

ON THE RELATIVE EXTRACTABILITY OF CHLOROPHYLL *a*685 AND CHLOROPHYLL *b* COMPLEXES BY TWO DETERGENTS

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SUMMARY

Treatment of *Phaseolus vulgaris* chloroplast fragments with 8 or 4 mM digitonin as well as 8 or 4 mM sodium dodecyl sulfate results in a proportionally higher solubilization of the chlorophyll *a*685 complex and a lower one of chlorophyll *b* as compared to the extractability of the remaining chlorophyll *a* forms. The results are discussed with respect to previous data suggesting the occurrence of a chlorophyll *b*-chlorophyll *a*685-protein complex. It may be that both pigment forms are bound to different polypeptides of this complex.

1. INTRODUCTION

In an earlier study KLEINEN HAMMANS & THOMAS (1976) observed a quantitative relationship between the amounts of chlorophyll *b* (C_b) and chlorophyll *a*685 (C_{a685}) in various species. In particular the absence of C_{a685} in the studied C_b -free algae suggests that these pigment forms are complexed with one and the same proteinaceous carrier. This suggestion, however, disagrees with results obtained by other investigators. THORNBER (1975), THORNBER & HIGHKIN (1974), KAN & THORNBER (1976) and KITAJIMA & BUTLER (1975) stated that all C_b occurs in the light-harvesting pigment (LHP)-protein complex together with a single accompanying C_a form showing its red absorption maximum around 670 nm. On the other hand, BROWN et al. (1975) concluded that, in addition to C_b , the LHP complex contains the four major C_a forms.

In the present study it is attempted to examine the reason for the mentioned divergence of the results. To this purpose the effects of the detergents digitonin and sodium dodecyl sulfate (SDS) on the relative solubilization of the C_b and C_{a685} complexes are investigated.

2. MATERIALS AND METHODS

Chloroplast fragment suspensions were prepared from fresh leaves of *Phaseolus vulgaris*, grown at the institute.

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The bean leaves were minced in a Sorvall Omnimixer in 0.02 M Tris-HCl buffer, pH 8.0, at 0°C. The homogenate was filtered through cotton wool and centrifuged at $12,000 \times g$ for 5 min. The sediment was suspended in the same buffer. To chloroplast fragment suspensions containing chlorophyll at a concentration of 1 mg/ml either digitonin or sodium dodecyl sulphate (SDS), in concentrations mentioned below, was added. The mixture was stored at room temperature for 20 min. Next, the preparation was centrifuged at $50,000 \times g$ for 30 min. yielding supernatant 1 and sediment 1. Supernatant 1 was centrifuged at $225,000 \times g$ for 2 hrs., resulting in sediment 2 and supernatant 2. The sediments were taken up in the mentioned buffer. Upon adding glycerol up to a concentration of 66% to the various preparations, absorption spectra at 77 K were established using a Cary Model 14R recording spectrophotometer.

Digitonin, BDH 'ANALAR', or SDS, BDH, was added up to a final concentration of 8 or 4 mM.

Difference spectra were obtained after equalizing the height of the main absorption band of the samples with that of the main maximum of the non-treated preparations.

3. RESULTS

An example of the *Phaseolus vulgaris* low-temperature absorption spectrum, analyzed by computer using the RESOLV program, cf. OUDSHOORN & THOMAS (1975), is shown in fig. 1. The exact location of the C_a685 , C_a680 , C_a670 and C_b forms occurs at 683 nm, 677 nm, 670 nm and 649 nm respectively.

The applied concentrations of each of the detergents used did not yield

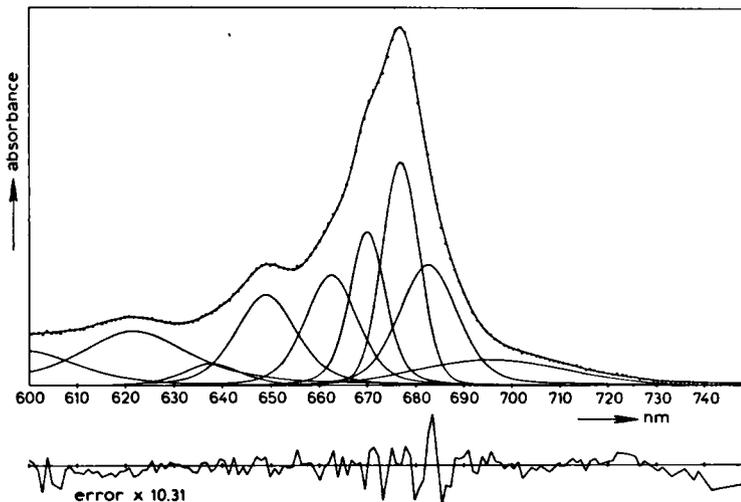


Fig. 1. Computer analysis of the red absorption band of *Phaseolus vulgaris* chloroplasts.

qualitatively different results. Therefore it was possible to choose the concentrations yielding optimal experimental conditions. Since a chloroplast fragment suspension containing 8 mM digitonin shows considerable light scattering, while the effect of 4 mM SDS is poor, the presented examples refer to experiments on the effects of 4mM digitonin and 8 mM SDS only. For each of these concentrations three experiments, showing a very satisfactory reproducibility, were performed.

