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ON THE QUANTITATIVE RELATIONSHIP BETWEEN CHLOROPHYLL *B* AND THE CHLOROPHYLL *A* FORM C_a685 IN THE LIGHT-HARVESTING PIGMENT-PROTEIN COMPLEX OF CHLOROPLASTS

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SUMMARY

The relative amounts of chlorophyll *b* (C_b) and chlorophyll *a*685 (C_a685) are determined for seven plant species. It is found that the amount of the C_b–C_a685 chlorophyll-protein complex is correlated with the relative amounts of both C_b and C_a685. However, this relationship differs for these chlorophylls.

The same relations are studied for a single species, *Phaseolus vulgaris*, grown under various light intensities. No changes are observed in plants grown at intensities ranging from 15 to 150 $W \cdot m^{-2}$. At 8 $W \cdot m^{-2}$ the amount of the C_b–C_a685 complex is hardly changed. However, the ratio of the relative amounts of both components is clearly increased.

The results are discussed.

1. INTRODUCTION

Comparison of the amounts of various chlorophyll-protein complexes from the studied seven plant species showed that, in contrast to the other chlorophyll *a* (C_a) complexes, the relative amount of the C_a form C_a685 is directly correlated with that of chlorophyll *b* (C_b), whereas in two C_b-free algae C_a685 is absent, see KLEINEN HAMMANS & THOMAS (1976). These results were interpreted to suggest that both pigments are complexed with one and the same protein carrier.

Experiments on the effect of detergents by THOMAS et al. (1977) showed that C_a685 and C_b can be readily separated from each other. The latter authors considered the possibility that these pigment forms are bound to different polypeptides constituting a single protein subunit of the light-harvesting pigment-protein (LHP) complex. This consideration was based on the results of KLEIN & VERNON (1974), who found that the absence of C_b coincides with the absence of two polypeptides. In this connection it seems worth while to obtain some more information about the mutual amounts of C_b and C_a685 in various LHP complexes. To this purpose the present study deals with the quantitative relationship between C_b and C_a685 in various plant species. Moreover, this

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relationship is examined in a single species, *Phaseolus vulgaris*, grown under different light intensities.

2. MATERIALS AND METHODS

From the algae *Vischeria stellata*, *Tribonema aequale*, *Euglena gracilis*, *Chlorella pyrenoidosa*, and *Ulva lactuca* as well as the higher plants *Aspidistra elatior*, *Phaseolus vulgaris*, *Spinacia oleracea*, and *Callitriche stagnalis* chloroplast fragment suspensions were prepared. The latter species was gathered from its natural habitat, while the remaining species were grown at the institute. For details about the preparation see KLEINEN HAMMANS & THOMAS (1976).

Phaseolus vulgaris was grown under four light intensities, namely 150, 60, 15, and 8 $W \cdot m^{-2}$. These intensities were obtained by adjusting small-mesh wire-netting as neutral filters in front of fluorescent daylight tubes.

Absorption spectra of the chloroplast fragment suspensions at 77 K, established in a Cary model 14R recording spectrophotometer, were analyzed in a CYBER-73 computer using a revision of the RESOLV program developed by Dr. D. D. Tunnicliff of the Shell Development Laboratory, Houston, Texas. For details about the scope of the program see FRENCH et al. (1969), FRENCH & LAWRENCE (1972), OUDSHOORN & THOMAS (1975) and KLEINEN HAMMANS & THOMAS (1976).

3. RESULTS

The ratio of the amounts of C_b and C_a685 differs for various values of the total amounts of these chlorophylls in the C_b-C_a685 complex. For the studied species it ranges from 1.5 to 0.5. It is obvious from *fig. 1* that this ratio declines at an increase of the total chlorophyll content of the complex. The decline is found to be due to the fact that the increase pattern of C_b at growing amounts of the chlorophylls in the mentioned complex, shown in *fig. 2*, differs from that of C_a685, depicted in *fig. 3*.

The effect of growing *Phaseolus vulgaris* under four light intensities on the pigment composition of the complex in question is summarized in *table 1*. It is obvious that a tenfold increase of the about-saturating intensity 15 $W \cdot m^{-2}$ is without any effect on the ratio of both pigment forms under consideration. The same holds for the relative amount of the C_b-C_a685 complex. At the lower light intensity 8 $W \cdot m^{-2}$ the latter amount stays about the same. However, the ratio C_b/C_a685 is clearly increased.

