Plant Science Letters, 1 (1973) 201—206

ABSORPTION AND CHLOROPHYLL *a* FLUORESCENCE CHARACTERISTICS OF TRIS-TREATED AND SONICATED CHLOROPLASTS

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(Received January 18th, 1973)

SUMMARY

An attempt was made here to examine the three-light-reactions scheme of photosynthesis presented by Knaff and Arnon challenging the conventional Z-scheme. Tris-treated, sonicated chloroplasts ($T_{20}S_2$) — as used by the earlier workers — were prepared and their absorption, fluorescence action and emission spectra at 77° K were measured. This was done to test whether this treatment causes preferential loss of pigments of pigment system (PS) I; the latter could have led to an action spectrum of cytochrome b559 photooxidation matching that of PS II in Knaff and Arnon's work, and thus to their scheme. Results of room temperature absorption spectra, difference absorption spectra of normal minus $T_{20}S_2$ chloroplasts, the ratios of chlorophyll *a* (Chl *a*) fluorescence intensity at 735 nm to that at 685 nm (F735/F685) at 77° K of both particles, and action spectra of Chl *a* fluorescence (F760) of thick samples, indicated that, indeed, there is a preferential loss of long wavelength forms of Chl *a* relative to other forms of Chl *a* in $T_{20}S_2$ particles. Thus, it is likely that system IIa (according to Knaff and Arnon's terminology) may simply be system I without the Chl *a* 690—700 (Chl *a* forms with "red" bands at 690—700 nm); this would make system I look like system II.

INTRODUCTION

An alternate model to the Z-scheme (or the two light reactions, in series) for green plant photosynthesis has been proposed by Knaff and Arnon [1].

Abbreviations: Chl, chlorophyll, Cyt, cytochrome; PS, pigment system.

They proposed the existence of three light reactions in photosynthesis: two system II short-wavelength photoreactions that operate in series and a third system I long-wavelength photoreaction that operates parallel to system II. Evidence claiming support of this proposal was presented [1,2]. Failure to confirm some of the observations in support of the proposed scheme was reported recently by Esser [3]. Among the several lines of evidence supporting the above-mentioned scheme was the necessity of addition of plastocyanin for photooxidation of Cyt b559 by what is claimed system II light (664 nm) in Tris-treated, sonicated chloroplasts [4]. If sonication of chloroplasts causes preferential loss of long-wavelength form of Chl *a*, as was shown in Chlorella [5] and in particles obtained by sonication and differential centrifugation [6] in addition to causing loss of plastocyanin then it is possible to explain (partially) the results obtained by Arnon's group without need to propose two system II light reactions.

Furthermore, in all experiments reported by Knaff and Arnon on Cyt b559 photooxidation by PS II, light of 664 nm wavelength is assumed to be exciting PS II only. This is incorrect as 664 nm excites both system I and system II almost equally [7,8]. At 664 nm, PS II is only 12% more excited than PS I (calculated from action spectra of PS I and PS II reported by Joliot *et al.* [9]).

These above-mentioned ideas considered together with the possible preferential destruction of the long-wavelength form of Chl *a* during sonication could account for an action spectrum of photooxidation of Cyt b559 which appears to match that of PS II in Knaff and Arnon's work [1,2,4].

With this in mind we checked the absorption and fluorescence characteristics of Tris-treated, Tris-treated and sonicated, and control chloroplasts to see whether these treatments actually caused any changes in the concentration and characteristic of the forms of the absorbing pigments.

Several experiments showed that no significant changes in the absorption spectrum of the Tris-treated chloroplasts were obtained, irrespective of the time of treatment. Hence in the results presented here, only data for Tris-treated and sonicated particles are compared with normal chloroplasts. Although Tris treatment did not have significant effect on absorption, only sonicated chloroplasts could be used. However, we used Tris-treated and sonicated particles in order to be consistent with the procedure of Knaff and Arnon [4].

MATERIALS AND METHODS

Chloroplasts were prepared from spinach according to Whatley and Arnon [10] followed by Tris treatment as was used by Knaff and Arnon [4] to study photooxidation of Cyt b559. Tris treatment was followed by sonication in a Branson Sonifier model W185D for either 1, 2 or 5 min. Throughout this study, chloroplasts treated this way will be referred to as $T_{20}S_2$ where T stands for the time of incubation of the chloroplasts in 0.8 M Tris buffer pH 8.0 and S refers to the time of sonication in minutes.

Chlorophyll concentration was determined according to Arnon [11]. Absorption measurements were made in the Bausch and Lomb (Spectronic 505) spectrophotometer.

