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FLUORESCENCE MEASUREMENTS AT CHENOPODIUM CHLOROPHYLL PROTEIN CP 668

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SUMMARY

1. The fluorescence spectrum of photoconverted CP 668 shows a high peak at 747 nm and a much lower one at 676 nm.

2. From the action spectrum of 747-nm fluorescence it is concluded that, in aqueous solution, energy is transferred from CP 668 to CP 740.

3. The influence of KCN and β-mercaptoethanol (reagents breaking S–S linkages) on the fluorescence spectrum of photoconverted CP 668 and on the action spectrum of 747-nm fluorescence was investigated.

INTRODUCTION

In a previous article[1] spectroscopic measurements of the red absorption band of Chenopodium chlorophyll protein CP 668 (refs. 2, 3) and its phototransformation into CP 740 have been described. The fluorescence measurements reported below were intended to supplement and extend the data obtained.

METHODS

Fluorescence spectra were recorded as described by GOEDHEER[4]. The sample was placed in either a 0.5- or a 1-cm cuvette.

Action spectra for 747-nm fluorescence were determined as follows: monochromatic light, obtained from a combination of a 100-W incandescent lamp and a Bausch and Lomb monochromator (blazed at 350 nm, slit width 1.5 mm) was used to irradiate a 0.5-cm sample cuvette. Fluorescence from the sample was focused on a liquid nitrogen-cooled Dumont 6911 photomultiplier provided with a Wratten 88A and a Schott UG 6 filter. This filter combination transmits a band with a maximum at 754 nm and band width of about 100 nm (Fig. 1). The photo-current was amplified and recorded as a function of wavelength of the exciting light on a Servogor recorder. The spectra were corrected for monochromator transmission and multiplier sensitivity.

The preparation of CP 668 was described in the preceding article[1]. All samples had been treated with carbowax (*cf.* ref. 1).

RESULTS AND CONCLUSIONS

The fluorescence yield of CP 668 appears to be relatively high as compared with that of chloroplast preparations of similar density. As CP 668 is rapidly photo-transformed into CP 740 upon irradiation, measurement of the fluorescence spectrum

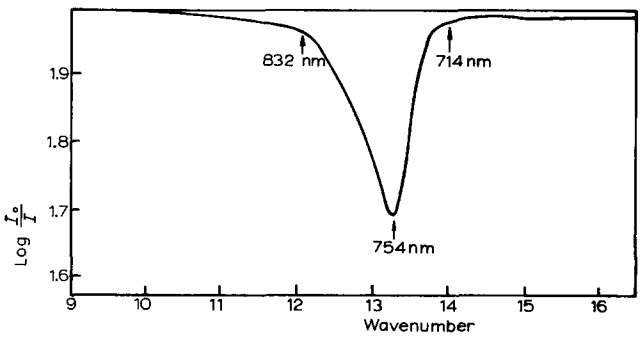
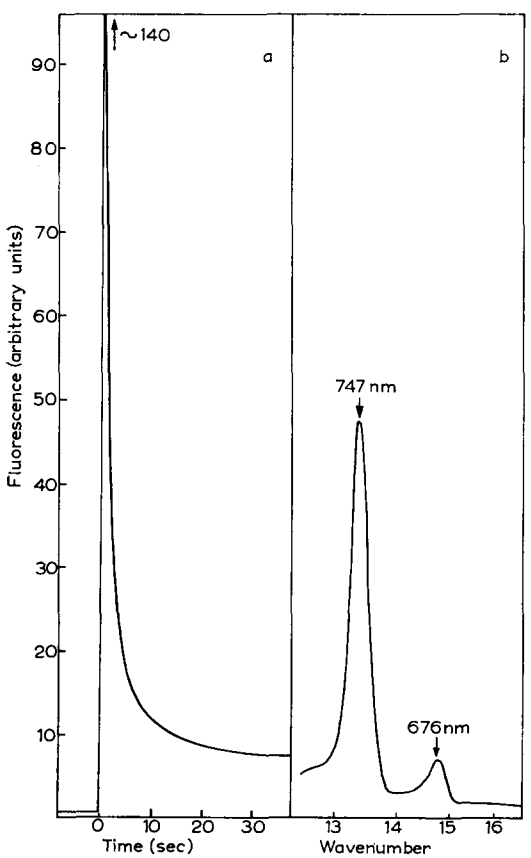


Fig. 1. Transmission of filter combination (*cf.* text).