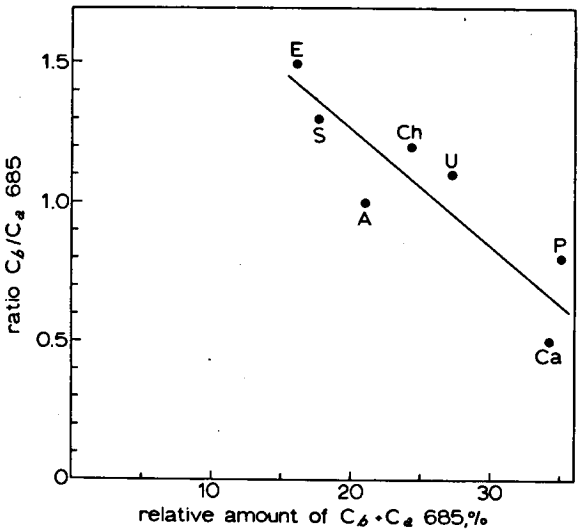

Fig. 1. Relationship between the C_b/C_a685 ratio and the total amount of these chlorophylls, expressed in per cents of the total area of the red absorption band between 620 nm and 710 nm, in seven species. A; *Aspidistra elatior*, Ca; *Callitriche stagnalis*, Ch; *Chlorella pyrenoidosa*, E; *Euglena gracilis*, P; *Phaseolus vulgaris*, S; *Spinacia oleracea*, U; *Ulva lactuca*.

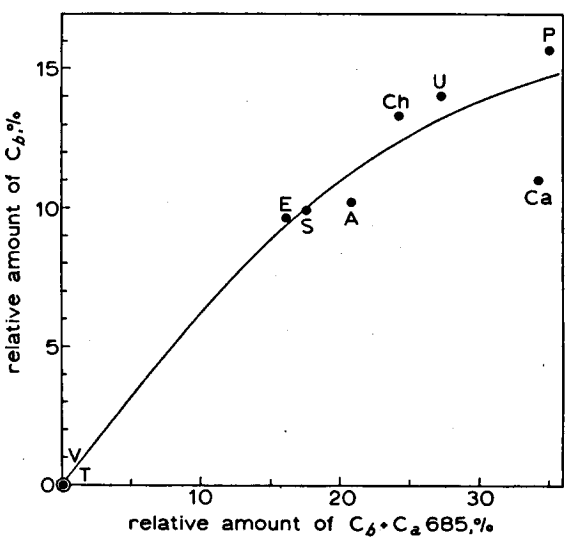

Fig. 2. Proportion of C_b in the total chlorophyll content of the C_b/C_a685 complex. T; *Tribonema aequale*, V; *Vischeria stellata*. For remaining symbols as well as for details see legends of *fig. 1*.

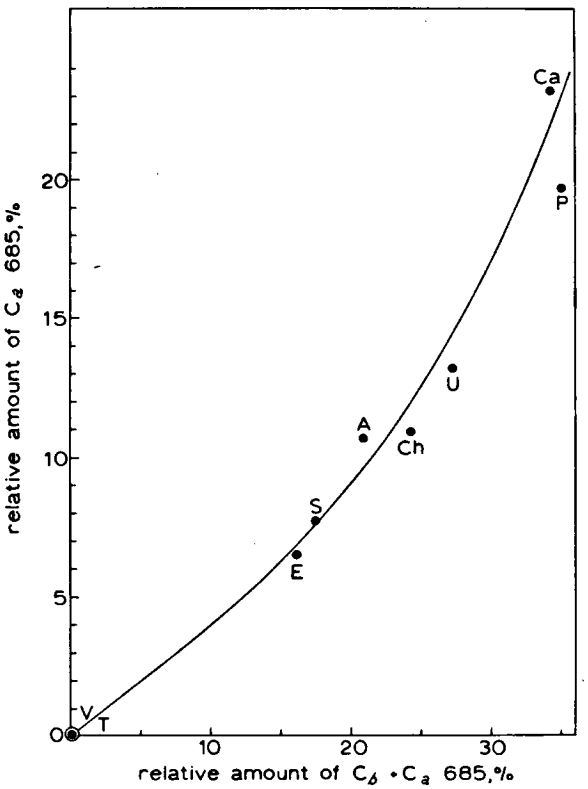

Fig. 3. Proportion of C_a685 in the total chlorophyll content of the C_b/C_a685 complex. For symbols and details see legends of *figs. 1* and *2*.

