

Action Spectra of Chlorophyll Fluorescence in Spinach Chloroplast Fractions Obtained by Solvent Extraction

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ABSTRACT

Action spectra of chlorophyll fluorescence in spinach chloroplast fractions obtained by solvent extraction. Das, M. and Govindjee (Dept of Physiology and Biophysics, University of Illinois, Urbana, Illinois 61801, U.S.A.). *Plant Biochemical Journal* 2(2) : 51-60 1975. Measurements of the absorption and the action spectra of chlorophyll *a* (Chl *a*) fluorescence of Chl *a*-containing complexes obtained from spinach chloroplasts with different acetone-water mixtures show that dilute solvents extract a complex with spectral properties similar to that of intact chlorophyll-carrier complex, while more concentrated solvents dissociate these complexes. This conclusion was drawn from a comparison of the various extracts and the residues with the intact chloroplasts with respect to: (1) the peak positions and the half-bandwidths of the red absorption and fluorescence excitation bands; (2) the relative quantum yields of Chl fluorescence of the extracts; and (3) the action spectra of Chl *a* fluorescence in the "red drop" region.

INTRODUCTION

Thomas and coworkers (1, 2) made attempts to separate, with different solvents, chlorophylls *in vivo* into different components. Usually treatments with detergents and ultracentrifugation are used to separate the pigment systems in photosynthesis (see Boardman, 3). Cederstrand *et al.* (4) used aqueous acetone and methanol of different concentrations to extract, from chloroplasts, fractions with different absorption and emission spectra. Their results suggested that dilute aqueous solvents extract more-or-less intact Chl-carrier complexes while concentrated solvents dissociate them. Low temperature (-196°C) fluorescence spectra suggested a partial separation of the two types of complexes, with the "long wave" component (Chl *a* in system I?) being extracted more by dilute solvents than the "short wave" component (Chl *a* in system II?). We report here data on the action spectra of Chl fluorescence in chloroplast extracts prepared with aqueous

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acetone of different strength, as well as the corresponding properties of residues after such extractions.

MATERIALS AND METHODS

Chloroplast suspensions were obtained from fresh spinach (*Spinacea oleracea*) leaves. Cut leaves were minced for 40 sec. in a solution containing 0.4 M sucrose, 0.05 M Tris buffer and 0.01 M sodium chloride (pH 7.2). The suspension was squeezed through eight layers of cheesecloth to remove the pulp. Ten ml aliquots of the filtrate were centrifuged for 10 min. at 12,000 xg. The supernatants were discarded and the precipitates suspended in 10 ml of aqueous acetone of different concentration (from 0 to 100% acetone, increasing in increments of 10%). After shaking, the solutions were left in the dark for 30 min, and centrifuged at 22,000 xg for 30 min. The supernatants were decanted, and the residues resuspended in equal volumes (10 ml) of Tris-sucrose buffer (pH 7.2).

Chlorophyll *a* was prepared from the blue-green alga *Anacystis nidulans* by extraction with 100% methanol. It was evaporated to dryness and then dissolved in various concentrations of acetone. (As all measurements were made in the red region of the spectrum, no attempt was made to remove carotenoids from Chl *a*.)

Absorption spectra were measured by means of a Bausch and Lomb recording spectrophotometer (Spectronic 505). The residues were placed in the integrating sphere, and the extracts in the regular sample compartment.

The excitation (or the action) spectra of Chl fluorescence were measured by means of a previously described spectrofluorometer (5, 6). The half-band widths of all the slits were 6.6 nm. The action (or the excitation) spectra were corrected for the variations in the incident quantum flux with wavelength.