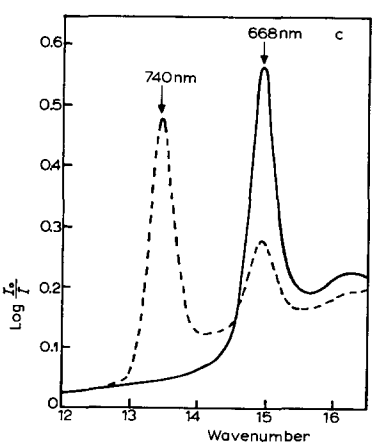


Fig. 2a. Fluorescence decrease at 676 nm during CP 668 → CP 740 photoconversion.
Fig. 2b. Fluorescence spectrum of photoconverted CP 668.
Fig. 2c. Absorption spectra of CP 668 before (———) and after (– – – –) photoconversion.

of the non-illuminated form requires special precautions. Fig. 2a shows the decline of 676-nm fluorescence during irradiation with the exciting light. In Fig. 2b the fluorescence spectrum of this preparation is given after photoconversion of CP 668 into CP 740. In this spectrum two maxima can be seen, a low one at 676 nm and a high one at 747 nm. These correspond with the 668-nm and the 740-nm absorption band, respectively. The red part of the absorption spectra of the same preparation, before and after photoconversion, is given in Fig. 2c.

Photoconversion was obtained by 5-min illumination with a 100-W incandescent lamp (distance about 10 cm, the sample being placed behind a 3-cm water cuvette). As can be seen from the figures, the absorption at 668 nm after photoconversion is still about 50–65% of that at 740 nm in carbowax-treated preparations. If we assume that CP 740 has no absorption band at 668 nm, then the ratio of specific absorption coefficients of CP 740 and CP 668, as estimated from absorption measurements, is roughly 1.5. This indicates that only half, or slightly more, of the CP 668 molecules is transformed into CP 740 by illumination. The fluorescence measurements, however, show that very little of the fluorescence of CP 668 is left. According to Fig. 2b, its amount is appreciably less than 10% of the initial intensity. This could be explained by the assumption that the remaining CP 668 molecules transfer their absorbed energy to CP 740 with quite a high efficiency. The occurrence of energy transfer was checked by (1) establishing the fluorescence spectra excited by two different wavelengths, and (2) determination of action spectra for CP 740 fluorescence.

Regarding (1): Using interference filters, exciting light of 566 nm and 437 nm

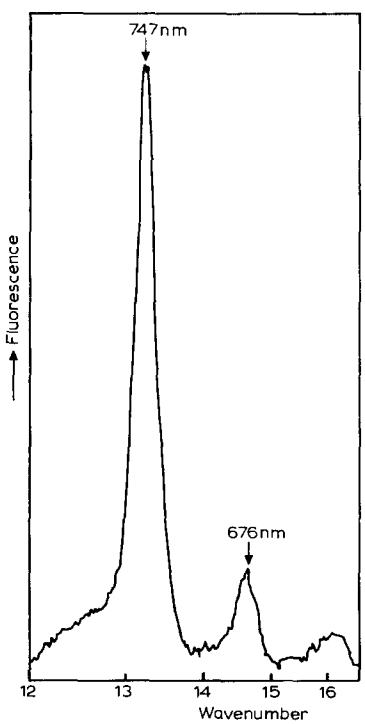


Fig. 3. Fluorescence spectrum of photoconverted CP 668 measured with 437-nm incident light.

was isolated from light of an incandescent lamp. The former wavelength is absorbed mainly in the 568-nm band belonging to CP 740, the latter in the 437-nm Soret band belonging to CP 668 (refs. 2, 3). With 566-nm incident light only the 747-nm fluorescence band is evident. With incident light of 437 nm the fluorescence is mainly

