THE LIGHT INDUCED EPR SIGNAL OF PHOTOCATALYST P700

II. TWO LIGHT EFFECTS*

Bessel Kok

RIAS, 7212 Bellona Avenue, Baltimore 12, Maryland

and

Helmut Beinert**

Institute for Enzyme Research, Madison, Wisconsin

Received October 8, 1962

We have reported evidence that the light dependent narrow EPR signal in photosynthetic material is due to the photooxidized form of photoconverter P700 (Beinert et al., 1962). If this were correct it would afford another possibility to study the kinetic behavior of this catalyst in addition to the so far solely available method of difference spectroscopy. Actually the EPR method has several advantages over the latter. There is no interference by the excessive amount of sensitizing chlorophyll and the effects can be studied without a monitoring illumination, itself, bound to provoke actinic effects. In whole Anacystis cells - the material used in this work - the P700 signal is practically absent in darkness at room temperature and can become very predominant in the light. Therefore, the second, broad EPR signal (Commoner, 1961; cf. also Allen, et al., 1961, 1962), and also the Mn^{++} signals which are present both in light and dark necessitate only a minor correction. In this paper we report some observations made at room temperature with the technique described before (Beinert et al., 1962). Agreement between the kinetic response

*This work was supported by grants from the National Institutes of Health (RG-5073, RG-6692, RG-6762), the Air Force Office of Scientific Research (AF49(638)-947), and by AEC contract AT (11-1)-909.

**Recipient of Research Career Award of the National Institutes of Health.

to different wavelengths of P700, observed spectroscopically (Kok and Gott, 1960), and the EPR signal further stresses that an identical species is involved and confirms its proposed mechanism of operation.

Whole Anacystis cells placed in a flat sample holder (0.3 mm layer thickness) in the resonance cavity were illuminated with monochromatic light obtained by placing interference filters in a light beam generated by a projector. Two wavelengths were used - one 635 mμ, largely absorbed by the accessory pigment phycocyanin, the other 713 mμ, largely absorbed by chlorophyll a. A copper sulfate filter and short wavelength cutoff filters (Schott OG3 and RG8, respectively) were in the light path. We estimated that the sample absorbed 80-90% of the incident light at 635 mμ and 25% or less at 713 mμ. Intensity was varied by changing lamp voltage and measured with a thermopile in relative units. Rather independent of lamp voltage, the 713 mμ light was of approximately half the intensity of the 635 mμ beam. Therefore, considering the difference in absorption, the 635 mμ beam provided the algae with about eightfold more absorbed light than the 713 mμ beam at any given lamp voltage. For expt. fig. 1 we used a split beam arrangement: half the beam passing through the 635, the other half through the 713 filter. By covering up one, both or neither of the filters, we could measure at each lamp voltage the effect of 713 mμ light, o about eightfold stronger 635 mμ light, and of both lights together.

Exposures to light were alternated with dark observations and in each case the signal was recorded during sufficient time to reach a steady value. Unless dark times of 30 seconds or shorter were used, the signal developed slowly in weak light in a fashion reminiscent of photosynthetic induction.*

In fig. 1 we plotted the observed EPR signals as a function of incident intensity. Conversion to absorbed intensity would require an

*In a recent paper Allen, et al., 1962, ascribe this induction to respiratory oxygen depletion in the dark. These authors also mention an antagonistic effect of two wavelengths which are possibly comparable to the one described in this paper.

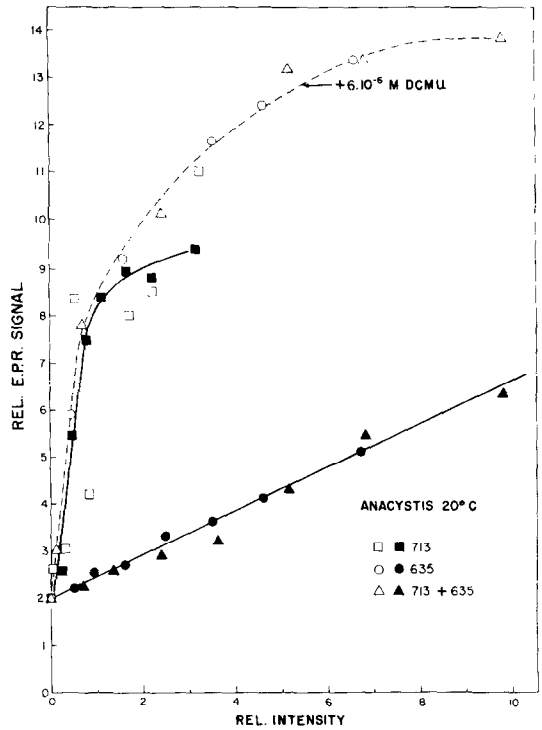


Fig. 1. Dependence of intensity of narrow EPR signal on light of different intensity at 713, 635 and 713 + 635 mμ. Whole cells of Anacystis nidulans were suspended in growth medium at a chlorophyll concentration of 4.9×10^{-4} M. They were placed in a flat EPR quartz cell of optical path 0.3 mm. The cell was introduced into the cavity and illuminated through the slotted window as described in the text (temperature 20°). Wavelength of illuminating light: squares 713 mμ, circles 635 mμ, triangles 635 + 713 mμ. Closed symbols and full lines: data obtained with unpoisoned algae. Open symbols and upper dashed line: data obtained with a suspension containing 6.10^{-6} M DCMU.

approximately fourfold decrease of the abscissa scale for the 713 mμ data, and a correction of about 30% for the (713 mμ + 635 mμ) data.