4. DISCUSSION

The present data as well as earlier ones, cf. KLEINEN HAMMANS & THOMAS (1976), deviate from a number of results in literature. First, FRENCH et al. (1972), using the RESOLV program as well, computed that the C_b-free alga *Tribonema* contains C_a685. Moreover it can be seen from their Table IV that the C_a685 content of different species is inversely related to that of C_b. The divergence of results probably is due to the fact that Dr. French and coworkers used a Gaussian curve as the single component, whereas in our case such a component consists of a mixture of a Gaussian and a Lorentzian curve, *cf.* OUDSHOORN & THOMAS (1975). This was done since the lower parts of absorption bands are better approximated by a Lorentzian curve than by a Gaussian one.

In a review THORNBER (1975) stated that it is generally agreed that the same amounts of C_a and C_b occur in the LHP complex. According to *e.g.* KITAJIMA & BUTLER (1975) and KAN & THORNBER (1976) the absorption bands of this

Table 1. Effect of light intensity on the amounts of chlorophyll *b* and chlorophyll *a*685 in the suggested LHP subunit, termed below 'complex', as well as on the relative amount of this complex.

Light intensity $W \cdot m^{-2}$	Experiment nr.	Area of complex in % of that of the red absorption band	Amount of chlorophyll forms in % of the complex		Ratio C_b/C_a685
			C_b	C_a685	
150	1	31	43	57	0.7
	2	30	41	59	0.7
	3	29	49	51	1.0
	mean	30.0	44.3	55.7	0.8
60	1	33	43	57	0.8
	2	31	43	57	0.8
	3	32	43	57	0.8
	mean	32.0	43.0	57.0	0.8
15	1	32	43	57	0.8
	2	33	44	56	0.8
	3	33	42	58	0.7
	mean	32.7	43.0	57.0	0.8
8	1	29	56	44	1.3
	2	29	57	43	1.3
	3	31	56	44	1.3
	mean	29.7	56.3	43.7	1.3

complex are located at about 650 nm and 670 nm. On the other hand, BROWN et al. (1975) concluded that, in addition to C_b, the four major C_a forms occur in the LHP complex. Moreover, conclusions about the site of occurrence of C_b are conflicting as well. The latter authors mentioned that all C_b is located in the LHP complex, while THORNBER & HIGHKIN (1974) and NAKAMURA et al. (1976) suggested that other locations are probable as well.

According to THORNBER & HIGHKIN (1974) and GENGE et al. (1974) the absence of C_b is correlated with that of part of the protein moiety of pigment-protein complexes. KLEIN & VERNON (1974) identified this part of the protein moiety to be 22 and 24 kdaltons polypeptides. As mentioned already, THOMAS et al. (1977) suggested that the C_b and C_a685 forms may consist of chlorophylls bound to one of these polypeptides each, whereas these complexes are combined so as to constitute a pigment-protein subunit of the LHP complex.

It might be that the above-mentioned divergency of results is due to different techniques applied for isolating the latter complex. In this way the proposed subunit complex. or part thereof, could be removed from the LHP complex. As it is indicated by the results of the latter authors such a possibility may be due to the use of detergents.

The present results show that the higher the relative amount of the suggested C_b-C_a685 pigment-protein complex the higher are the amounts of both pigment forms. However, such an increase differs for these forms in such a way

that the ratio C_b/C_a685 decreases from 1.5 to 0.5 at increasing relative amounts of the complex from 16 to 35, cf. *fig. 1*. The above data are obtained with the mentioned seven species, all of these grown under saturating light intensities.

For *Phaseolus vulgaris*, grown under different light intensities, it is found that at low light intensity, about 50% of the saturating one, the C_b/C_a685 ratio is raised to 1.3 from its value 0.7 at high intensities. However, the relative amount of the complex decreased only very little, namely from about 31.4 to 29.7, cf. *table 1*. From *fig. 1* one may expect the latter value to be about 19.

One may wonder whether the data obtained with the various species grown at saturating light intensities are indicative of different proportions of the C_b and C_a molecules in the C_b-C_a685 complexes of various relative amounts being due to either different quantities of these molecules bound to the carrier component, or to various amounts of the pigment-carrier complexes with a constant ratio: pigment molecules/carrier component. As outlined above, these components may be the 22 and 24 kdalton polypeptides observed by KLEIN & VERNON (1974). The experiments with *Phaseolus* grown under various light intensities may be in favour of the first possibility. However, to elucidate the problem with certainty as well as to obtain more insight into the structure of the suggested C_b-C_a685 pigment-protein complex, it is required to examine the variations of the amounts of the two polypeptides in various plant species grown at saturating as well as non-saturating light intensities. It is hoped that future research will provide more information.

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