Fluorescence measurements were made in a spectrofluorometer described earlier [12]. Emission spectra were corrected for the spectral variation of the monochromator and the photomultiplier (EMI 9558B) and excitation spectra were corrected for spectral variation of exciting monochromator. For measurements of emission spectra at room temperature and at 77°K, the procedure of Cho *et al.*[13] was followed. Measurements of excitation spectra of thick samples at 77°K was made as described by Govindjee and Yang [14].

RESULTS AND DISCUSSION

(1) Absorption spectra of Tris-treated and sonicated chloroplasts at 298°K

In Fig. 1 are presented the absorption spectra between 610—750 nm of suspensions of untreated chloroplast fragments and Tris-treated, sonicated chloroplast fragments ($T_{20}S_2$). In comparison with untreated fragments, $T_{20}S_2$ fragments show a 2-nm shift of the red band towards shorter wavelengths. In aerobic sonicates of Chlorella, Das and Govindjee [5] observed a larger shift (5 nm) of the red band towards short wavelengths in comparison to non-sonicated Chlorella suspensions. In order to observe the differences in

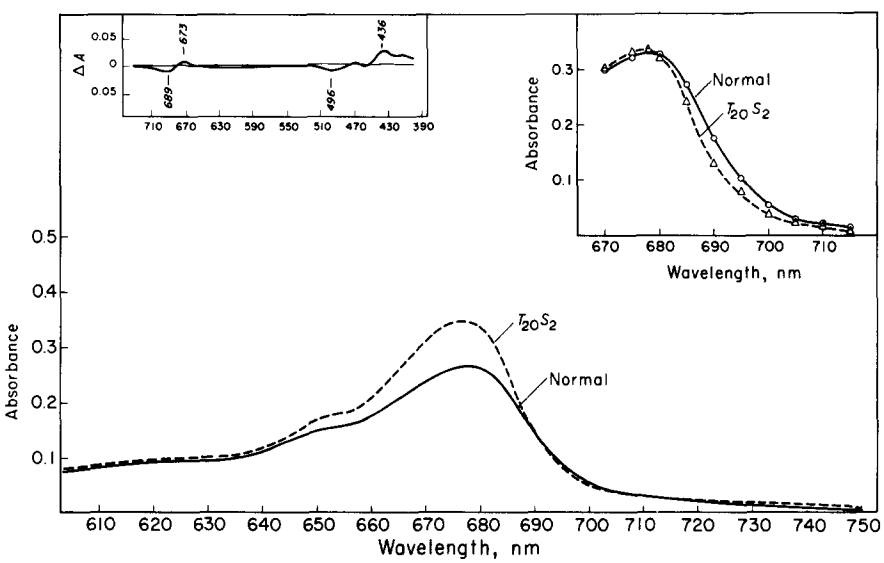

Fig. 1. Room temperature absorption spectra of $T_{20}S_2$ (see text) and untreated chloroplast suspensions. Top right insert, same spectra normalized at 678 nm. Top left insert, difference absorption spectra of (untreated minus $T_{20}S_2$) chloroplast fragments (note the direction of increasing wavelength is from right to left). Chloroplasts were suspended in Tris-HCl buffer (0.02 *M*, pH 7.8); sorbitol, 0.4 *M* and NaCl, 0.01 *M*.

absorption of the two samples, the two curves were normalized at 670 nm (Fig. 1 insert—top right). A decrease in the absorption between 678 nm and 715 nm relative to 670 nm is observed in $T_{20}S_2$ chloroplast compared to untreated chloroplasts. The cross-over of the two curves seems to be true because it was observed in several samples prepared on separate days.

The difference in absorption characteristics of $T_{20}S_2$ and untreated chloroplasts are further confirmed by measuring their absorption difference spectrum (insert, top left of Fig. 1). This shows that, in comparison to untreated chloroplasts, $T_{20}S_2$ have decreased amounts of a long wavelength form of Chl *a* with a peak maximum at 689 nm (Chl *a* 690) and increased amounts of Chl *a* 670 relative to other forms of chlorophyll. With this decrease in Chl *a* 690 relative to Chl *a* 670, there is a concomitant decrease in absorption at 496 nm relative to absorption at 430 nm. Since at 496 nm the main absorbing pigments are carotenoids, therefore, sonication seems to lower the amounts of carotenoids relative to other pigments, and of Chl *a* 690 relative to Chl *a* 670. This result agrees with that of Das and Govindjee [5] obtained in aerobic sonicates of Chlorella.

