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FURTHER DATA ON THE IDENTITY OF A PIGMENT ABSORBING AROUND 640 nm IN *ULVA LACTUCA*

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SUMMARY

A direct relationship exists between the amount of a pigment complex, absorbing around 640 nm, removed from *Ulva lactuca* chloroplast fragment preparations by mild acetone extraction, and the quantity of a pigment component, absorbing around 633 nm, occurring in the extract. It is concluded that both spectral components refer to one and the same pigment.

Evidence that this pigment is identical with chlorophyll *a* is presented. In view of earlier data, this result supports the suggestion that the reaction center pigment of Photosystem II consists of chlorophyll *a*.

INTRODUCTION

In a previous report [1] it was mentioned that a 3-h extraction with 40% acetone removed the major part of a pigment complex absorbing around 640 nm from chloroplast fragment preparations of *Ulva lactuca*. Concomittantly a pigment component absorbing around 633 nm, not present in the chloroplast fragments, was observed in the extract. The present study was designed to obtain more information about a possible relationship between both pigment components, as well as about the nature of the involved pigment.

MATERIALS AND METHODS

Batches of *Ulva lactuca* were obtained from the Netherlands Institute for Sea Research at Den Helder, and stored at −40 °C. Chloroplast fragments were prepared as described earlier [1]. Accordingly, the chloroplast fragments were taken up in demineralized water, in 30%, or in 40% reagent-grade acetone in demineralized water, and treated as indicated [1]. In case of storage of the supernatants of both latter preparations upon raising the acetone concentration to 80%, 0.02 M Tris buffer, pH 8.0, was used. Extraction and storage were performed under pure nitrogen. These precautions are required to prevent oxidation of dissolved chlorophyll *a*, cf. ref. 2.

Absorption spectra and absorption difference spectra were recorded in a Cary model 14R spectrophotometer. For chloroplast fragment suspensions this was done at 77 °K as previously described [1].

An attempt was made to determine the height of the 640-nm absorption bands by subtraction of a chlorophyll *b* spectrum free from the 640-nm component, as obtained earlier [3]. However, because of the considerable differences in the chlorophyll *a*/*b* ratios, as well as differences in scattering in fragments treated with 30 or 40% acetone, this procedure proved to be inappropriate. Instead, an estimation was made by drawing a tangent along the short-wave side of the chlorophyll *b* absorption band, and measuring the height of the 640-nm shoulder over this tangent. The same method was used for determining the height of the 633-nm bands in absorption difference spectra of the extracts. Though this procedure is only an approximation, it is an objective one, and the results are satisfactorily reproducible.

As acetone-containing preparations become snowy at 77 °K, the extract spectra were measured at room temperature. In order to compare spectra obtained at both temperatures, it was established from a mean of 10 experiments that the height of the main red absorption band of chlorophyll *a* at 77 °K is 1.39 time that at room temperature. This factor was used for correcting the spectra of the extracts. Thus, the assumption was made that the height of the 633-nm band showed the same temperature dependence as the main chlorophyll *a* band. Since it will be shown below that the 633-nm pigment is identical with chlorophyll *a* such an assumption may not be too risky.

RESULTS

Relationship between the 640- and 633-nm components in absorption spectra of chloroplast fragments and their acetone extracts, respectively

As discussed earlier [1], the 633-nm component in 40% acetone possibly represents a modification of the naturally occurring pigment–lipoprotein complex absorbing around 640 nm. For example, such a change may be due to removal of some lipids from this complex by acetone. If both components actually are related to each other, a linear relationship between the amounts of the 640-nm component, removed from the chloroplast fragments by 40% acetone, and the 633-nm component in the extract should occur.

A 3-h extraction period in 30% acetone does not markedly affect the ratio of the 640-nm component and the main red chlorophyll *b* absorption maximum [4]. Moreover, the refractive indices as well as the degree of scattering of preparations treated with 40% acetone, differ considerably less from those of samples treated with 30% acetone, than from those of non-treated preparations. Therefore, only the differences between preparations extracted with 30 and 40% acetone are considered below.

Fig. 1 shows the relationship between the amount of the 640-nm component removed from the chloroplast fragments, and that of the 633-nm component in the acetone extract. The relative amount of the 640-nm component varies in different *Ulva* fronds. Use was made of these naturally occurring differences to study the relationship under consideration. Fig. 1 demonstrates that the relation between the plotted data can be characterized reasonably by a straight line which passes through the origin upon extrapolation.

