Biochimica et Biophysica Acta, 305 (1973) 483–487

BBA Report

BBA 41233

Light-induced spectral changes of P700 in the 800-nm region in *Anacystis* and spinach lamellae

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(Received March 19th, 1973)

SUMMARY

The light-*minus*-dark difference spectra of Photosystem I fragments of *Anacystis* and spinach lamellae in the far-red region were measured in an attempt to find the spectrum of the oxidation product of reaction center chlorophyll, P700. The results indicated a low but distinct positive band at 815 nm with a round shoulder around 750 nm. This was interpreted to be due to a P700 cation, probably $P700^+$, from its similarity in band position and height to the chlorophyll *a* cation, recently observed *in vitro*. The molar extinction coefficient of this 815-nm band was estimated to be approximately $1.2 \cdot 10^4$ $M^{-1} \cdot cm^{-1}$.

The reaction center chlorophyll in Photosystem I of photosynthetic organisms, which was discovered spectrophotometrically by Kok[1,2], is called P700 because of its maximum absorption decrease at 700 ± 5 nm (refs 3–6). Data obtained *in vivo*[7,8] as well as *in vitro*[9,10] support the view that the primary photochemical act in Photosystem I is photo-oxidation of P700, resulting in the formation of a P700 cation. Recently, Borg *et al.*[11] succeeded in observing the absorption spectrum of the one-electron oxidation product of chlorophyll *a* in organic solvents and the spectrum showed a band at 820 nm in the far-red region. More recently, the use of β-ray pulses from a Van de Graaff generator was successfully applied by Seki *et al.*[12] in our institute to obtain chlorophyll anions and cations in organic solvents. The chlorophyll cation showed a band at 810 nm at room temperature and at 835 nm at 77 °K, and the molar extinction coefficient (ϵ) of this 835-nm band was $0.7 \cdot 10^4$ $M^{-1} \cdot cm^{-1}$, which is roughly one-tenth of the ϵ values of the red band of chlorophyll *a* in organic solvents. The weak band in the far-red region *in vitro* is worthy of notice, as light-*minus*-dark difference spectra[1–6] of P700 of various chloroplast preparations show a deep 700-nm trough with a large coefficient change of about $-\Delta\epsilon$ =

$6 \cdot 10^4 - 7 \cdot 10^4$ $M^{-1} \cdot cm^{-1}$, but do not show any marked maxima comparable in height with this minimum. The absorbance band of the oxidation product may be in a completely different spectral region, but the low absorbance band of the chlorophyll cation rather suggests that oxidized P700 might also possess a similar low band in the far-red region. In fact, the light-*minus*-dark spectrum recently observed[4,6] showed a trend of absorbance increase above 730 nm. The present study was undertaken to measure the light-induced spectral changes in the 800-nm region of intact lamella preparations of *Anacystis nidulans* and *Spinacia oleracea* (spinach) by a difference spectrophotometry technique developed in our laboratory.

Membrane fragments containing Photosystem I of *Anacystis nidulans* were prepared by differential centrifugation ($10^4 - 10^5 \times g$) of sonicated cells in 10 mM Tris buffer (pH 7.7) containing 400 mM sucrose and 10 mM NaCl. Photosystem I fragments of spinach were prepared from chloroplasts treated with Triton X-100 (ref. 13). The molar ratio of P700 to total chlorophyll in these *Anacystis* and spinach fragments were 1/(95±5) and 1/(90±5), respectively.

Light-*minus*-dark difference spectra were measured with a Shimadzu recording spectrophotometer UV-200 with a symmetrical double-beam system. The photomultiplier supplied with this instrument was replaced by the R-636, a far-red sensitive photomultiplier with a Ga–As(Cs) photocathode, newly developed by the Hamamatsu TV Co.

The base line was first measured with a sample of *Anacystis* lamella suspension in a 1.0-cm cuvette placed in the sample beam and the same suspension in the reference beam. The measurement was made in transmittance units so that, in this case of a dark sample in both beams, I_{ds}/I_{dr} was measured. I_{ds} represents the intensity of the light transmitted through the dark sample on the sample side and I_{dr} represents that on the reference side. The base line (Curve A in Fig. 1) thus observed with dense lamella suspensions showed a small peak and a trough on a nearly straight line. The light-*minus*-dark spectrum was then measured with the sample suspension under illumination against the reference suspension kept in darkness. The cuvette was illuminated on the side with the light (intensity on the cuvette surface = 6300 ergs $\cdot cm^{-2} \cdot s^{-1}$) from a 50-W tungsten lamp through an interference filter (the wavelength for maximum transmission = 444 nm, Nihon Shinkuh Kohgaku) and a 2.0-cm layer of 30% $CuSO_4$ solution. A Toshiba VO-56 filter, which transmits light at greater than 560 nm, was placed in an aperture after the sample suspension to absorb the actinic light. The light-*minus*-dark spectrum (Curve B) thus measured is an approximate one because in this case $I_{ls}^*/I_{dr} = (I_{ls} + I_f)/I_{dr}$ is measured. The intensity (I_{ls}^*) of the light measured for the sample suspension in the light is equal to the sum of the intensity (I_{ls}) of the measuring light transmitted through the suspension in the light and the intensity (I_f) on the fluorescence excited by the actinic light.