RESULTS AND DISCUSSION

After extractions with 10% and 20% acetone, the peak position of the absorption band in the residues (extracted chloroplasts) is the same (675 nm) as in intact chloroplasts (Fig. 1) but the half-band widths become smaller (23 nm instead of 27 nm). After extraction with 30% acetone, the absorption band of the residue again becomes wider (31 nm); this width remains almost constant for acetone concentrations above 30% while the peak position shifts toward the shorter wavelength, reaching 666.5 nm in residues from extraction with 100% acetone. The increase in half-band width with 30% acetone may be due to the introduction of water into chlorophyll antenna leading to the formation of some long wavelength-

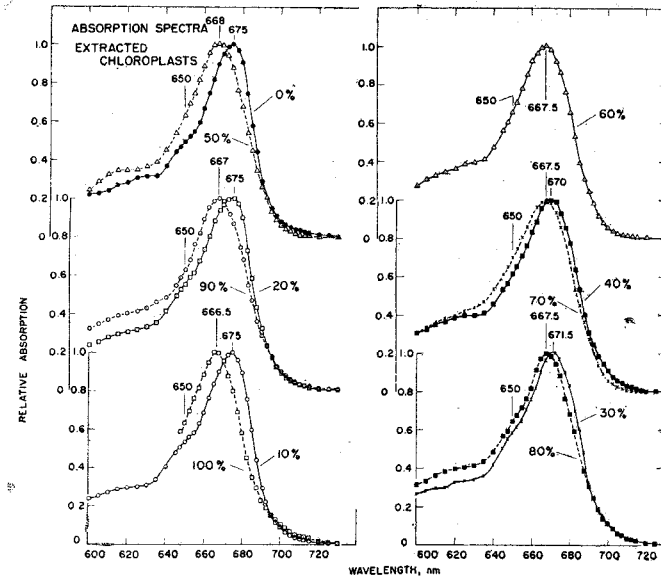


Fig. 1. Absorption spectra of chloroplasts after they have been extracted with aqueous acetone of different strengths, as indicated by percentage of acetone in acetone-water mixtures. Extracted chloroplasts (residue) were resuspended in aqueous buffer (see text). Absorption peaks in nanometers are also indicated in the figure.

absorbing form. However, with very high concentrations of acetone, short wavelength chlorophyll-acetone may be produced.

Analysis of the absorption spectra of the extracts from the chloroplasts revealed that, in 10% acetone, the peak position remains almost the same as in the intact chloroplasts (Fig. 2). In extracts with acetone higher than 20%, the peak begins to shift toward the shorter wavelength until it reaches 660 nm in 90% and 100% acetone extracts. The half-band width of the absorption band remains, in 10% and 20% acetone extracts, almost the same as in the intact chloroplasts (27-28 nm). The observed difference in the position of the absorption peak in intact chloroplasts (675 nm) and in 20% acetone extract (670 nm) may be due to differences in the dielectric properties of the aqueous buffer and of the dilute acetone-water mixture. The peak positions and the half-band width of the 60-100%

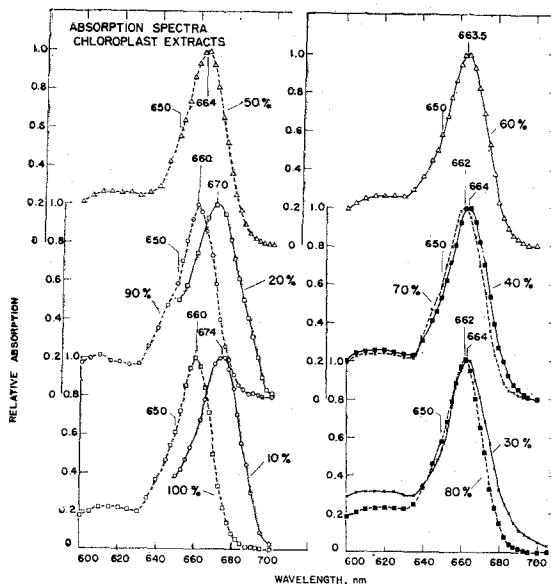


Fig. 2. Absorption spectra of chloroplast extracts. The percentage of acetone in various acetone-water mixtures used for extraction and the absorption peaks in nanometers are indicated in the figure.

acetone extracts are quite similar to those of the bands in Chl *a* solutions in the same solvents. These results suggest that, at acetone concentrations higher than 50%, Chl *a* breaks completely from the complex.

