photoreduction of pheophytin in the IR region fully fit the proposed scheme. Indeed, the presence in the structure of the molecule of the secondary photoreduced form of pheophytin of four imino groups instead of two in the structure of the initial pheophytin molecule should lead to substantial changes···

vibrations of these groups, which is in fact observed experimentally (see above). The absorption band at 1665 cm^{-1}, arising upon photoreduction of pheophytin, may be assigned to vibrations of the $C = C$ bonds formed in the molecule in place of the 18-membered cycle of conjugated bonds (Fig. 2). The lowering of the vibration frequency of the keto group $C = O$ upon photoreduction of pheophytin may be explained by an increase in the conjugation of this group. Indeed, in going from compounds in which the group

Fig. 2. Scheme of photoreduction of pheophytin: 0—the skeleton of the pheophytin molecule; 1—the skeleton of the molecule of the primary photoreduced form; 2—the skeleton of the molecule of the secondary photoreduced form. The bold line denotes the 18-membered cycle of conjugated bonds. The dashed line shows the electron uptake by the system of conjugated bonds of the light-excited pheophytin molecule according to the scheme proposed by Krasnovskii for chlorophyll ([2]).

$C = O$ is conjugated with a closed conjugated system, to compounds in which the $C = O$ group is conjugated with $C = C$ double bonds, the vibration frequency of the $C = O$ group, as a rule, decreases ([10]). The absorption band at 1584 cm^{-1} in the spectrum of the photoreduced form of pheophytin may be assigned to deformation vibrations of the four imine groups that characterize the structure of its molecule (Fig. 2).

When the photoreduced form of pheophytin is precipitated from solution on the walls of the cuvette, a light-yellow precipitate can be observed; apparently, this is sulfur formed as a result of the photoreaction. The separation of sulfur confirms the correctness of the reaction scheme for photoreduction that we have proposed.

Comparison of the curves in Fig. 1 and spectral measurements in the visible region show that, in air, the photoreduced form of pheophytin *a* largely regenerates the original pheophytin *a*. Certain differences between curves 1 and 3 in Fig. 1 are evidently due to partial irreversibility of the photoreduction reaction.

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Note: Figure translations are in progress. See original paper for figures.