From the ten experiments the mean ratio of the percentage of the 633-nm

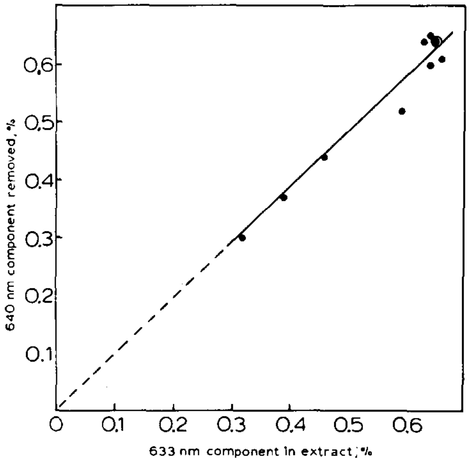

Fig. 1. Relationship between the amount of the 640-nm component removed from chloroplast fragments of *Ulva lactuca* by a 3-h extraction in 40 % acetone in the dark at 4 °C, and that of the 633-nm component obtained in the extract. The data are expressed in percentage of the main red chlorophyll *a* maximum of the original preparation.

component to the percentage of the 640-nm component was calculated to be 1.04 ± 0.01. This result seems to justify the earlier mentioned assumption about the temperature dependence of the 633-nm band. However, major emphasis is laid upon the fact that the relationship in question is expressed by a straight line which, upon extrapolation, passes through the origin.

It is concluded that the 633-nm component in 40% acetone extracts most likely represents a modification of the 640-nm component in chloroplast fragments.

Nature of the pigment from the 640- and 633-nm components

It has been shown earlier [1] that the 640-nm shoulder in absorption spectra of chloroplast fragments is related to a band peaking around 682 nm. As a consequence, such a band should be expected to occur in the 633 component containing extract as well, although its location may be expected to be somewhat shifted. Furthermore, it was suggested [1] that the 640-nm component is due to the reaction center pigment of Photosystem II [5]. The 633-nm component, then, is likely to represent a modification of the reaction center complex. As the reaction center pigment of Photosystem I [6] as well as reaction center bacteriochlorophyll [7] are suggested to be held in a rigid matrix, the same may be true of the pigment under consideration. If so, only prolonged extraction at high acetone concentrations may result in isolating this pigment from its reaction center complex. These considerations led to the following procedure.

Chloroplast fragment preparations were extracted in 40% acetone as described above. Next, the chloroplast fragments were spun down and discarded, whereas the acetone concentration of the supernatant was raised to 80%. For details see Materials and Methods. Absorption spectra were recorded at room temperature immediately upon raising the acetone concentration as well as after 18 h of storage in the dark, under nitrogen, and at 4 °C. It was checked in preliminary experiments that

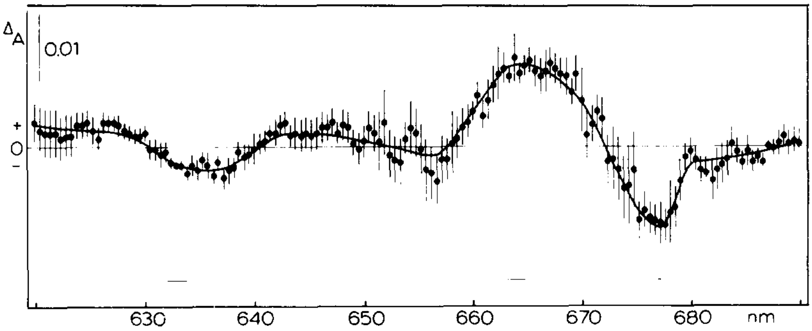

Fig. 2. Mean difference spectrum of ten 40 % acetone extracts of *Ulva lactuca* chloroplast fragments, obtained by subtracting the spectrum taken immediately after raising the acetone concentration to 80 % from that taken 18 h after the same procedure.

this period is sufficient. Of both sets of spectra difference spectra were computed. Ten experiments were done. An example is shown in Fig. 2. It may demonstrate that the absorbance around 664 nm, the red peak location for chlorophyll *a* in acetone, cf. ref. 8, is increased, whereas it is decreased around 676 nm and 633 nm. The horizontal bars indicate spectral ranges of 1.9 nm, the middle of which is located at the mentioned wavelengths. The means of the 5 measurements in each of these 3 ranges are taken to express the extent of the absorbance changes. They are summarized in Table I.

