ELECTRON SPIN RESONANCE STUDY OF MANGANESE AND IRON IN CHLORELLA PYRENOIDOSA

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In a previous communication, Treharne et al. (1960), relating Mn^{++} content with the light-induced ESR signal in Chlorella pyrenoidosa, reference was made to an unidentified, broad background signal upon which the characteristic six line peak of Mn^{++} was superimposed. We now have evidence that this unidentified signal is related to the presence of iron in the cell.

Fig. 1 presents the ESR spectra obtained from Chlorella pyrenoidosa cells grown with different levels of ferrous and manganous ions in Warburg-Burk medium. The "normal" ESR signal, Fig. 1a, shows the large light-induced ESR signal reported by other investigators, Commoner et al. (1956), Calvin et al. (1957). This light-induced signal is superimposed upon the characteristic six line peak of Mn^{++} which in turn rides on a broad background signal spanning over 600 gauss.

Fig. 1b shows the ESR signal obtained from cells grown in nutrient medium with no added Mn^{++} ion, but with 1.0 ppm Fe^{++}. Here the Mn^{++} signal is missing, the broad signal remains and the light-induced signal is markedly decreased, as reported previously. Growth in a medium containing Mn^{++} but no Fe^{++} yielded cells which exhibited only the usual Mn^{++} signal and a weak light-induced signal. The broad signal was not observed, as shown in Fig. 1c.

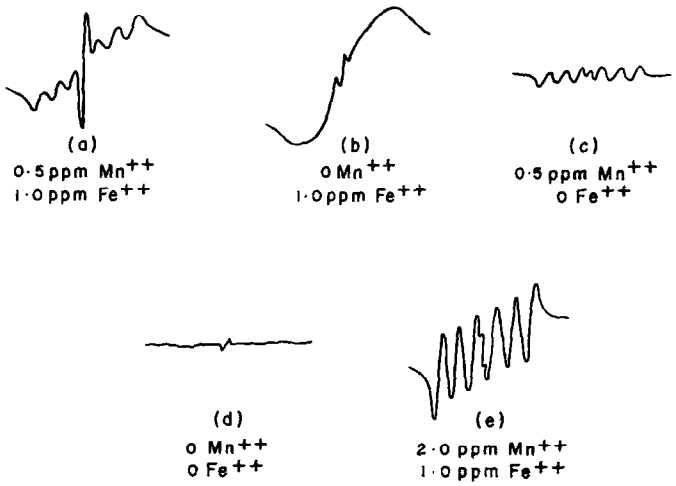

Figure 1. The effect of Fe^{++} and Mn^{++} on the ESR signals observed with Chlorella pyrenoidosa.

Different levels of iron and manganese were supplied in Warburg and Burk medium during growth. At time of harvest (5 days growth) the packed cell volume in microliters per milliliter for each case was (a) 9.33, (b) 2.0, (c) 4.5, (d) 2.0. Each trace spans approximately 600 gauss. Light-induced signals appear at g = 2.0.

For cells grown in the absence of Mn^{++} or Fe^{++} the ESR spectrum is nearly a straight line, as shown in Fig. 1d. Growth was observed under these deficient conditions, but the rate was less than one fourth the normal rate. It should be noted that such cells were "deficient" to the extent that the iron and manganese were below the detectable limits of the electron spin resonance spectrometer. Emission spectrograph measurements on these deficient cells still showed traces of these elements derived from the parent cells which were taken from normal medium.

The optimal Fe^{++} and Mn^{++} levels for both ESR signal magnitude and growth rate were 0.5 ppm Mn^{++} and 1.0 ppm Fe^{++}. With Mn^{++} in excess of 0.5 ppm, the characteristic six peaks of Mn^{++} were more prominent, as seen in Fig. 1e, but the light-induced signal was markedly decreased. Other experiments have shown that the effects of excessive Mn^{++} content

in Chlorella could be offset by increased Fe^{++} level. Maximal cell growth was obtained at high Mn^{++} levels only by increasing the Fe^{++} content of the medium.

The data shown in Fig. 1 indicate that the broad signal is related to the iron content of the cells. Ferrous ion solutions do not exhibit a signal in this region, but ferric ion solutions at 10^{-2} M do produce a signal very similar in g value and line width to that observed in the Chlorella cells. Emission spectrograph determinations show that the iron level within the cell is greater by a factor of 1000 than that of the nutrient medium. Therefore, a 10^{-2} M concentration of iron within the cell is not unreasonable. However, the present experiments give no information on the nature of the iron within the cell.

The electron spin resonance observations were made on a Varian Model 4500 EPR Spectrometer at 100 KC field modulation frequency. Chlorella cells were collected by centrifugation at 1000 x G. and packed in a flat 1 cm x 5 cm quartz sample holder of .05 ml capacity. The samples were mounted in a slotted cavity and illuminated by a 200 watt tungsten bulb through a 4 cm glass cell containing 2.5% $CuCl_2$ in water. The light intensity reaching the sample was 1300 foot-candles measured with a Weston Model No. 2445 light meter. This light intensity is below the saturating light intensity range for normal cell growth. At this intensity only one light-induced signal was observed at g = 2.0. At intensities above light saturation we were able to detect a second light-induced signal as reported by Commoner et al. (1956).

The microwave power level at which the spectra are run is very important for the optimal resolution of Mn^{++}, Fe^{+++} and light-induced signals. Although the light-induced signal shows little decrease in

amplitude out to 10 db attenuation of microwave power (0 db corresponds to 250 milliwatts), the Mn^{++} sensitivity falls off rapidly with decreasing microwave power indicating short relaxation time compared to the light-induced signal. Under our conditions, 3 db attenuation, or 125 milliwatts microwave power, is optimal for simultaneous observation of Mn^{++}, Fe^{+++} and the light-induced signal.

REFERENCES

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