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ACTION SPECTRUM FOR THE SHRINKAGE OF CHLOROPLASTS

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SUMMARY

The effect of monochromatic light on the rate of shrinkage of whole chloroplasts—a new response of the plastids to light, recently discovered by the present authors—was investigated. The relative rate of shrinkage of isolated spinach chloroplasts on illumination was determined using the Coulter counter. The action spectrum obtained showed two major peaks at 435 and 680 mμ with a minor peak and a shoulder at 720–740 and 490 mμ, respectively. The significance of this spectrum is discussed referring to the absorption spectrum of chloroplasts as well as the action spectra so far reported for other photosynthetic reactions.

INTRODUCTION

In a previous study[1], we have established that whole spinach chloroplasts in suspension shrink on illumination to approx. 50–80% of their volume in the dark. This shrinkage in the light is usually completed within several to 10 min and gradually reverses in the dark as observed with the Coulter counter. Electron micrographs of illuminated chloroplasts as compared with those in complete darkness revealed that the shrinkage is accompanied by a considerable change of axial ratio, which accounts for the observed increase of the light-scattering cross-sectional area of chloroplasts by illumination.

These changes in size and shape of chloroplasts could be obtained in phosphate buffer without any addition of cofactors of photophosphorylation such as Mg^{2+}, ADP, etc., or electron acceptors of the Hill reaction. Inhibition experiments indicated, however, that the shrinkage of whole chloroplasts undoubtedly has some correlation with photophosphorylation. PACKER's[2] observation of the change by illumination of light-scattering by chloroplast fragments, which was interpreted by him as a result of photophosphorylation, is therefore considered to be essentially related to our findings. In this communication, we describe the dependencies of the rate of shrinkage on the intensity and wavelength of light, which were investigated in order to have an insight into the nature of the photochemical site of the phenomenon.

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EXPERIMENTAL

Preparation of samples

The preparation of whole spinach chloroplasts with a sharp volume distribution (Fig. 1 and ref. 1) was made by the same procedure as described earlier[1,3], except that 0.002 M EDTA was added to 0.04 M phosphate buffer (pH 7.2), the medium used

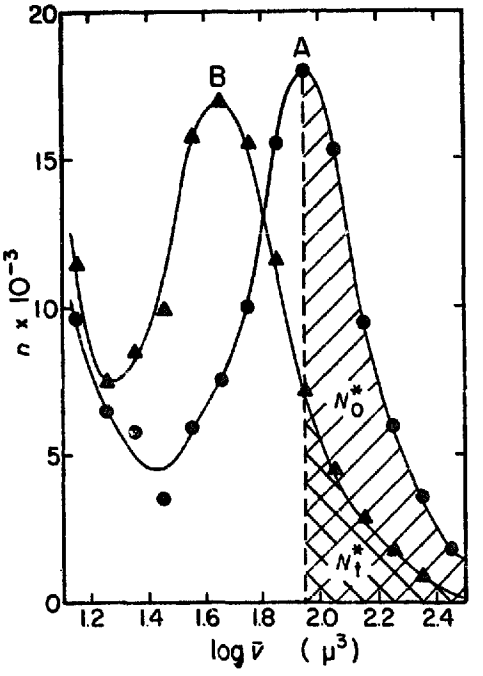

Fig. 1. The principle for the determination of the rate of shrinkage (reproduced from Fig. 1 of ref. 1). Curve A (●—●), the volume-distribution curve of whole chloroplasts in the dark; Curve B (▲—▲), the curve for the chloroplasts in the light.

previously for the preparation and for the measurements of shrinkage. It was found in a preliminary experiment of this study that the rate of shrinkage was approximately doubled by the addition of EDTA. This may be due to the elimination of traces of some toxic metal ions. Such a protective or stimulatory effect of EDTA has been observed on the Hill reaction[4,5]. A concentrated suspension of freshly prepared chloroplasts was preincubated in the dark at 0° for 1 h. The suspension was then diluted appropriately with the same phosphate buffer with EDTA, and was measured.