A Yasec data recorder was modified to correct for the change of I_{ds}/I_{dr} and for the effect of fluorescence. The base line (Curve A) and the difference spectrum (Curve B) were recorded in parallel on two separate channels of a magnetic tape in this recorder and their difference (Curve C), which is $(I_{ls}^*/I_{dr}) - (I_{ds}/I_{dr}) = (I_{ls} - I_{ds} + I_f)/I_{dr} = (\Delta I_s + I_f)/I_{dr}$, was taken to correct for the effect of the base line fluctuation. To correct for the

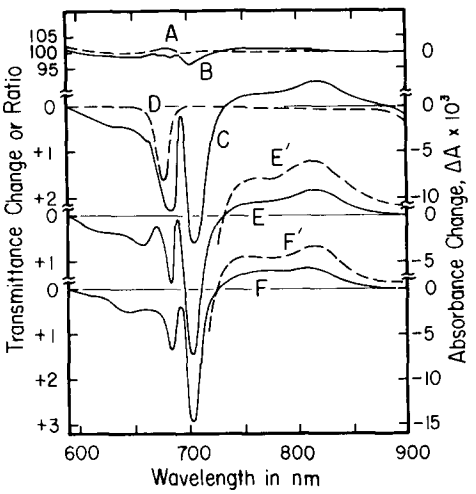

Fig. 1. The light-*minus*-dark difference spectra of Photosystem I fragments of *Anacystis* and spinach lamellae: Curves A to E and E' for *Anacystis* lamella fragments and Curves F and F' for spinach lamella fragments. The sample or reference suspension contained *Anacystis* or spinach lamella fragments at a concentration of 51.5 μM chlorophyll, 167 μM methyl viologen and 5 mM sodium ascorbate in 10 mM Tris buffer (pH 7.7). Curve A is the base line (the dark-*minus*-dark spectrum in I_{ds}/I_{dr}), and Curve B is the uncorrected light-*minus*-dark spectrum in $(I_{ls} + I_f)/I_{dr}$ (see text). Curve C is the spectrum in $(\Delta I_s + I_f)/I_{dr}$ corrected for the base line fluctuation which was obtained by subtraction of Curve A from Curve B. The corrected light-*minus*-dark spectrum (Curve E) was obtained after further correction by subtraction of Curve D (the spectrum in I_f/I_{dr} of the fluorescence excited by the actinic light) from Curve C. The light-*minus*-dark spectra thus corrected are shown by Curves E, E', F and F', of which Curves E' and F' were measured under stronger light illumination.

fluorescence effect, the spectrum (Curve D) measured in terms of I_f/I_{dr}, with the sample suspension illuminated with the actinic light but not with the measuring light beam against the dark reference suspension, was subtracted from the difference signals (Curve C) from the Yasec recorder. The resultant signals were recorded on a chart to obtain the difference spectrum (Curve E) in terms of $\Delta I_s/I_{dr}$ free from various artifacts. The value of $\Delta I_s/I_{dr}$ is equal to $-\Delta \ln I = -2.303 \times \Delta \log I = 2.303 \times \Delta A$ (absorbance difference), when this fractional change is very small. This condition was satisfied in the present experiment.

The corrected light-*minus*-dark spectrum (Curve E) thus observed agrees with the difference spectra recently observed[5,6], in that it has two marked negative peaks; the negative peaks on Curve E were at 682 and 703 nm. In addition to these negative peaks, Curve E clearly shows a positive band at 815 nm with a round shoulder around 750 nm, which may be regarded as the band of the oxidation product of P700. Upon illumination of a dark suspension on the sample side, the corrected absorbance value at 815 nm increased in parallel with the absorbance decrease at 703 nm, and an isobestic point was at 725 ± 3 nm. It took about 15 s to obtain the steady-state difference spectrum such as shown by Curve E. A similar experiment was conducted with a stronger actinic light transmitted through a Toshiba VB-46 band-pass filter (maximum transmission at 460 nm),

together with Toshiba VR-67 filter for elimination of the scattered actinic light. The corrected light-*minus*-dark spectrum (Curve E′) obtained under these conditions showed the 815 nm positive band more distinctly and indicated complete conversion of P700; the 700-nm negative peak on complete conversion is off the scale in Fig. 1. This filter choice made it, however, difficult to observe the spectrum below 690 nm. The degree of conversion in Curve E was estimated to be 53%, based on the conversion shown by Curve E′.

