EPR DETECTION OF DEUTERIOCHLOROPHYLL CATION-RADICALS ARISING AT ITS PHOTOREACTION WITH QUINONE AT LOW TEMPERATURE

A. P. KOSTIKOV, L. P. KAYUSHIN, L. N. CHEKULAEVA
Institute of Biological Physics, USSR Ac. Sci., Pushchino, Moscow Region, 142292, USSR

and

N. A. SADOVNIKOVA, V. B. EVSTIGNEEV
Institute of Photosynthesis, USSR Ac. Sci., Pushchino, Moscow Region, 142292, USSR

Received 30 August 1974

1. Introduction

The photosensitized oxidation of chlorophyll by different oxidizing agents is being investigated in several laboratories in order to provide guidelines for understanding the primary steps in photosynthesis [1–11]. Among the various oxidants, the reaction of chlorophyll with various quinones is of special interest. In the primary act of this reaction an electron transfer from excited chlorophyll to quinone is supposed to occur [1–6]. However, the literature contains some different opinions on this question [9,10]. It is well established that quinone anion-radicals are formed through this reaction [7–11]. In our previous papers [4,7] it was shown that in acidic ethanol both $Q^{\cdot-}$ and $Chl^{\cdot+}$ arise. Other authors [6] came to a similar conclusion.

On the other hand, it has been proposed by Tollin et al. [9,10] that in the reaction with Q, $Chl^{\cdot+}$ is not formed at all, but the electron transfer from the solvent to Q occurs, sensitized by Chl, and $Chl^{\cdot+}$ which can be observed in this system results from the independent electron transfer from singlet chlorophyll to alcohol. It is the purpose of the present communication to demonstrate that when illuminating an alcohol solution of Chl with Q a stationary concentration of Chl^{+} is produced which depends on the presence of Q and on the same sample temperature. We have used deuteriochlorophyll 'a' (D-Chl) whose EPR signal width of radicals is less than that of the corresponding signal of protonated chlorophyll (H-Chl) [12], which enables one to make identification of radicals considerably easier.

2. Materials and methods

Deuteriochlorophyll was obtained from Chlorella sp. K grown in 99% D_2O. Purification of chlorophyll 'a' was performed by standard methods [13]. Commercial ethanol was purified by double distillation. Benzoquinone was purified by double sublimation, tetrachlorquinone was recrystallized from alcohol. Deuteriochlorophyll concentration was 10^{-4} M, that of quinones was 10^{-3} M. All of the samples used were thoroughly degassed prior to investigation (residual pressure 10^{-5} torr). EPR spectra were obtained with a X-band 100 kc modulation spectrometer equipped with a Computer of Average Transients. For the illumination of the samples a 400 W Tungsten filament lamp was focused through a water bath and the red filter ($\lambda > 600$ nm) in the cavity of spectrometer. The temperature of the sample was adjusted by the rate of flow of nitrogen gas. As a standard for obtaining the g-factor we have used anion-radicals of 1,4 benzosemiquinone for which $g=2.0047\pm0.0001$ [14].

3. Results and discussion

Illumination of an ethanolic solution containing chlo-

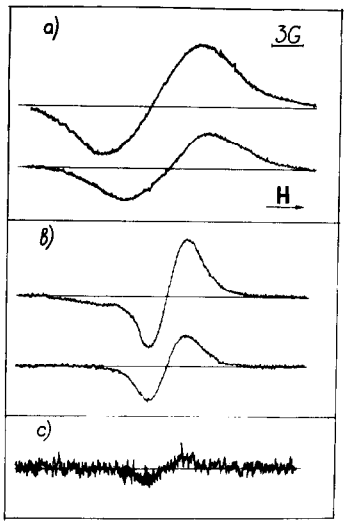

Fig. 1. EPR spectra at −120°C of a solution of H-Chl (a) and D-Chl (b) with p-benzoquinone observed under illumination (upper spectra) and after light turned off (lower spectra); (c) light induced EPR signal of D-Chl·⁺ arising in solution of D-Chl without quinone. The gain for last spectrum 4-fold larger. Modulation amplitude 2G, microwave power 1 mW.

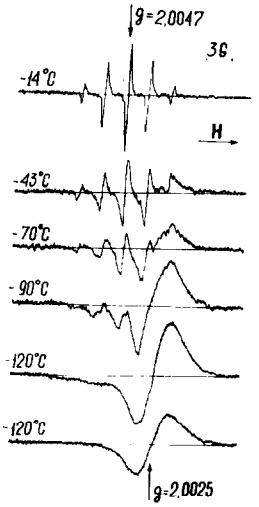

Fig. 2. Effect of temperature on the EPR spectrum in the deuteriochlorophyll–p-benzoquinone system. Lower spectrum after light turned off, the others under illumination. Further conditions see fig. 1.

rophyll and p-benzoquinone at low temperature produces an EPR spectrum, whose form and g-factor depends on the conditions of observation (light on or off) and on the nature of the pigment (H-Chl or D-Chl) Comparing EPR spectra in fig. 1a obtained for the solution of H-Chl with Q, one can see that the g-factor of the signal recorded after switching off the light is shifted in relation to that of the signal recorded at the time of illumination in the direction of smaller values. One could suggest that H-Chl·⁺ are responsible for the 'dark' EPR signal, and both H-Chl·⁺ with g=2.0025 and Q·⁻ with g=2.0047 contribute to the EPR spectrum observed under the sample illumination. It should be expected that the substitution of H-Chl by its deuterated analogue will result in the narrowing of the EPR signal of Chl·⁺ without affecting the form of EPR spectrum of Q·⁻. This effect is demonstrated in fig. 1b. One can see in this figure that besides a comparatively narrow line with ΔHpp=3.2G and g= 2.0025 another line of EPR absortion with larger g-factor is also observed under illumination. As is seen from fig. 2, Q⁻ are responsible for the latter.

