FORMATION OF A STABLE FREE RADICAL IN AN ILLUMINATED CHLOROPHYLL COMPLEX*

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Extensive recent work (Emerson et al., 1957; Govindjee and Rabinowitch, 1960; French, 1961; Kok, 1961; Witt et al., 1961; Duysens et al., 1961; Duysens and Amesz, 1962) indicates that two light reactions, catalyzed by different pigments which probably act through different complexes or aggregates of chlorophyll a, are required for photosynthesis. A chlorophyll complex which could be the catalyst of one of these reactions has recently been partially purified from Chlorella pyrenoidosa (Allen et al., 1963). The complex, obtained by breaking the cells in a carefully controlled manner, followed by differential centrifugation, has its red absorption maximum at 6720 Å and contains approximately equimolar amounts of chlorophylls a and b. It does not sediment in one hour at 144,000 g at 5°C. The heavier material from which it is separated has its absorption maximum at 6780-6800 Å.

The chlorophyll complex as originally described was obtained mixed with all the other cell constituents that did not sediment under these conditions. It has since then been possible to separate it from much of this material by centrifugation for one hour at 144,000 g at 25-28°C. It is not yet clear whether this separation depends upon aggregation of the green complex at this temperature or upon a change in viscosity of the medium, or both. The separated complex can be redispersed by homogenization in the cold. The

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material thus obtained will be referred to here as P-672, by analogy with Kok's (1961) terminology.

Illumination of P-672 results in the generation of a single line electron spin resonance signal similar to the fast decaying signal induced by red light in intact Chlorella pyrenoidosa (Allen, Piette, and Murchio, 1961, 1962. Cf. the latter paper for a description of the apparatus and techniques of observation). However, unlike the signal from whole cells, that in P-672 does not decay on subsequent darkening of the suspension (cf. Fig. 1,A). P-672 does not exhibit the complex slow decaying signal found in intact cells, nor does it show the manganese lines that are such a prominent feature of ESR spectra of whole cells. The heavier fractions give a signal similar to that of P-672. This signal, however, decays rapidly in the dark.

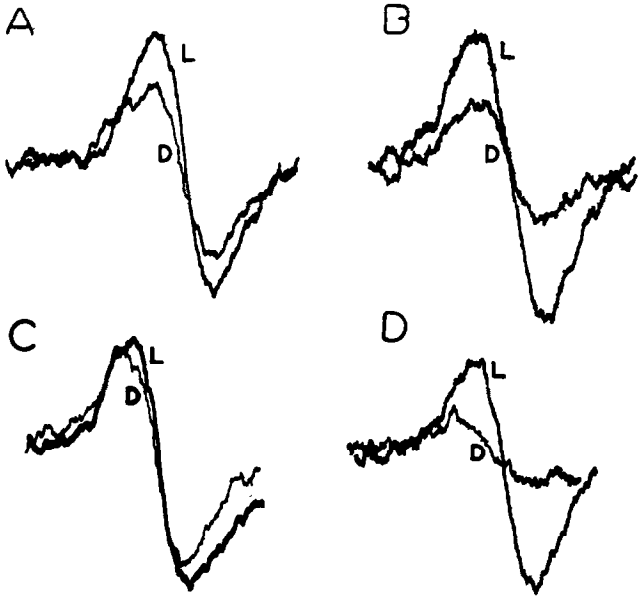

Figure 1. Light (L) and dark (D) ESR signals from (A) P-672; (B) P-672 mixed with supernatant liquid; (C) P-672 in 5×10^{-3} M $K_3Fe(CN)_6$; (D) P-672 in 5×10^{-3} M $K_4Fe(CN)_6$.