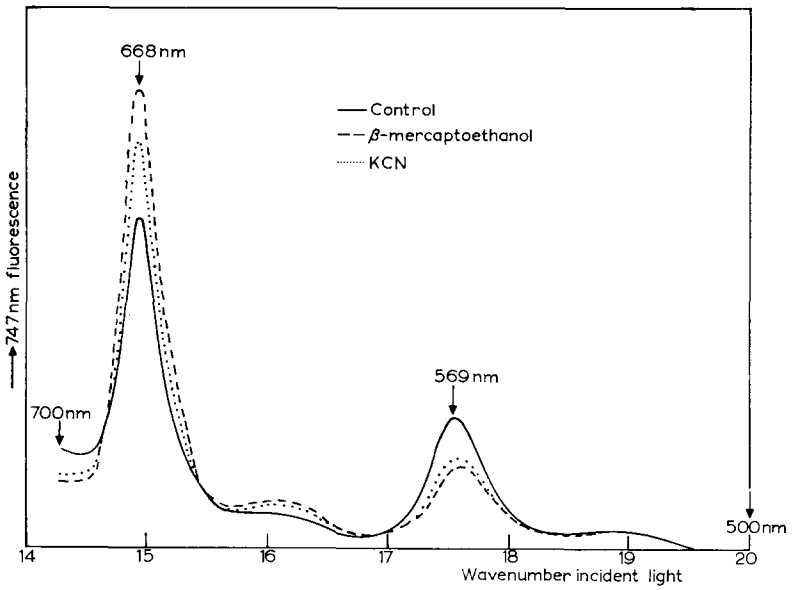


Fig. 4. Action spectra of 747-nm fluorescence.

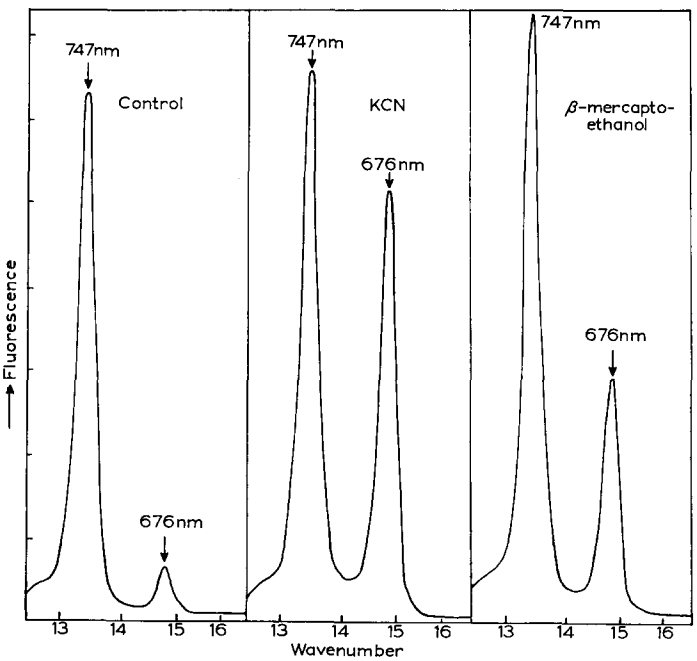


Fig. 5. Effect of KCN and β-mercaptoethanol, 0.05 M, on fluorescence of photoconverted CP 668.

that of CP 740 although the absorption of CP 668 is probably many times that of CP 740 (Fig. 3) at this wavelength.

Regarding (2): Fluorescence action spectra for the 747-nm fluorescence show bands belonging to both CP 668 (668 nm) and CP 740 (740 and 569 nm), in a ratio comparable with that in the absorption spectrum (Fig. 4).

Thus, energy transfer occurs between oscillators responsible for the 668- and 740-nm absorption bands.

