THE LIGHT INDUCED ELECTRON PARAMAGNETIC RESONANCE SIGNAL OF PHOTOCATALYST P700*

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Evidence reported earlier (reviewed in Hoch and Kok, 1961) or to be published elsewhere (Kok and Hoch, 1962) has yielded the following information about a pigment complex absorbing at 700 mμ, present in catalytic quantity (one per 300-400 chlorophyll molecules) in all aerobic photosynthetic material. In whole cells or chloroplasts it undergoes two photochemical conversions. One is a direct photochemical bleaching sensitized by surrounding chlorophyll molecules which brings the pigment in the oxidized state and an associated moiety in the reduced state. Sensitization experiments, to be reported, indicate this reduced moiety has a potential low enough ($E_o' \leq -.32$ volt) to reduce, e.g., pyridine nucleotide. A second photochemical conversion mediated by "dark" reactions regenerates the reduced state of the pigment. The activation spectrum for this conversion is different from that of the oxidative step. A particulate fraction can be obtained from chloroplasts or algae which contains the 700 mμ pigment complex in a more concentrated form, amenable to spectroscopic study, but in which only the first photochemical reaction remains intact. The pigment moiety of the complex in all likelihood is a chlorophyll. In the dark it behaves as a reversible, single electron transfer redox agent with an E_o' (pH 7.0) = +0.43 volt (Kok, 1961). The photochemical transfer of an electron from the pigment was found to occur at liquid nitrogen temperature as well as at +50°C. In well-washed preparations, this "charge separation" is quite stable – even at room temperature, the back reaction requires considerable time (minutes). Phenazin

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methosulfate (PMS) was found to be very effective in accelerating this back reaction.

Since P700 is a single electron transferring agent, the possibility exists that it will give rise to an EPR signal in either its oxidized or reduced form. To test this, we have carried out the following experiments.

A concentrated suspension of red algae (strain TX27, obtained from Dr. Jack Myers) was sonicated for five minutes. By repeated centrifugations, larger particles as well as water soluble material (e.g., phycocyanin) were removed. Brief treatment with cold 68% acetone removed two-thirds of the chlorophyll and left practically all original P700 in the precipitate. This precipitate, taken up in phosphate buffer pH 7.4 and dispersed by sonication showed an optical density of about 30 at 680 mμ (chlorophyll) and a reversible absorption change at 700 mμ of 0.3 O.D. units.

These data were obtained with known dilutions of the suspension; the absorption difference at 700 mμ was measured by chemical oxidation and reduction as well as by photochemical oxidation in the presence of PMS. If it is assumed that the red absorption band of P700 equals the intensity of the 680 band of chlorophyll a in solution (E_{molar} 9.10^4), the ratio chlorophyll a/P700 amounted to about 100 and the concentration of P700 to about 2.7×10^{-6}M. The assumed value of the extinction coefficient is a maximum value and thus yields a minimum concentration of the pigment.

This suspension was studied at low temperature and at 20° by EPR techniques with a standard Varian V4500-10A spectrometer. The magnetic field was monitored with a proton probe and proton resonance frequencies were measured with a Hewlett-Packard electronic counter to ± 100 cycles/sec. Diphenylpicrylhydrazyl (DPPH) was used as a field marker. The quantity of free radical formed was determined by double integration of the signal measured at -90°(to prevent a saturation effect) and a comparison to the integrated signal of a standardized solution of nitrosyldisulfonate observed at the same temperature.

Without any external additions, a weak Mn^{++} and a stronger Fe^{+++} (g = 4.3) signal were observed, neither of which were affected by illumination. At g = 2.00, a free radical signal of about 2×10^{-6} M. concentration was present. This signal was affected by light, ferricyanide and PMS in

a fashion identical to the absorption at 700 mμ. When 10^{-4} M. PMS was added in darkness and the sample then brought to liquid nitrogen temperature only the signal of a PMS radical was observed. Upon illumination (-176°C) the signal reappeared and reached a strength of 3.3×10^{-6} M. After thawing to room temperature in the light and refreezing in darkness, the radical concentration dropped to about one-third the maximal value. This follows the prediction that at room temperature the back reaction induced by PMS lowers the steady state concentration of oxidized P700 in moderate light. In order to eliminate any interference by the appearance of PMS radicals, similar experiments were also conducted in the presence of ferro- and ferricyanide. These one electron oxidation-reduction agents do not by themselves give free radical or iron signals. Addition of 10^{-4} ferricyanide (which brings all pigment in the oxidized state) produced a radical concentration very similar to that generated by light (in samples containing 10^{-4} M. ferrocyanide, 10^{-4} PMS or no addition at all). The value of the maximal EPR signal (3.3×10^{-6} M.) agrees reasonably well with that derived from the spectroscopic determination of P700 as discussed above (2.7×10^{-6} M.) if one considers the limits of error of both procedures (20-30%) and the unknown difference between the molar extinctions of the oxidized and reduced forms of P700. Addition of a mixture of ferro- and ferricyanide in the ratio 1 : 1 yielded a signal somewhat less than half of the maximal strength, which agrees with the established potential value E_o (pH 7.0) + 0.43 volt. A second extraction with a higher acetone concentration (80%) removed both the EPR signal and the light induced absorption change at 700 mμ.

The free radical signal that was observed in the preparation initially appeared to be of the same species as the radical that formed on illumination or addition of ferricyanide. It had the following characteristics: $g = 2.0025 \pm 0.0005$ with reference to the g value of DPPH, which was taken as 2.0036 ± 0.0003; peak to peak width at 2.5 gauss modulation, 7.2 ± 0.1 gauss; ready saturation with respect to microwave power and temperature; apparent symmetry and no fine structure observable at a modulation of 1 gauss at 20°; maximal signal strength, corresponding to a $3.3 \pm 1.0 \times 10^{-6}$ M. concentration of the active component if it is assumed that there is one unpaired electron per molecule.

Signals of very similar characteristics were observed on illumination of whole algae TX27 and Scenedesmus either at -176° or at room temperature. In addition to these light-induced signals, there were stronger Mn^{++} signals and broad (about 20 gauss) dark signals superimposed in the whole cells. It appears, however, probable that the component responsible for the light-induced signals in these materials is identical to that producing the signal in the extracted preparation enriched in P700.

Light-induced EPR signals in algae and chloroplasts have been reported earlier (reviewed in Hoch and Kok, 1961). The concentration of unpaired electrons found by all authors including ourselves is considerably lower than the chlorophyll concentration, but consistent with the concept of the photosynthetic unit. There seems to be no evidence against identifying the signal reported above with those noticed by others, although most authors indicate a width of 9 - 10 gauss for the light-induced signals and we consistently found 7 - 8 gauss. A long wavelength sensitization and temperature independence of spin formation noticed by Calvin (1961) and by Allen (1961) are to be expected if P700 were the conversion center involved. Commoner (1961) noticed two kinetically interrelated signals affected by light. The signal observed by this author in light at g = 2.002, half-width 9 gauss, might be identified with the one we reported on above. It is to us a particularly attractive hypothesis that P700 is the reactive component responsible for the EPR signals. This photocatalyst has other properties which one would expect of an ultimate energy converter in photosynthesis (Kok and Hoch, 1962) and is present at a concentration very nearly the same as that of the unpaired electrons which are detected on illumination at low temperature.

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