The absorption spectra of chloroplast extracts in 10% and 20% acetone (Fig. 2) and of Chl *a* solutions (Fig. 3) in the same solvents are strikingly different. Interpretation of these differences is difficult because the extracts are colloidal systems and the state of chlorophyll may be a complex function of the composition of the solvent which would be affected by the milieu of the chloroplast in the case of extracts. (A strong 740 nm band is observed in Chl *a* solutions in dilute acetone; this is due to chlorophyll-water adducts (for a review of recent literature on chlorophyll, see Katz, J. J., and Norris, J. R., 7).

Fig. 4 shows action (or excitation) spectra measured for fluorescence at 720 nm of chloroplast residues after extraction with various concentrations of acetone. The action peaks are at similar locations as the absorption peaks (Fig. 1).

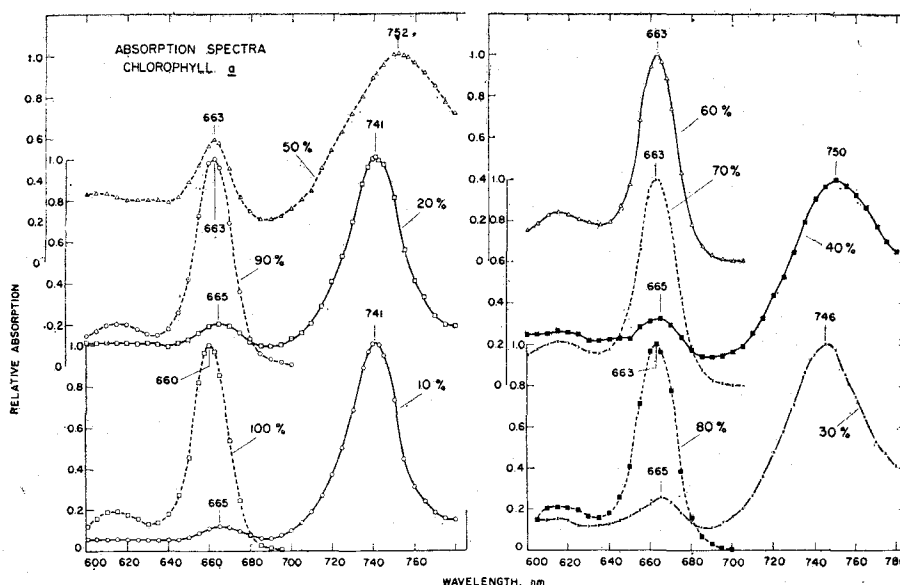


Fig. 3. Absorption spectra of Chlorophyll *a* dissolved in different concentrations of acetone-water mixtures. See legend of Fig. 2.

The peak positions of the excitation bands of the residues extracted by 10% and 20% acetone are similar to those of the intact chloroplasts (675 nm), but the half-band widths are narrower (23 nm instead of 27 nm). The half-band-widths become wider (30 nm) in chloroplast residues after extraction with 30% acetone. With increasing concentrations of acetone, the half-band widths of the excitation bands of the residues remain almost constant while the peak position shifts from 673 to 668 nm.

Fig. 5 shows action (or excitation) spectra of Chl fluorescence for chloroplast extracts in various concentrations of acetone. As in the absorption spectra, here too, the peak position (675 nm) and the half-bandwidth (27 nm) of the extract in 10% acetone is similar to those in the intact chloroplasts. In the 20% acetone extract, the half-bandwidth remains unchanged, but the peak position is shifted from 675 to 668 nm. (Different dielectric properties of the solvents may be the reason for this shift too, as shifts in excitation peaks were observed in Chl *a* solutions, see Fig. 6.) In extracts with 30% and higher acetone the excitation band becomes narrower (23 to 19 nm) and the peak position shifts continuously from 665.6 to 660 nm, showing that Chl *a* is being increasingly dissolved (*c. f.* Fig. 2).