In the preceding paper[1] the influence of β-mercaptoethanol and KCN on CP 668 → CP 740 photoconversion was described. In order to obtain more insight into the action of these reagents, fluorescence spectra and fluorescence action spectra of preparations treated with β-mercaptoethanol or KCN (both at about 0.05 M) were determined. The results were qualitatively the same for both reagents. In the fluorescence spectrum an increase of the 676-nm fluorescence is noted, while the 747-nm fluorescence remains the same or is slightly increased (Fig. 5). In the action spectrum of the 747-nm fluorescence band the 668-nm band increases while the 569-nm band, which is considered to be an indicator of the contribution of the 740-nm absorption band in 747-nm fluorescence, decreases (Fig. 4). The spectra will be discussed in the next section.

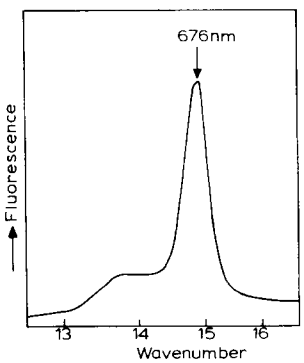


Fig. 6. Fluorescence spectrum of non-photoconvertible 668-nm absorbing material.

Fig. 6 shows a fluorescence spectrum of the "soluble", non-photoconvertible substance (absorbing at about 668 nm) that was extracted from Chenopodium leaves (*cf.* the preceding article[1]). Its fluorescence maximum at 676 nm corresponds closely with the same maximum measured with CP 668. No fluorescence band around 747 nm was found with these preparations.

DISCUSSION

The fluorescence measurements show once more[1] that CP 740 is not formed by aggregation of CP 668 chlorophyll.

From fluorescence spectra measured with exciting light absorbed mainly by the CP 668 pigment form and from the action spectrum for 747-nm fluorescence, it was concluded that excitation energy is transferred from the oscillator causing the 668-nm absorption band to that of the 740-nm absorption band. Two different mechanisms may occur: (a) CP 740 has two absorption bands in the red part of the spectrum,

a minor one at 668 nm and the main one at 740 nm; energy transfer is intramolecular. (b) CP 740 has only one absorption band in the red part of the spectrum, at 740 nm; transfer of intermolecular energy occurs, in solution, between CP 668 and CP 740.

Possibility (a) is considered improbable. No single chlorophyll complex has so far been detected which exhibits such a multi-peaked absorption spectrum as is shown by an irradiated CP-668 preparation. Possibility (b) is considered much more likely. It implicates either that in CP 668 more than one chlorophyll molecule is present for each protein molecule, or that at least two protein molecules are combined, with their chlorophyll-carrying sites close together in solution.

Energy transfer by inductive resonance between two chlorophyll molecules might provide an explanation for the above-mentioned fact that hitherto no complete transformation of CP 668 into CP 740 has ever been found. After the formation of a sufficient number of CP 740 molecules, energy absorbed by the remaining CP 668 pigment molecules might easily be transferred to CP 740 before being able to initiate photo-oxidation.

There is, however, another possible explanation for the incomplete photoconversion of CP 668: part of the chlorophyll-carrying soluble protein might still be in its native form[1], thus protecting the chlorophyll in some way against photo-oxidation. However, as the percentage of CP 668 conversion in different carbowax-treated preparations was roughly the same in all cases, this explanation seems less likely at present.

The results obtained with preparations containing β-mercaptoethanol or KCN cannot be easily explained. If these reagents merely inhibited the CP 668 $\rightarrow$ CP 740 photoconversion, presumably by acting on the protein (*cf.* ref. 1), the 747-nm fluorescence might be expected to be appreciably weaker than in the control not containing these inhibitors. This does not appear to be true. Fluorescence action spectra indicate that energy transfer from the 668-nm absorbing form to the 740-nm absorbing form is not blocked in the presence of β-mercaptoethanol or KCN. In order to explain the high 747-nm fluorescence in the treated preparations we suggest, tentatively, that the reagents, by disrupting S–S linkages in the protein molecule, affect the protein configuration in such a way that not only is the photo-oxidation of the chlorophyll moiety inhibited, but also that the excitation energy absorbed by this chlorophyll part is less easily dissipated.

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