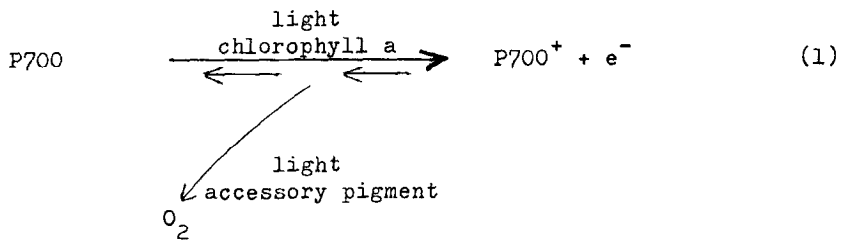
Six curves of signal vs. intensity are shown obtained with two parallel samples of algae, to one of which 6×10^{-6} M DCMU (3(3,4-dichlorophenyl) -1, 1-dimethylurea) was added. In non-poisoned algae the 713 mμ light proves manyfold more effective in provoking the EPR signal than the 635 mμ light (cf. Allen, et al., 1961; Calvin, 1961). We observed that at intensities higher than were attainable in the experiment of fig. 1, both 635 mμ and

713 mμ light yielded about the same saturation level of the signal. This showed that the difference between the two beams concerns the low light range only, where the rate of photosynthesis is determined by intensity.

Strikingly, if 635 mμ light is added to 713 mμ light, the resulting signal is nearly as low as if no 713 mμ light at all were present. Correction for absorbed instead of incident intensity would raise the slope of the (713 mμ + 635 mμ) curve by 20-40% but still leave it much lower than the 713 mμ slope.

Addition of DCMU in a concentration high enough to abolish photosynthetic activity makes 635 mμ light some fifteenfold more effective in producing the EPR signal. Considering absorbed intensities, 713 mμ light alone (and probably also the mixture) is still more effective than 635 mμ light, but the difference is far less pronounced than in the absence of DCMU. The data further indicate that the maximum (saturation) signal in strong light is higher in the presence of DCMU than in its absence.

An obvious interpretation of the data of fig. 1 can be given if it is assumed that the photooxidized form of P700 is the species yielding the EPR signal. We have proposed earlier (Kok and Hoch, 1961) that in photosynthesis two photoreactions occur, sensitized by different pigment groups. One of these photooxidizes P700, while the other causes its reduction (cf. eq. (1)).



In continuous white light both effects occur simultaneously and at equal rate. The higher the intensity, the faster the cycle will run until one of the two reactions reaches its maximum velocity and the process attains

its saturation rate. In addition to the reductive step driven by light, a dark reduction reaction of P700 exists - which, of course, is not accompanied by oxygen evolution as the light step.

According to this picture, reaction (1) will proceed all the way to the right in long wavelength light (713 mμ), which itself is practically unable to provoke photosynthesis. The result will be a high concentration of oxidized P700, observed spectrophotometrically and with the EPR method. Short wavelength light induces both conversions simultaneously and the steady state level of $P700_{ox}$ (the EPR signal) cannot simply be predicted. As long as photosynthesis is induced efficiently, oxidation and reduction rates balance each other and only at high (saturating) intensities will the equilibrium shift to an extreme side - depending upon where the rate limiting step is located. The fact that strong 635 mμ light induces the same (saturation) signal as strong 713 mμ light indicates that the rate limiting step is located in the reductive reaction ("photostep II"). In a way, the experiment of fig. 1 resembles an "enhancement" experiment, in which an excess of (effective) short wave light is added to (ineffective) long wave light. However, instead of observing the increase of the yield of oxygen evolution by the 713 mμ beam (e.g. Myers and French, 1960), we observe the decrease of the ratio $P700_{ox}/P700$ to a level corresponding to efficient mediation of electron transport.

Fig. 1 also shows that in the presence of DCMU - which inhibits oxygen evolution and the second (reductive) step - 635 mμ light, which sensitizes both reactions, is far more effective in producing the EPR signal. Still, under all conditions including the presence of DCMU, the EPR signal returns quickly to zero after the light is switched off and relatively strong intensities are needed to produce the maximum signal. This confirms the earlier finding, with the difference spectroscopic method, of a dark reduction path $P_{ox} \longrightarrow P_{red}$. We have no ready explanation for the possibly significant increase of saturation signal by DCMU.

In summary, the described EPR data support our earlier proposition that the species involved is the oxidized form of photoconverter P700 and our suggestion concerning the metabolic role of this intermediate.

REFERENCES

Allen, M. B., L. R. Piette and J. C. Murchio, Biochem. Biophys. Res. Comm., 4, 271 (1961).
Allen, M. B., L. R. Piette and J. C. Murchio, Biochim. Biophys. Acta, 60, 539 (1962).
Beinert, H., B. Kok and G. Hoch, Biochem. Biophys. Res. Comm., 7, 209 (1962).
Calvin, M., in Light and Life (McElroy, W. D., and Glass, B., Eds., Johns Hopkins Press, Baltimore, 1961), pp. 346-355.
Commoner, B., in Light and Life (McElroy, W. D., and Glass, B., Eds., Johns Hopkins Press, Baltimore, 1961). pp. 356-377.
Kok, B., and W. Gott, Plant Physiol., 35, 802 (1960).
Kok, B., and G. Hoch, in Light and Life (McElroy, W. D., and Glass, B., Eds., Johns Hopkins Press, Baltimore, 1961), pp. 397-416.
Myers, J., and S. French, J. Gen. Physiol., 43, 723 (1960).