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The photoreduction of C-550 in chloroplasts and its inhibition by lipase

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SUMMARY

Photochemical reactions mediated by Photosystem II at liquid-nitrogen temperature, *i.e.* the photoreduction of C-550 and the photooxidation of cytochrome b_{559}, are eliminated if spinach chloroplasts are digested with pancreatic lipase. The digestion destroys C-550 so the absorption band of this component can be observed in the difference spectrum of untreated-*minus*-lipase-treated chloroplasts. Comparison of such difference spectra and after irradiation at $-196°$ show that the photochemical electron transfer results in a shift of the absorption band of C-550 from 546 to 544 nm.

Irradiation of intact leaves, algae or chloroplast preparations at $-196°$ results in the reduction of C-550 and the oxidation of cytochrome b_{559} [1-3]. Both of these reactions are closely related to the primary photochemistry of Photosystem II. The absorbance changes of C-550 were shown to be related isomorphically to the fluorescence yield changes of chlorophyll, a reduction of C-550 being accompanied by an increase of fluorescence yield[4]. Thus, C-550 appears to be the primary electron acceptor of Photosystem II. The oxidation of cytochrome b_{559} at low temperature was ascribed to a reaction with the oxidized form of the primary electron donor to Photosystem II[4]. The photooxidation of cytochrome b_{559} does not occur when C-550 is in the reduced state prior to the irradiation presumably because the primary electron donor is not photooxidized when no electron acceptor is available.

One advantage of the low-temperature photoreaction is that it isolates a very limited region of the electron transport chain around Photosystem II and thus provides an assay procedure to determine whether given inhibitory treatments affect the reaction center of Photosystem II directly. For instance, neither 3-(3,4-dichlorophenyl)-1,1-dimethylurea, which inhibits electron transport on the Photosystem I side of C-550, or washing with high concentrations of Tris buffer, which inhibits between water and Photosystem II, have any effect on the light-induced absorbance changes at low temperature.

In the present work we examined the inhibitory action of lipase. Okayama[5] showed previously that lipase inhibited oxygen evolution by chloroplasts but not the dichlorophenol-indophenol–ascorbate supported photoreduction of $NADP^+$ and lowered the fluorescence yield of the chloroplasts. Similar effects have been shown to result from treatments such as heat[6],

Tris washing[7], or incubation with chaotropic agents[8] which inhibit electron transport between water and Photosystem II. The lipase treatment, however, was shown in the present work to inactivate the primary photochemistry of Photosystem II by destroying C-550.

The destruction of C-550 permitted its absolute absorption band to be observed in difference spectra between normal and lipase-treated chloroplasts. The absorbance change due to the reduction of C-550, although originally described as a bleaching, on closer scrutiny appeared to be a band shift to shorter wavelength[2] in that the bleaching at 547 nm was accompanied by an increase of absorbance at 542 nm. Difference spectra showing the absolute absorption bands of the reduced and oxidized forms of C-550 confirm the band shift.

The lipase treatment consisted of incubating chloroplasts (0.2 mg chlorophyll/ml) with lipase (0.25 mg/ml) (Tokyo Kasei Kogyo Co.) for 1 h at 20° in 50 mM Tricine–KOH buffer, pH 7.6. The incubation mixture was centrifuged for 10 min at 15 000 x *g* and the pellet was resuspended in buffer. Absorption spectra were measured in 0.5 ml samples of chloroplasts (100 μg chlorophyll/ml) and frozen to −196° with a single beam spectrophotometer on line with a PDP 8/I computer[4]. Absolute spectra are the differences between the spectrum of the sample and that of 0.5 ml of frozen buffer.

Fig. 1A shows the low-temperature absolute absorption spectra of control chloroplasts before (Curve 1) and after (Curve 2) irradiation at −196° and of lipase-treated chloroplasts before (Curve 3) and after (Curve 4) irradiation. Fig. 1B presents difference spectra between the various absolute spectra. Curve 2–1 is a typical light-induced difference spectrum for chloroplasts at −196° except that a greater bleaching at 556 nm, due to the photooxidation of cytochrome b_{559}, is generally observed. The comparable difference spectrum for the lipase-treated chloroplasts (Curve 4–3) shows no absorbance changes

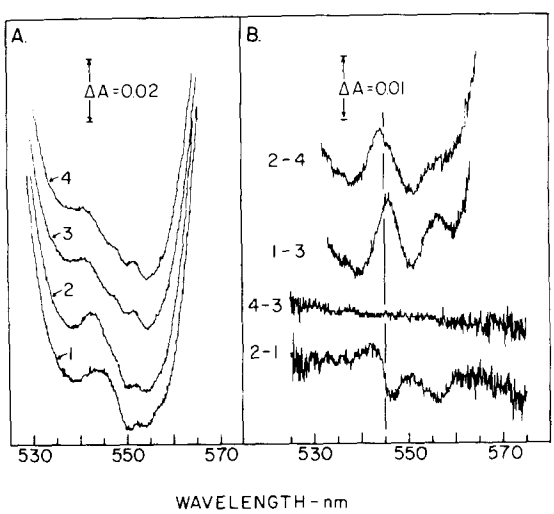

Fig. 1. (A) Absolute absorption spectra of spinach chloroplasts at −196°. Curve 1, normal chloroplasts before irradiation; Curve 2, normal chloroplasts after irradiation at −196° with red light (10^4 ergs • cm^{-2} • sec^{-1}) for 30 sec; Curve 3, lipase-treated chloroplasts before irradiation; Curve 4, lipase-treated chloroplasts after irradiation. (B) Difference spectra between the various absolute spectra of (A), as indicated.

The difference spectrum between the nonirradiated control chloroplasts and the non-irradiated lipase-treated chloroplasts (Curve 1–3) shows that a component, the oxidized form of C-550, with an absorption band at 546 nm is missing in the lipase-treated sample and that cytochrome b_{559} is either absent or oxidized. The difference spectrum between the two irradiated samples (Curve 2–4) shows that the absorption band of C-550 in the control sample shifts to 544 nm on irradiation and that cytochrome b_{559} in the control sample is photooxidized. Since Curves 3 and 4 are essentially identical in this spectral region the difference spectrum of Curve 2–4 *minus* Curve 1–3 would be the same as the direct light-induced difference spectrum (Curve 2–1).

The small band shift of C-550 from 546 to 544 nm which accompanies the primary electron transfer step is more reminiscent of a band shift due to a change in the environment around C-550 than of a chemical redox change of the molecule. C-550 is probably a membrane-bound component whose absorption band could be affected by energy-induced membrane changes. Such changes could also alter the fluorescence yield of the chlorophyll. However, the redox titration curves for the fluorescence yield[9] and the light-induced absorbance changes of C-550 [4] follow a Nernst equation for a one-electron transfer with a midpoint potential of about 0 V. It seems unlikely that such a close stoichiometry would be maintained if C-550 and fluorescence yield merely reflected the state of a membrane. Furthermore, the observed changes occurred at liquid-nitrogen temperature where gross changes of the membrane or the environment should be prevented. Nevertheless, it is still possible that C-550 is an indicator of the primary photochemical electron transfer rather than the actual acceptor. Until more information is at hand, however, it would seem reasonable to assume as a working hypothesis that C-550 is the primary electron acceptor of Photosystem II.

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