M. AVRON, *Proceedings of the Third International Congress on Photosynthesis*
September 2-6, 1974, The Weizmann Institute of Science, Rehovot, Israel
Elsevier Scientific Publishing Company, Amsterdam, The Netherlands, 1974

ACTION SPECTRA FOR LIGHT-INDUCED pH CHANGES IN CHLOROPLAST SUSPENSIONS FROM THE YELLOW-GREEN ALGA BUMILLERIOPSIS FILIFORMIS

Eckhard Loos

Fachbereich Biologie der Universität Regensburg
84 Regensburg, Germany

1. Summary

The action spectrum for light-induced proton uptake by Bumilleriopsis chloroplasts is shifted towards longer wavelengths in the presence of pyocyanine. For measuring the action spectra chloroplasts were illuminated with alternating beams of a reference and a variable wavelength yielding equal action as judged from the absence of any modulated pH signal. The relative action was obtained from the intensity ratio of reference to variable wavelength.

2. Introduction

Action spectra for proton uptake are reported to have relative high activity in the far red region, indicating a predominant action of photosystem I[1,2]. The red rise of the quantum yield for proton uptake points in the same direction[3]. However, evidence has also been presented for the participation of both pigment systems, based on the agreement of action spectra for proton uptake and NADP reduction with water as electron donor whereas a greater activity in the far red has been found only for NADP reduction with DCIP/ascorbate (+DCMU) as electron donor[4]. It was suggested previously[4] that in the cases where a photosystem I activity for proton uptake had been observed, this might have been due to the routinely added pyocyanine. To test this explanation, action spectra for light driven proton uptake were measured in the presence and absence of pyocyanine.

3. Material and methods

Growth conditions for Bumilleriopsis and the procedure for isolating chloroplasts were as described previously[4,5]. The chloroplasts were suspended in the same reaction mixture as used earlier[4]; stirring during the measurements, however, was omitted. Details on

light sources and the glass electrode used for pH measurements are to be found elsewhere[4].

4. Results and discussion

A. Experimental procedure

For obtaining action spectra for proton uptake the following principle was employed: The chloroplasts were illuminated with alternating beams of a fixed reference wavelength and a variable wavelength. If the effect of the two beams is unequal, a modulated pH signal results, which was detected by a lock-in amplifier. The intensities of the beams were adjusted for equal action, i.e. for zero output of the lock-in amplifier. The relative action of variable/reference wavelength then is given by the intensity ratio of reference/variable beam.

Fig. 1 shows the set-up used to realize this principle. The beam of variable wavelength is chopped at 0.15 Hz and causes - when given

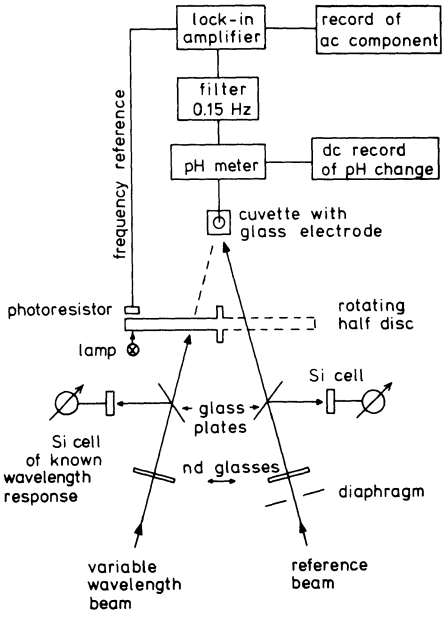

Fig. 1. Diagram of set-up for measuring action spectra. The reference beam was passed through a heat-reflecting filter, a red filter (Schott RG 665) and a 6 cm layer of water. The phase was adjusted by sliding the lamp-photoresistor unit along a circular track coaxial with the rotating half disc.

alone - a modulation in Δ pH to which the output of the lock-in amplifier corresponds. If the reference beam is alternated with the first beam, the average light intensity increases and with it the Δ pH. The modulated signal, however, decreases to zero if the two beams are equal in action (zero output of lock-in amplifier), or, it reappears, phase-shifted by 180° if the reference beam is stronger in action (negative output of lock-in amplifier). The exact matching of the beams is time consuming because of the relative long damping time of the lock-in amplifier (τ = 15 sec). For practical purposes, sufficiently accurate results are obtained by interpolating from two intensity settings yielding signals on each side of the zero line and close to it.