Measurements

The shrinkage of chloroplasts was measured with the Coulter counter model A, using a 100-μ orifice. The details of the technique are described in previous papers[1,3,6]. Fig. 1 (reproduced from Fig. 1 in ref. 1) illustrates the basic principle for the measurement of the rate of shrinkage. When chloroplasts are illuminated, the distribution curve shifts towards smaller volumes (from Curve A to B). This accompanies a decrease of N^* in the figure from N_0^* to N_t^*; N^* stands for the number of chloroplasts per ml having volumes greater than the volume at the distribution maximum in the dark. Thus, the plot of N^* against time produces a curve. In the present study, the slope of the linear decay of N^* in the first few minutes was taken as a measure of the rate of shrinkage (Fig. 2), and was expressed in $-\Delta N^*$/min. The concentration of chloroplasts in suspension suitable for the measurement of N^* was N(total number of

chloroplasts per ml of suspension) = $(5 \pm 2) \cdot 10^5$. The concentration of this order was so low that the color of chloroplasts in the suspension could hardly be recognized.

A chloroplast suspension in a rectangular cuvette (the front surface perpendicular to the light beam, 1.5 × 2.3 cm^2; optical path 5.0 cm) was placed on the Coulter counter, and was illuminated by the light from the monochromator of a Beckman DU spectrophotometer through a 2.0-cm layer of 0.65% $CuSO_4$ solution. The lamp of the monochromator was run at 8 V (a.c.), and illumination was continued during the measurement of N^*. The light intensity was controlled with the slit of the monochromator to give 65–250 ergs/cm^2·sec of monochromatic light to the sample. A 30-W incandescent lamp was used to illuminate the sample with white light, and thermal radiation was eliminated by the same filter solution of $CuSO_4$. Because of the low concentration of chloroplasts, the effect of mutual shading of chloroplasts under illumination was negligible and the chloroplasts in suspension were illuminated uniformly. Light energies were measured with a Kipp E2 thermopile calibrated to a standard lamp distributed by the National Bureau of Standards (U.S.A.). The absorption spectra of chloroplast suspensions were observed by the opal glass transmission method[7–9] for translucent materials with a Cary recording spectrophotometer model 14M.

RESULTS AND DISCUSSION

Examples of the time course of the decay of N^* during illumination are shown by the curves in Fig. 2, which were obtained with red and blue light beams of 680 and 435 mμ,

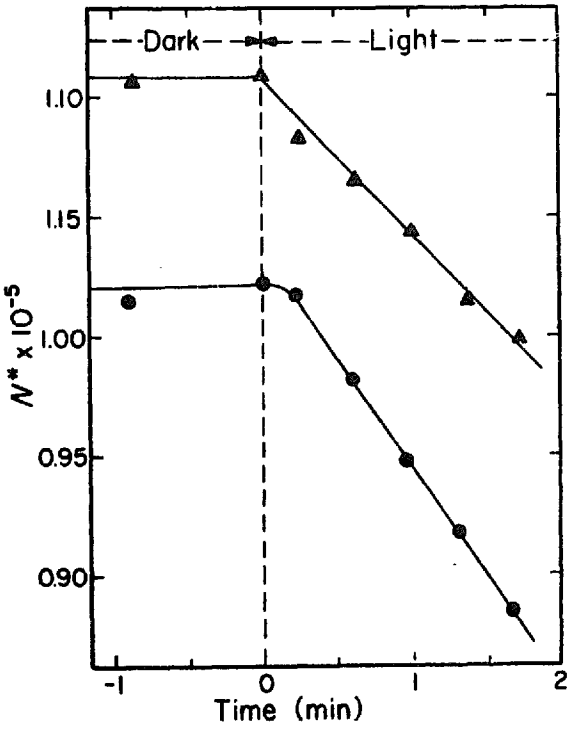

Fig. 2. The time course of the change of N^* during the illumination with red (680 mμ, ●—●) and blue (435 mμ, ▲—▲) light. The intensities of these light beams at the sample were 207 and 86 ergs/cm^2·sec.

