Physical Chemistry

V. E. KARPITSKAYA, I. A. DOLIDZE, M. S. ASHKINAZI

1962

SovietRxiv

Fig. 1. Absorption curves of an ethanolic solution containing diphenylamine, chlorophyll a, and oxygen. 1—before irradiation, 2—after irradiation with red light.

Figure 1: Fig. 1. Absorption curves of an ethanolic solution containing diphenylamine, chlorophyll a, and oxygen. 1—before irradiation, 2—after irradiation with red light.

Abstract

Full Text

Physical Chemistry

V. E. KARPITSKAYA, I. A. DOLIDZE, M. S. ASHKINAZI

ON THE FORMATION OF FREE RADICALS IN AUTOOXIDATION REACTIONS PHOTOSENSITIZED BY CHLOROPHYLL AND PHEOPHYTIN

(Presented by Academician A. N. Terenin, May 30, 1962)

A number of authors have shown ([1]) that free radicals are formed in the course of photosynthesis in plant organisms. In this connection, it is of interest that free radicals are also formed in photochemical reactions sensitized by chlorophyll. Experiments in which this effect was demonstrated in the photosensitized chlorophyll autoxidation of diphenylamine and paraphenylenediamine are described below. The preparations used—diphenylamine, paraphenylenediamine hydrochloride, and solvents—were subjected to appropriate purification. Irradiation was carried out with 1000-watt lamps under thermostatic conditions at a temperature of 20–22°. The sensitizers chlorophyll *a* and pheophytin *a* were prepared by methods described in the literature ([2]). A spectrophotometric method was used in the work, since colored products are obtained during photooxidation of diphenylamine and paraphenylenediamine. Absorption curves were recorded on SF-4 and SF-5 spectrophotometers. The concentrations of sensitizers were 10^{-5} mol/l, and those of the amines 10^{-2} mol/l.

Fig. 1. Absorption curves of an ethanolic solution containing diphenylamine, chlorophyll *a*, and oxygen. 1—before irradiation, 2—after irradiation with red light.

Upon irradiation with red light (KS-10 light filter) of an ethanolic solution of diphenylamine in the presence of chlorophyll *a* and oxygen, after only 10–15 min the color of the reaction mixture changes: the green solution first turns dark brown and then brown. This is manifested in a considerable increase in

Fig. 2. Absorption curve of the product of chlorophyll-photosensitized autooxidation of diphenylamine in ethanol

Figure 2: Fig. 2. Absorption curve of the product of chlorophyll-photosensitized autooxidation of diphenylamine in ethanol

Fig. 3. EPR spectra of products of photosensitized autooxidation: a — diphenylamine, b —paraphenylenediamine

Figure 3: Fig. 3. EPR spectra of products of photosensitized autooxidation: a —diphenylamine, b —paraphenylenediamine

absorption in the region 400–560 mμ, a certain increase in the blue maximum, and a decrease in the red maximum (Fig. 1). The same is observed when pheophytin *a* is used as the sensitizer. In this case, however, the intensity of the red band does not change. The product of the photoreaction was obtained in pure form. Unlike the initial diphenylamine, it dissolves excellently in water, owing to which it could be separated from unreacted diphenylamine from the mixture remaining after extrac-

chlorophyll with toluene. Its aqueous and alcoholic solutions are pink, and at high concentration purple. In ether the color acquires an orange tint. The absorption spectrum of the photoreaction product is characterized by two intense maxima located at 460 and 260 mμ (Fig. 2). The absence in the spectrum of the maximum at 285 mμ characteristic of diphenylamine indicates that the isolated photoproduct contains no traces of the starting diphenylamine.

Prolonged irradiation of a solution of diphenylamine with the addition of chlorophyll, thoroughly degassed under high-vacuum conditions, produces no changes in the absorption spectrum. The spectrum likewise does not change during prolonged storage, in the dark, of a mixture containing oxygen. These data convincingly indicate that the isolated pink compound is not the product of sensitized photooxidation of diphenylamine by oxygen. This conclusion is confirmed by experiments on the interaction of the pink product with reducing agents. Introduction of ascorbic acid (hydrazine, hydroquinone) into a solution of this product causes instantaneous regeneration of diphenylamine. The solution is thereby decolorized, and the maxima at 460 and 260 mμ disappear from the spectrum. To identify the diphenylamine formed, the reaction mixture was treated with benzene, in which ascorbic acid is insoluble. In the spectrum of the alcoholic solution of the residue obtained after evaporation of the benzene solution to dryness*, a maximum characteristic of diphenylamine at 285 mμ is clearly observed.

Fig. 2. Absorption curve of the product of chlorophyll-photosensitized autooxidation of diphenylamine in ethanol.

Fig. 3. EPR spectra of the products of photosensitized autooxidation: **a** — diphenylamine, **b** —*p*-phenylenediamine.