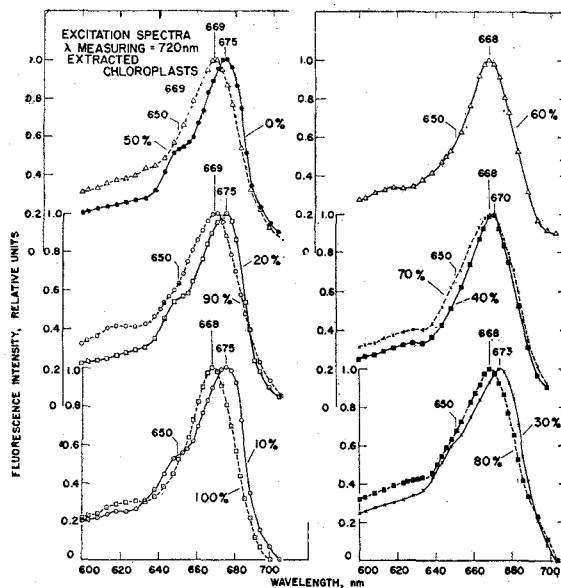


Fig. 4. Action (or excitation) spectra of Chlorophyll *a* fluorescence of chloroplasts (residue) after extraction with aqueous acetone of different strengths, resuspended in aqueous buffer. measuring $\lambda = 720$ nm.

Fig. 7 is a summary figure of the location of absorption and excitation peaks of the extracted chloroplasts and chloroplast extracts as a function of acetone concentration.

Relative quantum yields of Chl fluorescence of the acetone extracts and of the resuspended chloroplasts, calculated from the action spectra of Chl fluorescence, are plotted against acetone concentration in Fig. 8. In the extracts, the quantum yield of Chl fluorescence measured at 720 nm, increases with increasing concentration of acetone by up to a factor of 10. The quantum yield of Chl fluorescence in 10-20% acetone extracts of chloroplasts is about 3% [the same as found in intact cells: *c. f.* Latimer *et al.* (8) and Szalay *et al.* (9)] suggesting that the dilute acetone extracts from chloroplasts may contain the natural chlorophyll-carrier complex. The fluorescence yield of resuspended extracted chloroplast is almost the same as that of the intact chloroplasts (i. e., 3%); even in chloroplasts extracted with 90% acetone, the fluorescence yield is only increased by a factor of 2. This suggests that in chloroplast residues, Chl *a* remains attached to its carrier.

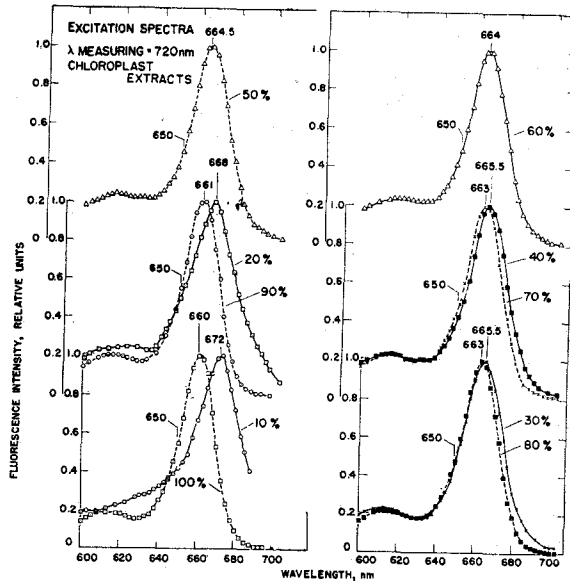


Fig. 5. Action (or excitation) spectra of chlorophyll fluorescence of chloroplast extracts in aqueous acetone of different strength, measuring $\lambda = 720$ nm.