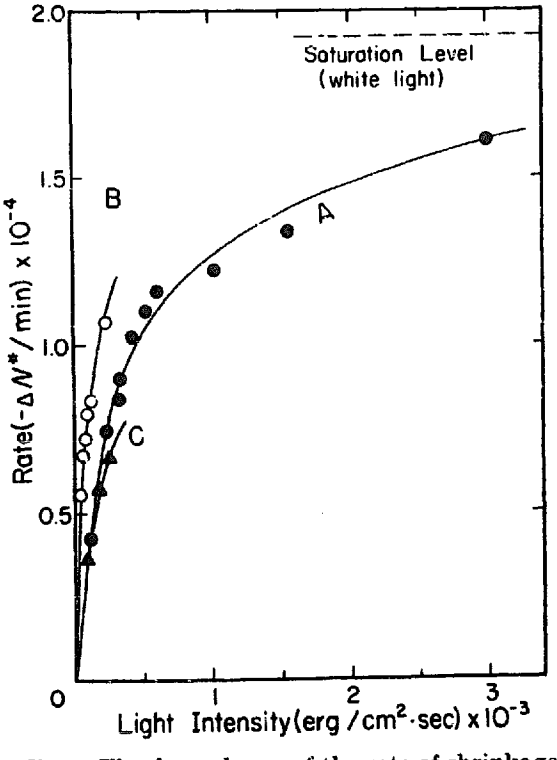

Fig. 3. The dependency of the rate of shrinkage on the intensity of white (Curve A, ●—●), red (680 mμ, Curve B, ○—○) and green (550 mμ, Curve C, ▲—▲) light.

the positions of two major peaks, respectively, in the action spectrum shown later. These curves show a linear decrease of N^* during the first 2 min of illumination, from which the rate of shrinkage was estimated. Curves A, B and C in Fig. 3 represent the rates plotted against the intensities of white, red (680 mμ) and green (550 mμ, the position of a minimum in the action spectrum) light, respectively. The rates for these different light beams increased proportionally as light intensity increases to approx. 250 ergs/cm²·sec. Above this intensity, these curves were more or less bent, and the curve for white light approached a rate level of $1.9 \cdot 10^4$ min^{-1}, which was obtained at light intensity of 5000 ergs/cm²·sec and is indicated by a dashed horizontal line in the figure. The highest intensities of the red and green light obtainable from the monochromator were 500 ergs/cm²·sec, which covered the proportionality range, but limited the observations at higher intensities.

The action spectrum was estimated from the rates measured between 65 and 250 ergs/cm²·sec in the proportionality range, and the result is shown by Curve A in Fig. 4, in which the ordinate on the left hand side indicates the rate per erg/cm²·sec of monochromatic light. Curve B in the same figure is the absorption spectrum of the same preparation of chloroplasts shown for comparison. The chloroplast concentration used for this absorption measurement was roughly 40 times that employed for the measurement of the action spectrum. The action spectrum shows two distinct peaks at 435 and 680 mμ with a shoulder at 490 mμ and a minor peak at 720–740 mμ, while the absorption spectrum shows two peaks at 435 and 678 mμ with a shoulder at 480 mμ. The following facts are evident or deduced from these action and absorption spectra.

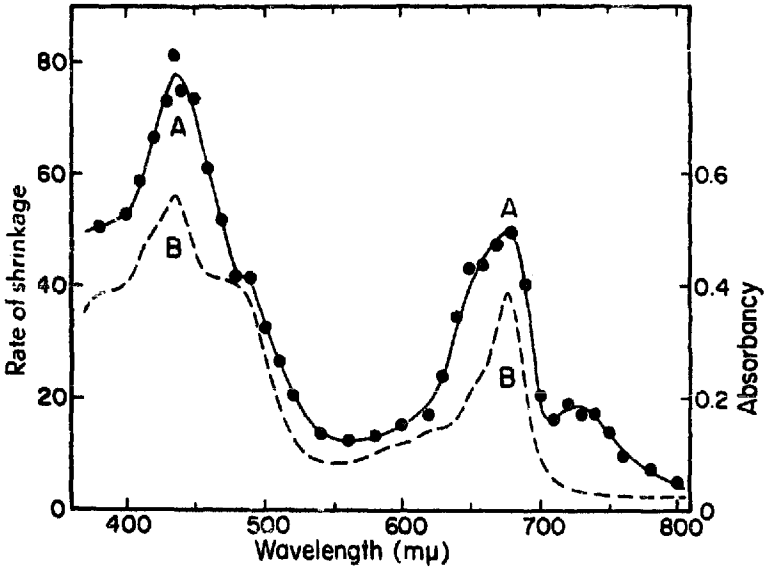

Fig. 4. The action spectrum (Curve A) for the shrinkage of chloroplasts as compared with the absorption spectrum (Curve B) of the same preparation of chloroplasts.