The same measurements were made for the spinach preparation under the same conditions as adopted for the measurements of Curves E and E′. The results are shown by Curves F and F′, respectively, which show two negative bands and a positive band and shoulder at the same wavelengths as those found for *Anacystis*. The ratio in height between the 682- and 703-nm peaks of these organisms was approximately 1:2, which agrees with the ratio determined previously for spinach preparations[5,6]. The ratio of $+\Delta\epsilon$ at 815 nm to $-\Delta\epsilon$ at 703 nm was estimated from the curves in Fig. 1 to be 0.20 for *Anacystis* and 0.15 for spinach. The extinction decrease at 700 nm so far determined as $3.6 \cdot 10^4$, $4.2 \cdot 10^4$ and $6.4 \cdot 10^4$ $M^{-1} \cdot cm^{-1}$ for spinach preparations[6,14,15], and $7.0 \cdot 10^4$ and $12.0 \cdot 10^4$ $M^{-1} \cdot cm^{-1}$ for *Anabaena*[6,16]. If we assume the most recently determined values[6] for *Anabaena* and spinach, the extinction increase at 815 nm is calculated to be $(6.4–7.0) \times 10^4 \times (0.15–0.20)$ $(1.2 \pm 0.3) \times 10^4$ $M^{-1} \cdot cm^{-1}$, which is not widely different from the ϵ value at 835 nm, $0.7 \cdot 10^4$ $M^{-1} \cdot cm^{-1}$ determined for the chlorophyll cation[12]. From the similarity in band position and height, compared to the chlorophyll cation spectrum found recently by Borg *et al.*[11] and Seki *et al.*[12], the *in vivo* 815-nm band with the 750-nm shoulder is very likely to be the band of $P700^+$. *In vivo* measurements with light pulses in the far-red region will bring more conclusive evidence for this interpretation than do the steady-state measurements in the present study.

The technical assistance of Miss Asayo Suzuki is gratefully acknowledged. The present study was supported by research on grants on Photosynthesis and on Chlorophyll formation, given by the Ministry of Education, and by a grant for the study of Life sciences at the Institute of Physical and Chemical Research (Rikagaku Kenkyusho).

REFERENCES

1 Kok, B. (1956) *Biochim. Biophys. Acta* 22, 399–403
2 Kok, B. (1957) *Nature* 197, 583–584
3 Kok, B. and Hoch, G. (1961) in *Light and Life* (McElroy, W.D. and Glass, B., eds), pp. 397–416, The John Hopkins Press, Baltimore
4 Rumberg, B. and Witt, H.T. (1964) *Z. Naturforsch.* 19b, 693–707
5 Döring, G., Bailey, J.L., Kreutz, W., Weikard, J. and Witt, H.T. (1968) *Naturwiss.* 55, 219–224
6 Hiyama, T. and Ke, B. (1972) *Biochim. Biophys. Acta* 267, 160–171
7 Kok, B. (1961) *Biochim. Biophys. Acta* 48, 527–533
8 Beinert, H. and Kok, B. (1963) in *Photosynthetic Mechanisms of Green Plants* (Kok, B. and Jagendorf, A.T., eds), pp. 131–137, Natl. Acad. Sci. Natl. Res. Counc., Washington D.C.
9 Linschitz, H. and Sarkanen, K. (1958) *J. Am. Chem. Soc.* 80, 4827–4832
10 Seifert, K. and Witt, H.T. (1968) *Naturwiss.* 55, 222–223
11 Borg, D.C., Fajer, J., Felton, R.H. and Dolphin, D. (1970) *Proc. Natl. Acad. Sci. U.S.* 67, 813–820
12 Seki, H., Arai, S., Shida, T. and Imamura, M. (1973) *J. Am. Chem. Soc.*, in the press
13 Vernon, L.P., Shaw, E.R. and Ke, B. (1966) *J. Biol. Chem.* 241, 4101–4109

14 Schmidt-Mende, P. and Rumberg, B. (1968) *Z. Naturforsch.* 23b, 225–228
15 Schliephake, W., Junge, W. and Witt, H.T. (1968) *Z. Naturforsch.* 23b, 1571–1578
16 Ke, B., Ogawa, T., Hiyama, T. and Vernon, L.P. (1971) *Biochim. Biophys. Acta* 226, 53–62