During the measurements the chloroplasts are kept under continous illumination; i.e., one gets an action spectrum for proton uptake in the steady state when proton influx equals proton efflux. Generally, the method should be applicable to any light-dependent process which is reversed in the dark.

The accuracy of the method depends on the signal to noise ratio when the signal results from any single beam. The amplitude of the signal is determined within a certain range by the chopping frequency and the decay constant of the process in question. In the best cases here the signal to noise ratio was around 30, corresponding to an estimated accuracy of $\pm$ 1.7 %. By matching a reference and a variable wavelength beam no corrections need be made for slow changes in chloroplast activity. This fact was realized already previously. Action spectra were accordingly measured for Hill reactions leading to nonreversible transmission changes[6]. In that case, however, two continous light beams had to be used and with them two separate samples.

B. Action spectra

The action spectrum for the light-induced pH change is shifted towards longer wavelengths in the presence of pyocyanine (fig. 2). It is concluded from this shift that photosystem II is involved in the proton pump with no cofactor added, the effect of pyocyanine is to catalyze an electron transport through photosystem I coupled with proton translocation.

This work was supported by Deutsche Forschungsgemeinschaft.

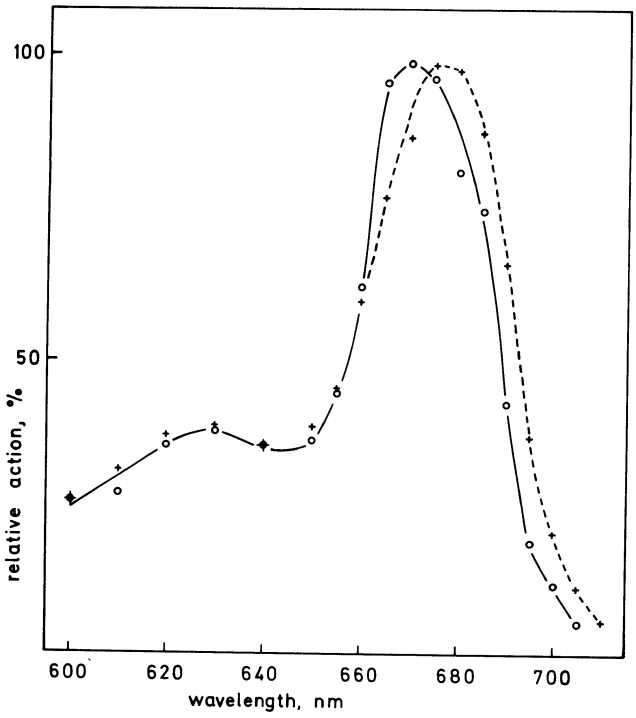

Fig. 2. Action spectra for the light-induced pH change; without pyocyanine (circles, full line) and with 10 µM pyocyanine (crosses, broken line).

5. References

1. Dilley, R.A., 1967, in: Energy conversion by the photosynthetic apparatus. Brookhaven Symp. Biol. 19, 258.
2. Heath, R.L., 1972, Biochim. biophys. Acta 256, 645.
3. Dilley, R.A. and Vernon, L.P., 1967, Proc. nat. Acad. Sci. U.S.A. 57, 395.
4. Loos, E., 1974, Photosynthetica 8, 109.
5. Böger, P., 1969, Z. Pflanzenphysiol. 61, 85.
6. Halldal, P., French, C.S. and Lawrence, M., 1971, in: Carnegie Institution Yearbook 69, 670.