SHORT COMMUNICATIONS

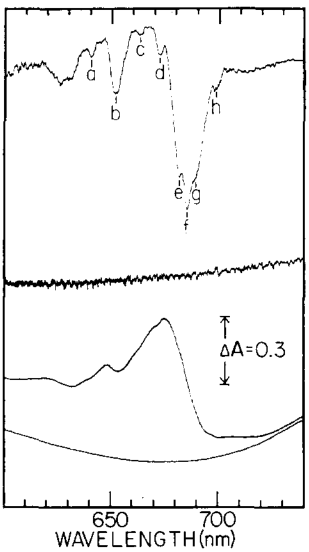
BBA 43192

Further resolution of chlorophyll pigments in photosystems 1 and 2 of spinach chloroplasts by low-temperature derivative spectroscopy

The physical separation of digitonin-treated chloroplasts into fractions enriched in photosystem 1 (PS 1) and photosystem 2 (PS 2) activities has permitted a comparison of the composition of the two systems[1]. Differences in pigment composition have been related to the different photochemical activities of the two systems. Chlorophyll *b* and chlorophyll *a* 670 which were implicated in PS 2 activity by action spectroscopy were found preferentially in the heavier-particle fraction (sedimenting at 10000 $\times$ *g*) while the longer wavelength-absorbing chlorophyll pigments associated with PS 1 action, chlorophyll *a* 680 and P_{700} (or C_{700}) were found with the lighter particles (sedimenting at 144000 $\times$ *g*)[1–4]. Additional absorption bands have been observed in the derivative absorption spectra of plant and algal tissue at low temperature[5]. In this communication, we report that such additional bands, observed in spinach chloroplasts at —196°, are also distributed unequally between the light (PS 1) and heavy (PS 2) particle fractions derived from digitonin-treated chloroplasts.

Particle fractions enriched in PS 1 and PS 2 were obtained from digitonin-treated spinach chloroplasts by the method of ANDERSON AND BOARDMAN[1]. The PS 2 particles evolved oxygen when ferricyanide was added as the electron acceptor. The PS 1 particles did not evolve oxygen but, in the presence of added 2,6-dichlorophenol-indophenol, ascorbate, ferredoxin, $NADP^+$ reductase, and plastocyanin, these particles would reduce $NADP^+$ at a rate about two-thirds that of intact chloroplasts. Chlorophyll *a*/*b* ratios determined with the equations of VERNON[6] were typically 3.0 for the intact chloroplasts, 2.6 for the PS 2 particles, and 4.6 for the PS 1 particles. Absorption spectra were measured with a single-beam spectrophotometer and the first derivative of the absorption spectrum was obtained by electrical (resistance-capacitance network with an operational amplifier) differentiation. The spectral resolution of the present measurements (0.5-nm passband) has been improved over those made previously[7,8].

The absorption and first-derivative absorption spectra of intact chloroplasts at —196° are shown in Fig. 1. Eight absorption bands are detected in the derivative spectrum between 630 and 700 nm. These absorption bands which have both maxima and minima in the derivative spectrum are labeled a through h at their minima. This high degree of spectral complexity occurs only at low temperature and only when the chlorophyll is complexed into the photosynthetic apparatus. At room temperature the derivative spectrum of the chloroplasts shows only the presence of chlorophyll *b* and two forms of chlorophyll *a*, generally referred to as chlorophyll *a* 670 and chlorophyll *a* 680 (refs. 9, 10). We were not able to confirm the complex chlorophyll spectra seen at room temperature by THOMAS[11] and METZNER[12]. The derivative spectrum of a dilute 80 % acetone extract of chloroplasts at —196°, however, shows no structure beyond one band for chlorophyll *a* and a smaller band for chlorophyll *b*. Thus, the structure seen in the derivative spectrum of Fig. 1 is not due to a splitting of the chromophore absorption band at low temperature. The absorption bands are not due to physical artifacts associated with disruption of the chloroplast structure. The same absorption bands are observed with a spinach leaf which has been frozen with liquid nitrogen and pulverized. (Such spectra are measured with the frozen leaf powder diluted with dry calcium carbonate powder.) Treatment of the chloroplasts with 0.5 % digitonin for 30 min has essentially no effect on the absorption spectrum; the same eight bands are seen in the derivative spectrum of digitonin-treated chloroplasts at —196°.



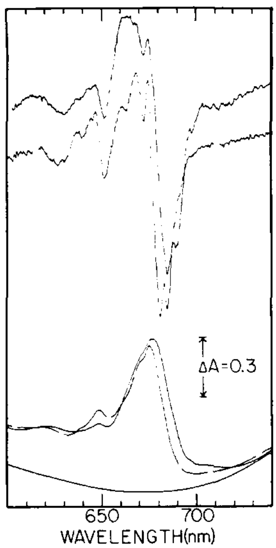


Fig. 1. Absorption spectrum (lower) and derivative absorption (upper) of spinach chloroplasts at —196°. Baselines for absorption and derivative spectra shown below respective spectral curves. Spectral passband, 0.5 nm. The spectral curves are the direct recorder traces. Minima of the derivative spectrum (in nm): a (641), b (652), c (663), d (673), e (682), f (686), g (690), h (698).

Fig. 2. Absorption and derivative absorption spectra at —196° of PS 1 (solid) and PS 2 (dashed) particles obtained from spinach chloroplasts by digitonin treatment.

The low-temperature spectra of the PS 1 and PS 2 fractions are compared in Fig. 2. Gross differences in pigment composition are readily apparent in the absorption spectra. The PS 2 particles contain most of the chlorophyll *b* and two bulk forms of chlorophyll *a*, one absorbing at about 670 nm which was previously denoted chlorophyll *a* 670, the other absorbing at 676 nm at —196°. The PS 1 particles contain a longer wavelength-absorbing form, denoted chlorophyll *a* 680, as the bulk pigment.

The derivative spectra at low temperature reveal much more structure. The pigments giving rise to derivative bands a, b, c, d, and e are associated with PS 2 particles. The presence of bands b (due to chlorophyll *b*) and d (due to chlorophyll *a* 670) in the derivative spectrum of the PS 1 particles may indicate an incomplete separation of PS 2 from the PS 1 particles. The derivative band f which appears in the spectra of both PS 1 and PS 2 particles is more difficult to assign. In the PS 1 fraction derivative band f can be ascribed to chlorophyll *a* 680 which has an absorption maximum at about 678 nm at —196°. In the PS 2 fraction, band f is due to a pigment which appears as a small shoulder in the absorption spectrum between 680 and 685 nm. Derivative band f in the PS 2 particles thus appears to be due to small amounts of a PS 2-associated pigment with an absorption maximum near 684 nm, rather than to a contamination by PS 1. Derivative bands g and h are readily assigned to PS 1. In addition, there is a broad absorption band between 700 and 720 nm which is associated with the PS 1 particles.

Not all of the absorption bands found in spinach are ubiquitous to green photosynthetic systems. Low-temperature derivative spectra of Scenedesmus and Chlorella do not show bands a, c, e (data not shown).

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Light-induced spectroscopic changes in the 600-mμ region in leaves of a higher plant and in Chlorella

We have previously observed[1] light-induced absorbance changes in the 600-mμ region in green plants and algae which were attributed to variations in the redox state of the copper-protein plastocyanin discovered by KATOH *et al.*[2]. In these experiments we have made measurements *in vivo* on leaves of a higher plant and on