ELECTRON SPIN RESONANCE STUDIES OF MANGANESE IN CHLORELLA PYRENOIDOSA

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Wet chloroplast preparations exhibit a light induced steady state electron spin resonance signal suggesting that unpaired electrons are formed as a primary act in photosynthesis (Commoner et al. 1956). A qualitative correlation between chloroplast luminescence and the ESR signal has been noted (Calvin et al. 1957) but until now no relationship of the ESR signal to any other light induced alteration in a photosynthetic system has been demonstrated. Studies at this laboratory comparing whole cell suspensions of normal and manganese deficient Chlorella pyrenoidosa have revealed a very interesting dependence of a light induced ESR signal at g = 2.0 on the presence of manganous ions. At the same time a progressive and reversible light induced decrease in the manganous ion signal is observed.

Chlorella cells containing a normal amount of manganese, and cells manganese deficient to a degree that they had no Hill reaction activity, were obtained by previously described procedures (Eyster et al. 1958). The cells were concentrated by centrifugation and packed in flat 1 cm. x 5 cm. quartz sample holders of 0.05 ml. capacity. The resonance observations were made on a Varian Model 4500 EPR Spectrometer at 100 Kc. field modulation frequency. The samples were mounted in a slotted cavity and illuminated by a 200 watt tungsten bulb through a 3 cm. glass cell containing 0.25% $CuCl_2$ in water. The light flux reaching the sample surface was 0.4 lumen, measured with a General Electric DP-9 light meter.

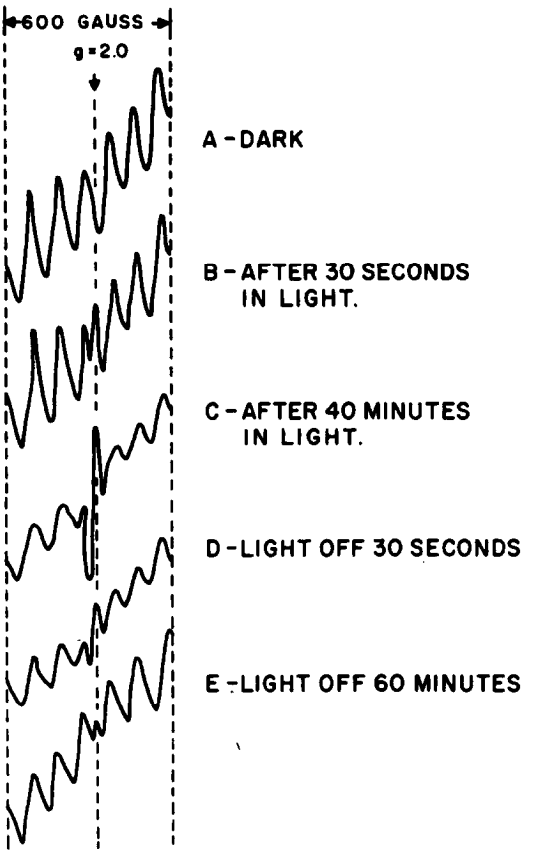

FIG. 1 - ESR SIGNAL - NORMAL Mn CHLORELLA CELLS

Figure 1A shows the signal from normal Chlorella cells in the dark with the characteristic six peak signal of the manganous ion. The signal strength was comparable to a concentration of around 3×10^{-6} molar Mn^{++} in water. Within a few seconds after the light is turned on a "free radical" spin signal at g = 2 becomes apparent as shown in Figure 1B. Continued illumination strengthens the free radical signal and the Mn^{++} signal decreases as shown in Figure 1C. The Mn^{++} signal strength in Figure 1C corresponds to approximately 1×10^{-6} molar Mn^{++}.

When the light is removed, the free radical signal disappears rather abruptly as in Figure 1D and the Mn^{++} signal returns slowly to its original value as indicated in Figure 1E. Over a period of hours the Mn^{++} signal reaches its original value. The cycle can then be repeated.

The manganese deficient Chlorella gave the ESR spectrum shown in

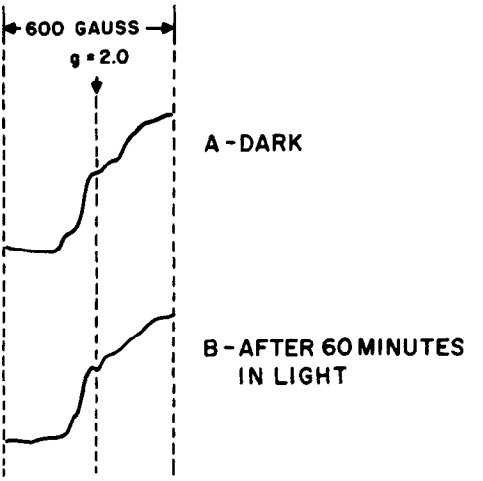

FIG.2-ESR SIGNAL-Mn DEFICIENT CHLORELLA CELLS

Figure 2. The six peak Mn^{++} signal does not appear. The weak free radical signal may reflect traces of Mn^{++} in the cells or it may indicate a low steady state radical concentration in the absence of Mn^{++}.

The broad signal spanning over 600 gauss about the g = 2.0 value which is very prominent in the manganese deficient curve seems to be characteristic of all ESR spectra of intact Chlorella cells. The same type of broad signal is present in the normal Chlorella spectrum, but is less obvious there because the characteristic six-line signal of manganese is superimposed. The source of this broad signal is not as yet identified.

Other work at this laboratory has shown that manganese is an essential factor for the Hill reaction (Brown et al. 1958) and that it is not required for photophosphorylation (Eyster et al. 1960). This indicates that the free radical ESR signal is characteristic of a photosynthetic process other than photophosphorylation.

The sample of normal Chlorella cells irradiated for 40 minutes, Figure 1C, would have evolved 10^{-6} moles O_2 under these conditions, based on measurements on similar cells. The observed decrease in the Mn ESR signal corresponds to a loss of 10^{-10} atom equivalent which is 10^4 smaller than the photosynthetic process. The signal decrease must therefore reflect a non-stoichiometric change, such as pH or the concentration of a metabolite. The

free radical signal appears and disappears much faster than the Mn signal and could be stoichiometric to the photosynthetic rate. Further studies of these relationships are underway.

References

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