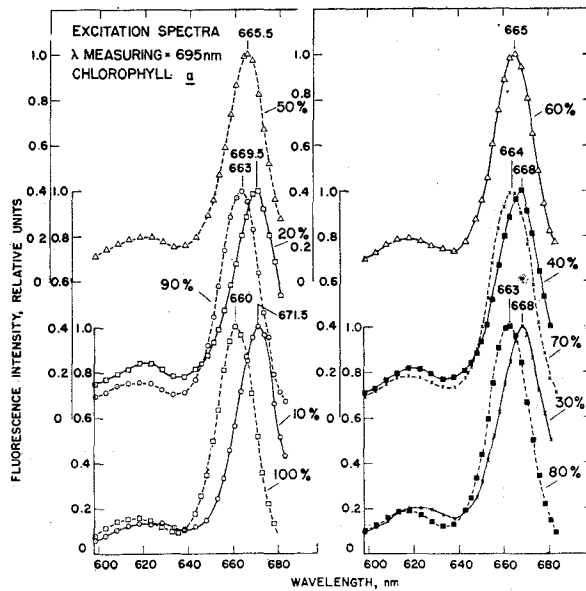


Fig. 6. Action (or excitation) spectra of Chlorophyll *a* dissolved in different concentrations of acetone-water mixtures, measuring $\lambda = 695$ nm.

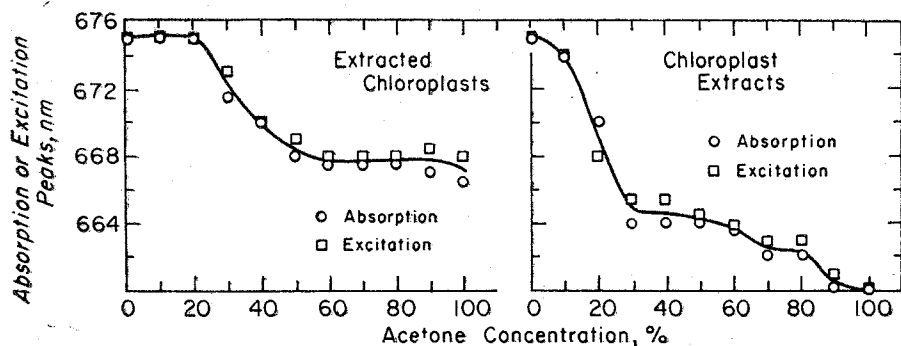


Fig. 7. Location of the peaks, in the red region, of absorption spectra and of excitation spectra of Chlorophyll *a* fluorescence as a function of different concentrations of acetone-water mixtures. Left : Chloroplasts (resuspended in aqueous buffer) after extraction with different acetone concentrations, as indicated. Right : Chloroplast extracts in different acetone-water mixtures.

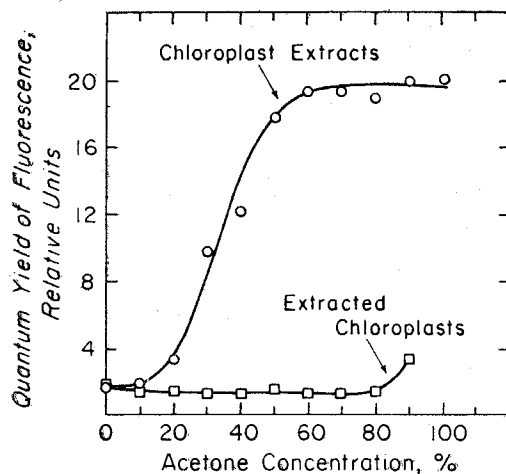


Fig. 8. Relative quantum yield of chlorophyll fluorescence of chloroplasts resuspended in buffer after extraction with various concentrations of aqueous acetone (squares) and of chloroplast extracts (circles) as a function of the acetone concentration.