The marked dependency of the rate on the wavelength of light indicates that the reactions inducing the shrinkage involve photochemical reactions. The positions of the two major peaks in the action spectrum agree with those of the Soret and red absorption bands of chlorophyll *a*, respectively. This bears evidence that the shrinkage is effected by the light absorption by chlorophyll *a*. The position of the shoulder on the

long wavelength side of the Soret peak is also in approximate agreement with that of the shoulder in the absorption spectrum. However, its height relative to the Soret peak is appreciably smaller than that in the absorption spectrum. BARER[10] suggested a contribution of chlorophyll *b* to the absorption around this wavelength, and MYERS AND FRENCH[11] obtained two distinct peaks due to chlorophyll *b* absorption at 483 and 653 mμ in the action spectra for the Emerson and the Blinks effects on Chlorella. On the other hand, the spectral changes observed by SAUER AND CALVIN[12] for spinach quantasomes upon bleaching the pigments revealed that the absorption due to carotenoids possesses maxima at 485, 455 and 428 mμ and the Soret band of chlorophyll *b* lies at 470 mμ. Considering these findings, the shoulder in the action spectrum may be due to the absorption by chlorophyll *b* or carotenoids or both, and the smaller relative height indicates their lesser contribution to the shrinkages as compared with chlorophyll *a*. The action spectrum obtained by BLACK *et al.*[13] for the photophosphorylation with spinach chloroplasts showed a shoulder at 500 mμ, and FULLER, BERGERON AND ANDERSON[14] demonstrated that light absorbed by Chromatium carotenoids is capable of catalyzing phenazine methosulfate-supported phosphorylation. These results are compatible with our data, and suggest a close correlation between the shrinkage and photophosphorylation.

A remarkable difference is found between the action and absorption spectra in the far-red region. A minor but distinct maximum is observed in the action spectrum at 720–740 mμ, whereas the absorption spectrum shows no band in the same spectral region. The action spectra obtained by JAGENDORF *et al.*[15] and by BLACK *et al.*[13] for the photophosphorylation with chloroplasts showed a peak at 675 and 680 mμ, respectively. These wavelengths agree with the wavelength of the major red maximum reported here. However, their spectra showed no peak above 700 mμ. The action spectrum for the photophosphorylation observed by KOK AND HOCH[16] also showed a single peak but its maximum was found at the longer wavelength of 710 mμ, which is in between 680 and 720–740 mμ, the positions of the two peaks in our action spectrum. An interesting observation made by the same authors is the absorption change on the addition of phenazine methosulfate to a chloroplast suspension. The difference spectrum has two peaks at 680 and 710 mμ, and the peak at the longer wavelength is lower than the other, so that the curve resembles our curve for the shrinkage, except in the difference in position between their lower peaks. These comparisons with previous data indicate that the shrinkage involves photochemical reactions in common with the photophosphorylation.

Several other reported observations are possibly related to the far-red peak for the shrinkage. DUYSENS[17], from his extensive studies on the energy transfer between pigments in photosynthesis, postulated the presence of a very low concentration of a pigment which has an absorption band in the region of the fluorescence band of chlorophyll *a*. RABINOWITCH *et al.*[18,18] working with Chlorella and Porphyridium observed the inhibition of photosynthesis by additional far-red illumination and found the inhibition maximum at 745 mμ. Later, GOVINDJEE, CEDERSTRAND AND RABINOWITCH[20], by means of a special spectrophotometric technique, identified one or two peaks in the 740–760 mμ region of the absorption spectra of these algae as well as of Anacystis. These results as well as the effects of far-red light on the fluorescence of green leaves[21] and on the photoconductivity of chloroplasts[22] indicate the presence of a pigment absorbing far-red light. According to BUTLER *et al.*[23], on illumination