Effect of digitonin, 4 mM: Examples of difference spectra obtained by subtraction of low-temperature absorption spectra, equalized at the maximum peak heights, are given in *fig. 2*. It is shown in *fig. 2a* that sediment 1 from the digitonin-treated sample contains proportionally less *C_{a685}* than the non-treated, reference, sample. *Fig. 2b* demonstrates that the relative content of this pigment form is higher in the particles from sediment 2, termed below 'small particles', than those from sediment 1, called 'large particles'. On the other hand, the latter particles contain relatively more *C_b* than the small ones. The negative band around 667 nm is ascribed to chlorophyll dissolved in the detergent micelles. Comparison of the differences in the proportional pigment composition between the reference and the small particle sample, *fig. 2c*, shows that the latter one contains more *C_{a685}* but less *C_b* than the reference sample. Moreover, it contains 'dissolved' chlorophyll.

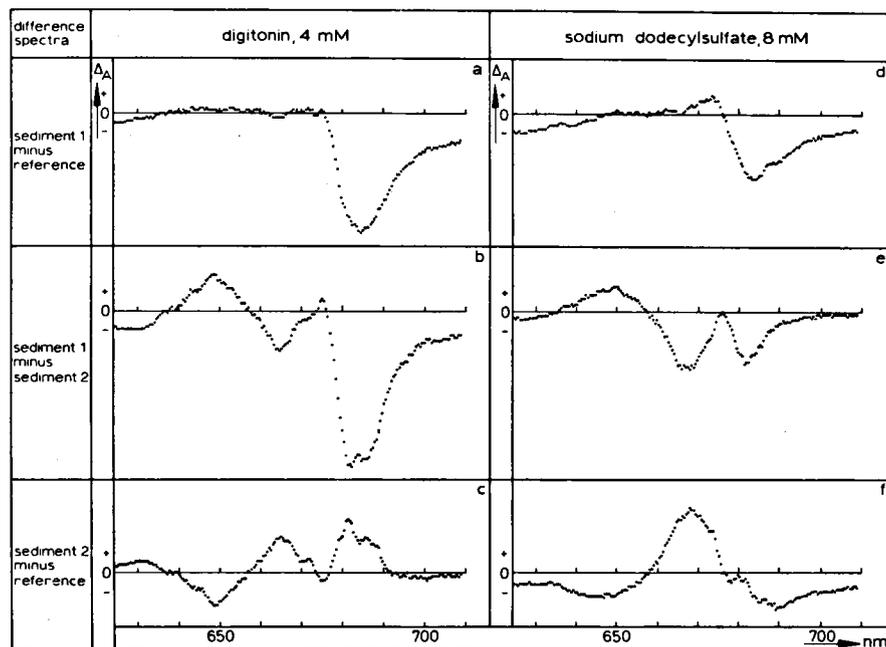


Fig. 2. Effects of digitonin and SDS on the distribution of *C_b* and *C_a* forms over large- and small-particle preparations from *Phaseolus vulgaris* chloroplast fragment suspensions. For details see text.

Effect of SDS, 8 mM: As it can be seen from *figs. 2d, e, and f*, the proportional differences in pigment composition of the various samples are less pronounced than those of the digitonin-treated preparations. The differences regarding C_b and C_a685 in *fig. 2f* are considerably smaller than those shown in *fig. 2c*. The relative enrichment of C_a685 in the small particle fragments from SDS-treated samples, *fig. 2f*, only shows up as a weak shoulder at the short-wave side of a negative band around 692 nm. The presence of this C_a band can also be observed in *fig. 2d*, where it is responsible for a reduction of the long-wave slope of the negative C_a685 band.

From the above data it can be concluded that the C_b and C_a685 complexes are differently affected in the present experiments. The effect of the used concentrations of the detergents in question consists of a relatively higher content of C_b in the large particles, whereas C_a685 preferentially occurs in the small ones.

4. DISCUSSION

The described experiments demonstrate that the distribution of C_b over large and small particles from detergent-treated *Phaseolus vulgaris* chloroplast fragments differs from that of C_a685 . This result is unexpected with regard to the earlier (1) suggestion holding that C_b and C_a685 are complexed with one and the same carrier.

At present there seem to be two possibilities. According to the first one, the mentioned suggestion is not true. However, the observed correlation of occurrence of both pigment complexes remains hard to understand in this case.

The second possibility may be that the detergents cause a cleavage of the carrier complex, common for both pigments, into at least two components. C_b and C_a685 , then, may be bound to different species of these components. It may be reminded that various authors observed a coincidence of the absence of C_b and the amount or composition of its apoprotein (THORNBER & HIGHKIN (1974), GENGE et al. (1974), ANDERSON & LEVINE (1974a, b), HENRIQUES & PARK (1975), LEVINE et al. (1972), and KLEIN & VERNON (1974a, b)). If C_b is absent two major polypeptides, of 22 and 24 kdaltons respectively, are absent as well (THORNBER & HIGHKIN (1974) and KLEIN & VERNON (1974b)). It may be that both polypeptides are part of a single subunit of the LHP complex, the C_b and the C_a685 forms being carried by one of these polypeptides each. In this case the mentioned coincidence of occurrence of both pigment forms can be understood. Future research is needed to solve this problem.

Additionally it may be remarked that, as mentioned under the Introduction, various authors found the pigment form accompanying C_b in the LHP complex to be C_a670 rather than C_a685 . Such a result may well be due to the fact that, as it is demonstrated by the present experiments, C_a685 is the preferentially removable C_a form by detergent activity.

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