Figure 4: Absorption spectra of the positive ion of paraphenylenediamine: a—obtained in the photosensitized autoxidation of paraphenylenediamine, b—taken from work ( )

Figure 4: Figure 4: Absorption spectra of the positive ion of paraphenylenediamine: a—obtained in the photosensitized autoxidation of paraphenylenediamine, b—taken from work ( )

The instantaneous regeneration of the initial diphenylamine upon interaction of the product of its photooxidation with reducing agents indicates that, in chlorophyll- (or pheophytin-) sensitized autooxidation of diphenylamine, a product of only very slight oxidation is formed. Pro-

* The need to replace benzene with alcohol is due to the strong absorption of benzene itself in the ultraviolet region.

this product, as the experiments showed, is capable of further oxidation. When its solution is exposed to ultraviolet light (in the presence of oxygen), or when oxygen is passed through its alcoholic–toluene alkaline solution while heating to 70°, a compound is formed whose spectrum differs sharply from that of the initial compound—the maxima at 460 and 260 mμ are completely absent. A compound obtained in the dark oxidation of diphenylamine itself under the same conditions has an entirely identical spectrum. According to the data of works (^3), it is regarded as a radical with tetravalent nitrogen, $(C_6H_5)_2N = O^{\cdot}$, diphenylnitrogen oxide. We suppose that the product obtained by us in the chlorophyll- (pheophytin-) photosensitized autoxidation of diphenylamine is a stable free radical of diphenylnitrogen, $(C_6H_5)_2N^{\cdot}$. In favor of the fact that this is a free radical are the data obtained by us by the electron paramagnetic resonance method*. Fig. 3a illustrates the EPR spectrum obtained for a solution of diphenylamine in toluene after irradiation with red light for 1.5 h in the presence of pheophytin while oxygen was being passed through**. The spectrum is a triplet with an intensity ratio of the components 1 : 1 : 1 and a splitting between them of 11 oersted. In the control experiment, carried out in the dark, the colored product is not formed. An analogous EPR spectrum was obtained in works ([4,5]) for the product of the interaction of diphenylamine with various peroxides and oxidants, and also in the photochemical oxidation of trimethylheptane in the presence of diphenylamine upon irradiation with ultraviolet light. The authors of the cited works attribute this spectrum to the free radical diphenylnitrogen.

Fig. 4. Absorption spectra of the positive ion of paraphenylenediamine: *a*—obtained in the photosensitized autoxidation of paraphenylenediamine, *b*—taken from work (^6).

The formation of a free radical was also detected by us during the pheophytin-photosensitized autoxidation of paraphenylenediamine. When red light acts on an alcoholic solution of paraphenylenediamine in the presence of pheophytin

and oxygen, a reddish-yellow photoproduct is formed (after separation of the sensitizer). Its absorption curve in the visible region is characterized by two maxima—at 462 and 480 mμ (Fig. 4a). Under dark conditions, and also upon irradiation of a solution freed from oxygen under high vacuum, this product is not formed.

EPR measurements showed that this photoproduct is a free radical. Fig. 3b illustrates a spectrum having the form of a singlet with a line width of $\sim$ 14 oersted, obtained for an alcoholic solution of paraphenylenediamine after irradiation for 30 min with red light while oxygen was being passed through in the presence of pheophytin. The radical formed under these conditions remains unchanged for only 1.5–2 h. On longer storage it is gradually destroyed, as was detected by us by spectrophotometric measurements. We suppose that this radical is the positive ion of paraphenylenediamine. According to the literature data, this ion is obtained as the result of one-elec-

* The EPR measurements were carried out by V. D. Pokhodenko, to whom the authors express their gratitude.

** The oxygen was removed from the ampoule with the solution before it was placed in the EPR spectrometer by pumping.

···oxidation of paraphenylenediamine by bromine ([6]), during electrolysis ([7]), and in anodic polarography ([8]), and is a comparatively stable free radical. Its radical nature has been proved by EPR measurements ([5,7,9]). The absorption spectrum shown in Fig. 4b was taken from ([6]) and, as can be seen, coincides with the spectrum of the photoproduct we obtained (Fig. 4a).

As far as we know, the formation, observed by us, of free radicals of the oxidized substance in chlorophyll-photosensitized autoxidation has not previously been reported.

We express our deep gratitude to Prof. B. Ya. Dain for his constant interest in the work and for his help in discussing the results.

Institute of Physical Chemistry
named after L. V. Pisarzhevskii
Academy of Sciences of the Ukrainian SSR

Received
22 V 1962

REFERENCES

1. B. Commoner, J. Heise et al., Science, **126**, 57 (1957); M. Calvin, P. B. Sogo, Science, **125**, 499 (1957); N. N. Bubnov, A. A. Krasnovskii, et al., Biophysics, **5**, no. 2, 121 (1960).

2. F. Zscheile, C. Comer, Bot. Gaz., **102**, 463 (1941); H. Fischer, A. Stern, *Die Chemie des Pyrrols*, 2, Leipzig, 1940, p. 329.

3. R. Hoskins, J. Chem. Phys., **25**, 788 (1956); J. R. Thomas, J. Am. Chem. Soc., **82**, 5955 (1960).

4. A. L. Buchachenko, M. B. Neiman, K. Ya. Kaganskaya, *Trudy po khimii i khim. tekhnol.*, **1**, 31 (1961).

5. A. L. Buchachenko, M. B. Neiman, Ya. S. Lebedev, *Trudy po khimii i khim. tekhnol.*, **1**, 39 (1961).

6. L. Michaelis, M. P. Schubert, S. Granick, J. Am. Chem. Soc., **61**, 1981 (1939).

7. M. T. Melchior, A. H. Maki, J. Chem. Phys., **34**, 471 (1961).

8. R. E. Parker, R. N. Adams, Anal. Chem., **28**, 828 (1956).

9. S. I. Weissman, J. Chem. Phys., **22**, 1135 (1954).

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