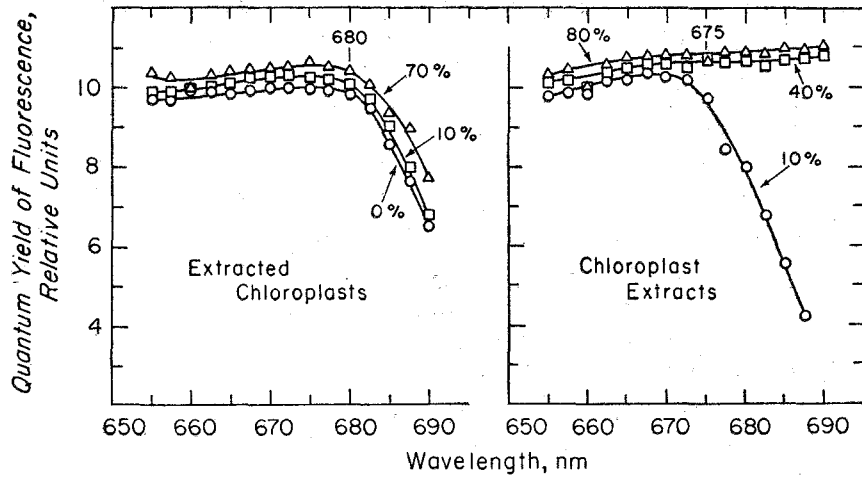


Fig. 9. Relative quantum yield of chlorophyll fluorescence of resuspended chloroplasts (left) and chloroplast extracts (right) as a function of the wavelength of excitation.

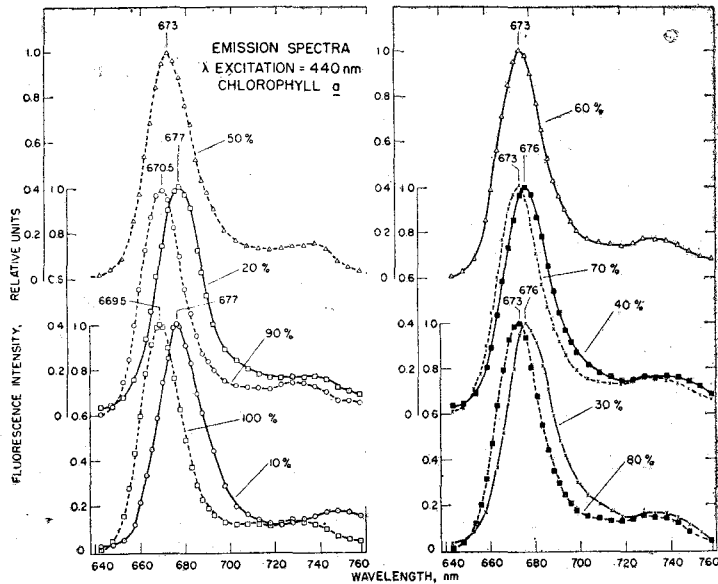


Fig. 10. Emission spectra of Chlorophyll *a* fluorescence as excited by 440 nm. Chlorophyll *a* was dissolved in different concentrations of acetone-water mixture as indicated.

(The slightly higher fluorescence yield of Chl in residues from extraction with 90% acetone may be due to some Chl *a* solution being left in the preparation.) Relative quantum yields of Chl fluorescence of Chl *a* dissolved in acetone showed low yield for 10-40% acetone due to the presence of non-fluorescent aggregates. The yield was, however, maximum for 70-100% acetone (data not shown).

Fig. 9 shows the (relative) quantum yield of Chl fluorescence, as a function of the wavelength of excitation, in chloroplast residues and in extracts. The "red drop" in Chl fluorescence yield (*c.f.* refs. 10-12) begins at the same position (680 nm) in intact chloroplasts and in resuspended residues after extraction. (Fig. 9 shows only the curves for residues after extraction with 10% and 70% acetone.) The "red drop" is present only in dilute acetone (10% and 20%) extracts where it begins at 675 nm. In extracts with 30% or higher acetone, there is no "red drop" indicating that the pigments are present in "true" solution.

Emission spectra of chloroplast extracts and extracted chloroplasts were earlier presented by Cederstrand *et al.* (4). However, some of the minor shifts observed in the extracts were, perhaps, due to differences in the emission spectra of Chl *a* in different percentages of acetone (see Fig. 10).

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