It should be noted that a weak photoinduced EPR signal with g=2.0025 could be also observed in the absence of Q, but the amplitude of this signal is much smaller than the corresponding amplitude in the system chlorophyll–quinone (fig. 1c). The mechanism of formation of this signal is not understood. One can assume that responsible for it are the cation-radicals of chlorophyll formed due to photoreaction of the latter with a solvent or with impurities.

Fig. 2 illustrates the temperature effect on the form of photoinduced EPR spectrum, arising in solutions of D-Chl containing p-benzoquinone. At temperatures close to room temperature only the EPR signal of Q·⁻ with the constant of hyperfine interaction 2.37±0.03 gauss was observed. The decrease of the sample temperature resulted in gradual widening of the lines of hyperfine structure of Q·⁻, apparently, due to anisotropy of g-factor of the latter and the singlet signal of gaussian form of D-Chl·⁺ simultaneously increased. The value of g-factor of D-Chl·⁺ (g=2.0025±0.00015) obtained from these spectra, is in good agreement with the value of g-factor reported by Borg et al. [15] for H-Chl·⁺. After switching off the light, the signal of Q·⁻ rapidly disappeared and the EPR signal of D-Chl·⁺ simultane-

ously decreased to a definite value, depending on the temperature. The residual signal of D-Chl$^{\cdot+}$ was retained at low temperature for a long time and disappeared only after heating the sample to room temperature. Similar results were obtained for the case when tetrachlorquinone was used as an acceptor of electrons.

Thus, on the basis of the results obtained one can state that when illuminating alcohol solutions of Chl with Q, the electron transfer from Chl to Q occurs with the formation of Q$^{\cdot-}$ and Chl$^{\cdot+}$, the stationary concentration of Chl$^{\cdot+}$ increasing with the decrease of the sample temperature. From the kinetic measurements it follows that a part of Chl$^{\cdot+}$ remains in the immediate vicinity from Q$^{\cdot-}$(they are likely to be in the 'cage' of the solvent) and when switching off the light these radicals can rapidly recombine even at low temperature. The part of Chl$^{\cdot+}$ which has probably come out of the solvent 'cage', remains stable after switching off the light.

Acknowledgements

The authors wish to thank G. D. Olovyanishnikova and N. I. Perepelkina for their assistance in isolating deuteriochlorophyll.

References

[1] Evstigneev, V. B. (1968). J. Chim. Phys. Physicochim. Biol. 65, 1447.
[2] Chibisov, A. K. (1969). Photochem. Photobiol. 10, 331.
[3] Kelly, J. M. and Porter, G. (1970). Proc. Roy. Soc. 319A, 319.
[4] Evstigneev, V. B., Sadovnikova, N. A., Kostikov, A. P., Gribova, Z. P. and Kayushin, L. P. (1971). Biofizika, 16, 431.
[5] Evstigneev, V. B., Sadovnikova, N. A., Kostikov, A. P. and Kayushin, L. P. (1971). Dokl. Akad. Nauk SSSR, 203, 1343.
[6] Bobrovsky, A. P. and Kholmogorov, V. E. (1973). Dokl. Akad. Nauk SSSR 208, 1472.
[7] Tollin, G. and Green, G. (1962). Biochim. Biophys. Acta 60, 524.
[8] Tollin, G., Chaterjee, K. K. and Green, G. (1965). Photochem. Photobiol. 4, 593.
[9] White, R. A. and Tollin, G. (1971). Photochem. Photobiol. 14, 15; 43.
[10] Harbour, J. and Tollin, G. (1972). Proc. Natl. Acad. Sci. U.S. 69, 2066; Photochem. Photobiol. 19 (1974), 69.
[11] Hales, B. J. and Bolton, J. R. (1972). J. Am. Chem. Soc. 94, 3314.
[12] Norris, J. R., Uphaus, R. A. and Katz, J. J. (1972). Biochim. Biophys. Acta 275, 161.
[13] Strain, H. H. and Svec, W. A. (1966) in: The Chlorophylls (Vernon, L. P. and Seely G. R. eds.) p.22 Academic Press, New York.
[14] Blois, M. S. Jr., Brown, H. W. and Maling, J. E. (1961) in: Free Radicals in Biological Systems (Blois, M. S. Jr., Brown, H. W., Lemmon, R. M., Lindblom, R. O. and Weissbluth, M., eds) p. 121, Academic Press, New York.
[15] Borg, D. C., Fajer, J., Felton, R. H. and Dolphin, D. (1970). Proc. Natl. Acad. Sci. U.S. 67, 813.