of maize seedlings containing phytochrome with far-red light the difference absorption spectrum has a negative peak at 735 mμ and a positive peak at 655 mμ. The position of the far-red peak for the shrinkage agrees within the experimental errors with this far-red peak of phytochrome. It should be noted also that microcrystals or monolayers[24-27] of chlorophyll *a* have an absorption band around 735 mμ. These facts suggest the identity of the pigment for the shrinkage with phytochrome or microcrystals of chlorophyll *a*. The fact that the pigment could not be detected by common absorption spectrophotometry indicates that the light energy absorbed by the pigment is much more efficiently utilized for the shrinkage than is the energy absorbed by chlorophylls or carotenoids.

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REFERENCES

1 M. ITOH, S. IZAWA AND K. SHIBATA, *Biochim. Biophys. Acta*, 66 (1963) 319.
2 L. PACKER, *Biochem. Biophys. Res. Commun.*, 9 (1962) 355.
3 M. ITOH, S. IZAWA AND K. SHIBATA, *Biochim. Biophys. Acta*, 69 (1963) 130.
4 A. T. JAGENDORF AND M. EVANS, *Plant Physiol.*, 32 (1957) 435.
5 S. IZAWA, *Plant Cell Physiol. (Tokyo)*, 1 (1960) 269.
6 W. H. COULTER, *Proc. Natl. Electron. Conf.*, 12 (1956) 1034.
7 K. SHIBATA, A. A. BENSON AND M. CALVIN, *Biochim. Biophys. Acta*, 15 (1954) 461.
8 K. SHIBATA, *J. Biochem. (Tokyo)*, 45 (1958) 599.
9 K. SHIBATA, in D. GLICK, *Methods of Biochemical Analysis*, Vol. 7, Interscience, New York, 1959, p. 77.
10 R. BARER, *Science*, 121 (1955) 709.
11 J. MYERS AND C. S. FRENCH, *J. Gen. Physiol.*, 43 (1960) 723.
12 K. SAUER AND M. CALVIN, *Biochim. Biophys. Acta*, 64 (1962) 324.
13 C. C BLACK, J. F. TURNER, M. GIBBS, D. W. KROGMANN AND S. A. GORDON, *J. Biol. Chem.*, 237 (1962) 580.
14 R. C. FULLER, J. A. BERGERON AND I. C. ANDERSON, *Arch. Biochem. Biophys.*, 92 (1961) 273.
15 A. T. JAGENDORF, S. B. HENDRICKS, M. AVRON AND M. B. EVANS, *Plant Physiol.*, 33 (1958) 72.
16 B. KOK AND G. HOCH, in W. D. MCELROY AND B. GLASS, *Light and Life*, Johns Hopkins Press, Baltimore, 1961, p. 397.
17 L. N. M. DUYSENS, *Thesis*, Utrecht, 1952.
18 E. RABINOWITCH, GOVINDJEE AND J. B. THOMAS, *Science*, 132 (1960) 422.
19 GOVINDJEE, E. RABINOWITCH AND J. B. THOMAS, *Biophys. J.*, 1 (1960) 91.
20 GOVINDJEE, C. CEDERSTRAND AND E. RABINOWITCH, *Science*, 134 (1961) 391.
21 W. L. BUTLER, *Biochim. Biophys. Acta*, 64 (1962) 309.
22 S. ICHIMURA, *Biophys. J.*, 1 (1960) 99.
23 W. L. BUTLER, K. H. NORRIS, H. W. SIEGELMAN AND S. B. HENDRIKS, *Proc. Natl. Acad. Sci. U.S.*, 45 (1959) 1703.
24 E. E. JACOBS, A. E. VATTER AND A. S. HOLT, *J. Chem. Phys.*, 21 (1953) 2246.
25 E. E. JACOBS AND A. S. HOLT, *J. Chem. Phys.*, 22 (1954) 142.
26 E. E. JACOBS, A. S. HOLT AND E. RABINOWITCH, *J. Chem. Phys.*, 22 (1954) 142.
27 E. E. JACOBS, A. S. HOLT AND E. RABINOWITCH, *J. Am. Chem. Soc.*, 76 (1954) 142.