The results, then, indicate that together with the weak 633-nm band a higher one around 676 nm is decreased by extraction in 80% acetone. Concomittantly, as

TABLE I

ABSORBANCE CHANGES AT 18 h AFTER RAISING THE ACETONE CONCENTRATION OF EXTRACTS OF *ULVA LACTUCA* CHLOROPLAST FRAGMENTS, OBTAINED BY A 3-h STORAGE IN 40% ACETONE, TO 80%

The values represent percentages of the height of the 664-nm band immediately upon raising the acetone concentration. The data are means of 5 measurements in each of the spectral ranges indicated by horizontal bars in Fig. 2.

Expt No.	Reference height at 664 nm (log I_0/I)	Percentage absorbance changes		
		Around 664 nm	Around 676 nm	Around 633 nm
1	0.60	+0.54	−0.80	−0.13
2	0.75	+0.80	−0.47	−0.33
3	0.52	+1.07	−0.94	−0.27
4	0.52	+1.34	−0.94	−0.40
5	0.67	+1.27	−1.34	−0.33
6	0.72	+0.60	−0.67	−0.27
7	0.90	+0.60	−0.80	−0.06
8	0.83	+1.27	−1.07	−0.27
9	0.41	+0.47	−0.80	+0.13
10	0.17	+1.74	−1.14	−0.20
Mean		+0.97±0.14	−0.90+0.08	−0.19±0.05

evidenced by the absorbance increase at 664 nm, the amount of dissolved chlorophyll *a* is increased.

DISCUSSION

The linear relationship between the 640-nm component removed from the *Ulva* chloroplast fragments by extraction in 40% acetone, and the amount of the 633-nm component obtained in the extract suggests that the latter component is a modification of the former one. Since the 640-nm absorbance shoulder is correlated with a 5–6-times higher band at 682 nm [1], whereas storage in 80% acetone results in an absorbance decrease around 633 and 676 nm, but not around 682 nm, cf. Fig. 2, it seems likely that the 676-nm band in the extract represents the shifted 682-nm band in chloroplast fragments. The more so, since, except for Expt 9, Table I, the reduction of the 676-nm band upon storage in 80% acetone is considerably more than that of the 633-nm one. The scattering of results renders it hard to estimate the value of such a ratio from a single experiment. The means of 10 experiments suggests that the absorbance change around 676 nm is about 5 times larger than that around 633 nm.

The considerable extent of the required extraction period, 18 h, indicates that the described phenomenon is due to isolation of chlorophyll *a* from a carrier complex rather than dissolving of colloidal aggregates of this pigment.

From the above the conclusion seems warranted that, most likely, extraction in 40% acetone for 3 h results in isolation of a pigment complex believed [1] to represent the reaction center pigment of Photosystem II, be it that the absorption bands of the isolated complex are shifted about 6 nm to the short-wave side.

Upon raising the acetone percentage of the original, 40%, extract to 80%, followed by an 18-h storage in the dark, under nitrogen, and at 4 °C, the absorption bands around 676 and 633 nm are greatly, if not totally, reduced, whereas the absorbance at the location of the red absorption maximum of chlorophyll *a* dissolved in acetone, around 664 nm, is increased. This increase is about as large as the absorption decrease around 676 nm, or slightly larger. Consequently, these results strongly suggest that the pigment of the extracted pigment complex is identical with chlorophyll *a*.

The above-mentioned data all fit into the following picture. A chlorophyll *a*–carrier complex, which is likely to be the reaction center complex of Photosystem II [1], can be extracted in 40% acetone, be it that the complex is somewhat affected by this procedure. Long-term storage in 80% acetone liberates the chlorophyll *a* from this particular carrier. The latter fact may indicate that the reaction center chlorophyll *a* of Photosystem II is relatively strongly bound to its carrier, as it is the case with this pigment in reaction center particles of Photosystem I [6], as well as reaction center bacteriochlorophyll [7].

Döring et al. [5] suggested that the reaction center pigment of Photosystem II consists of chlorophyll *a*. This suggestion is based on the fact that the location of the absorption bands is not far removed from that of the Photosystem I reaction center pigment, shown by Kok [9] to be identical with chlorophyll *a*. However, the life time of the excited reaction center complex of Photosystem II was found to be 100 times shorter than that for Photosystem I by Döring et al. [5]. The latter authors explained this phenomenon in terms of differences in the mode of action of the reaction center pigments of both photosystems. They pointed out that the reaction center pigment of

Photosystem II acts as a sensitizer, whereas that of Photosystem I is involved in the electron transport system itself. The pigments from both complexes, therefore, might be different. The present data, however, support the suggestion [5] that the reaction center pigment of Photosystem II consists of chlorophyll *a*.

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