(2) Emission spectra of Tris-treated and sonicated chloroplasts at 77° K

From measurements of the difference absorption spectrum of both chloroplast samples (Fig. 1, insert—top right), it is suggested that the amount of Chl *a* 690 decreases relative to short-wavelength forms of Chl *a* in $T_{20}S_2$. Chl *a* 690 is known to be weakly fluorescent at room temperature but it fluoresces strongly at low temperatures with bands at 720 nm in Chlorella and at 735 nm in isolated chloroplasts. In all likelihood, there is a shift of this band to 700—705 nm at 77° K. To test that there was preferential loss of long-wavelength form of Chl *a*, the emission spectra of both samples were measured at 77° K (Fig. 2). Both curves showed typical three-banded structure with peaks at 735 nm (F735), 685 nm (F685), and 695 nm (F695) characteristic of emission spectra of chloroplasts at 77° K. However, the two curves differed in the ratios of the heights of the bands. F735 has been attributed to emission mostly from pigments of PS I while F685 is emitted mainly from PS II, and F693 is attributed to the emission from (or near) the trap of PS II. (For further discussion see Govindjee *et al.* [15].) In $T_{20}S_2$ the ratio of F735/F685 is 2.1 compared to 3.2 in untreated chloroplasts, thus confirming the difference observed in the absorption spectra of $T_{20}S_2$ and untreated chloroplasts. There is also slight decrease of F695 relative to F685 in $T_{20}S_2$ compared to untreated chloroplasts.

(3) Excitation spectra of chlorophyll a fluorescence at 760 nm of thick suspensions of Tris-treated and sonicated ($T_{20}S_2$) chloroplasts at 77° K

The change observed in the absorption spectrum of $T_{20}S_2$ chloroplasts at long wavelengths is observed more clearly in the fluorescence excitation spectrum of Chl *a* measured at long wavelengths. Since absorption between 680 nm and 720 nm relative to absorption at 670 nm is lower in $T_{20}S_2$ than in

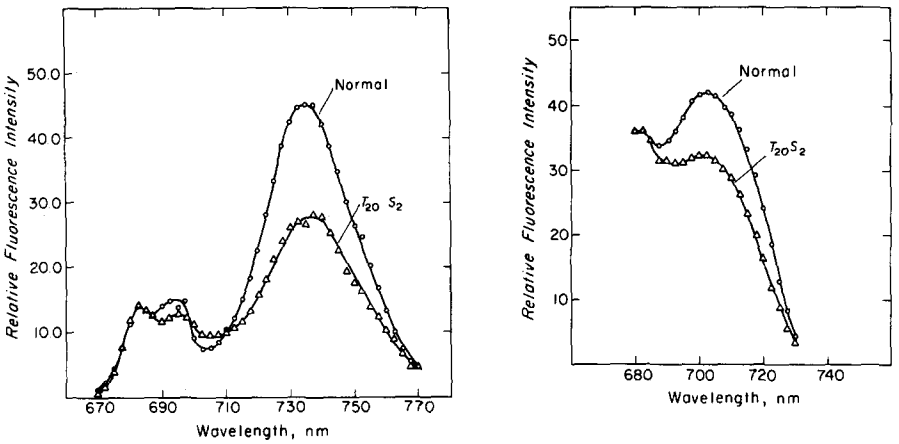

Fig. 2. Fluorescence emission spectra at 77° K of normal (○) and $T_{20}S_2$ (△) chloroplasts, normalized at 685 nm. 1-ml sample, containing 3 μg Chl, was suspended as described in the legend of Fig. 1. Emission spectra were measured (1/2 band width 3.3 nm) with an excitation at 440 nm (1/2 band width 9.9 nm). Corning (C.S. 2-73) filter was placed before the photomultiplier to eliminate stray exciting light. Samples were frozen in light.

Fig. 3. Excitation spectra of fluorescence measured at 760 nm (F760), at 77° K, of thick samples (100% absorption at 680 nm) of normal and $T_{20}S_2$ chloroplasts (see text). For details, see the legend of Fig. 1.

untreated chloroplasts, one would expect to observe a decrease in the excitation band of F760 excited by 705 nm in the former compared to the latter. (For further discussion, see ref. 16.)

To observe the excitation band at 705 nm, thick samples were used. Results of fluorescence excitation measured at 760 nm at 77° K, are presented for both chloroplast samples in Fig. 3. Because of the use of thick suspensions, the two samples showed equal amounts of Chl *a* fluorescence excited by any wavelength lower than 680 nm. However, the intensity of Chl *a* fluorescence excited at 705 nm differs greatly in both samples. The ratio of fluorescence intensity at 760 nm, as excited by 705 nm (F705/F760) to F680/F760 in untreated chloroplasts is 1.17 compared to 0.89 in $T_{20}S_2$ chloroplasts. This result is consistent with that of the absorption spectra at room temperature, and emission spectra at 77° K, in that preferential loss of Chl *a* 690—700 is observed in $T_{20}S_2$. Thus, it is likely that Arnon and coworker's system II *a* may simply be system I without the Chl *a* 670—700 that makes system I look like system II.

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