When the P-672 is mixed with the supernatant liquid from which it has been separated, the light induced signal partially disappears in the dark* (cf. Fig. 1,B). It is presumed that this is due to the presence of an as yet unidentified electron transfer agent in the soluble fraction, since the same effect can be obtained with artificial electron transport systems. Addition of 10^{-4} M 2,6-dichlorophenol indophenol (DCPIP) or 5×10^{-3} M potassium ferrocyanide results in the virtually complete disappearance of the signal in the dark, whereas ferricyanide may slightly increase the dark signal, as shown in Figure 1,C,D. With mixtures of ferri- and ferrocyanide the dark signal stabilizes at a level dependent on the redox potential of the mixture, as shown in Figure 2. Comparison of the discharge of the radical by the natural electron transport agent with that by the ferro-ferricyanide system permits an estimate of the redox potential of the natural electron carrier, as shown in Figure 2. It may be of interest that the value obtained is close to that of cytochrome f (0.38 v., cf. Clark, 1960).

P-672 carries out a Hill reaction with DCPIP, as evidenced by photoreduction of the dye+, but does not do so with quinone. This is consistent with its postulated role as catalyst of one of the two light reactions, since the Hill reaction with quinone is considered to involve both light reactions and that with DCPIP only one (Govindjee, Thomas, and Rabinowitch, 1960; Witt et al., 1961; Losada et al., 1961).

In spite of the difference in absorption spectrum and in putative photochemical role, certain properties of P-672 resemble those reported

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* ESR spectra are recorded as first derivative curves. A double integration of these curves is required to obtain the number of unpaired electrons. However, for a given signal the difference between the upper and lower peaks of the trace will be proportional to the number of unpaired spins. The latter measure has been used in this paper.

+ Measured by change in absorbance at 600 mμ in the reaction mixture of Holt et al. (1951). A typical preparation showed an absorbance change of 0.42 OD units/min/mg chlorophyll when illuminated; no change in the dark.

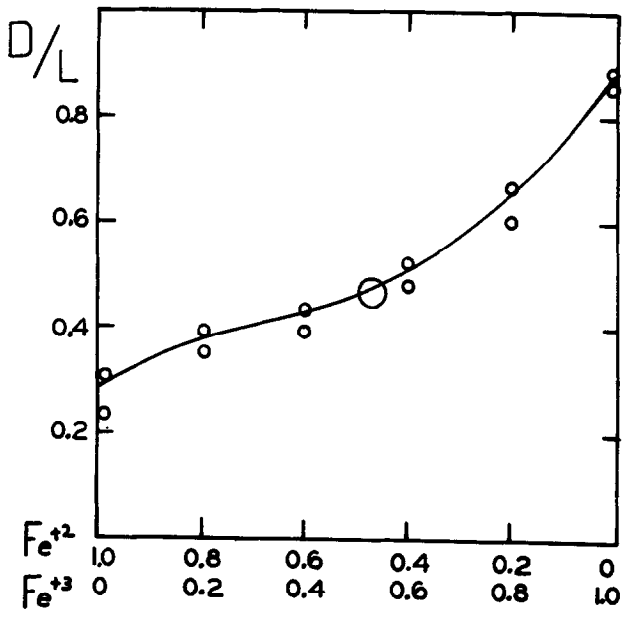

Figure 2. Ratio of dark to light signal as a function of the proportions of ferri- and ferrocyanide in the system. An equimolar mixture of ferri- and ferrocyanide has a potential of 0.43 v. at pH 7 (Clark, 1960). The large circle indicates the light/dark ratio with the natural electron carrier in the supernatant liquid.

for photocatalyst P-700 (Kok, 1961, Beinert et al., 1962), which is presumably also a chlorophyll complex. Both the ESR signal formed on illumination and the redox potential of the two complexes are similar. Since neither has as yet been obtained completely pure, it may be that this similarity reflects a common component responsible for these reactions in both systems. Alternatively, it may be that this type of ESR signal and redox potential are characteristics of several, perhaps all, chlorophyll complexes. In this case, the long wave length peak in the action spectrum of the activation of this type of ESR signal in intact cells (Allen et al., 1961, 1962) may be ascribed, not to a specific association of this signal with P-700 (Beinert et al., 1962), but rather to the lack of formation at these wave lengths of other photoproducts that can react with the radical and thus reduce its steady state concentration.

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