

ON THE CORRELATION BETWEEN THE AMOUNTS OF CHLOROPHYLL *B* AND CHLOROPHYLL *A* FORMS IN VARIOUS PLANTS

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SUMMARY

The relative amounts of chlorophyll *b* (C_b) and various chlorophyll *a* (C_a) forms in nine plant species were determined by computer analysis of low-temperature absorption spectra.

The results show that (a) the amount of C_b is inversely related to the sum of the amounts of the bulk C_a from both photosystems, (b) a direct correlation is likely to occur between the amounts of C_b and the C_a bands peaking around 685 nm and 640 nm, and (c) no correlation exists between the amounts of C_b and the C_{a662} and C_{a695} forms.

These data are interpreted to suggest that C_b is capable of taking over the antenna function of the bulk C_a , whereas it may occur in a complex carrying the C_{a685} form as well.

1. INTRODUCTION

Various studies deal with the problem whether chlorophyll *b* (C_b) functions in the photosynthetic apparatus in a way additional to its role of antenna pigment. The results, however, are rather contradictory. GOODCHILD et al. (1966), BOARDMAN & HIGHKIN (1966), and HIGHKIN et al. (1969) concluded that C_b functions in the formation of chloroplasts as well, whereas VELEMINSKY & RÖBBELEN (1966), SAGROMSKY & DÖBEL (1974), and HENRIQUES & PARK (1975) did not succeed in obtaining such-like indications. A C_b deficit was reported to be correlated with a decrease of the chlorophyll *a* (C_a) amount in *Hordeum* mutants by SAGROMSKY (1974) and THORNBER & HIGHKIN (1974). However, such a correlation was not observed in all cases for maize mutants by GAVAZZI et al. (1975).

Furthermore it was found that the absence of C_b coincides with the lacking of certain polypeptides, cf. THORNBER & HIGHKIN (1974), GENGE et al. (1974), ANDERSON & LEVINE (1974a, 1974b) and HENRIQUES & PARK (1975). According to the latter authors a variation in the peptide composition does not result in a change in density or size of the intramembranous particles. GENGE et al. (1974) stated that the presence and amount of the pigment-protein complex II, and of its apoprotein correlates with the C_b content.

By far the majority of the mentioned results are obtained from experiments

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with mutants. In complex systems, such as chloroplasts, it may well be that properties without any relation to each other are changed in mutants. Such a situation may be the reason why so many contradictory conclusions are drawn.

AKOYUNOGLU & MICHELINAKI-MANETA (1975) studied the effect of flashes as well as continuous light on the development of the photosynthetic activity in wild-type bean leaves. These authors concluded that the chlorophyll-protein complex II and C_b are likely to be related to the stacking of the lamellae.

KLEIN & VERNON (1974) stated that certain polypeptides are associated with C_b . Moreover, they obtained a light-harvesting C_b -protein complex containing C_a as well.

Both latter studies indicate that a relation between the presence of C_b and the composition of C_a pigment complexes is possible. If so, it might be that certain phenomena, ascribed to the presence of C_b , in fact are related to C_a . Since such a possibility regards general properties of the pigment complexes it seemed interesting to examine whether a quantitative relation between C_b and C_a forms occurs in wild-type species of a number of taxonomically differing plants. For this reason computer analyses of low-temperature absorption spectra of nine plant species were performed. The results are presented below.

2. MATERIAL AND METHODS

Chloroplast fragment suspensions were prepared from the algae *Vischeria stellata*, *Tribonema aequale*, *Euglena gracilis*, *Chlorella pyrenoidosa*, and *Ulva lactuca*, as well as from the higher plants *Spinacia oleracea*, *Aspidistra elatior*, *Phaseolus vulgaris*, and *Callitriche stagnalis*. Except for the latter one, which was gathered from its natural habitat, these species were grown at the institute.

The fragment suspensions from *Vischeria* and *Tribonema* were obtained by sonication, cf. THOMAS & BRETSCHNEIDER (1970), from the two nextmentioned species by grinding in a mortar cf. THOMAS & DE ROVER (1955), from *Aspidistra* by mincing in a Braun Multipress, cf. THOMAS (1962), and from the remaining species by macerating in a Sorvall Omnimixer, cf. THOMAS (1971).

Absorption spectra at 77°K were established in a Cary Model 14R recording spectrophotometer. The spectra were analyzed in a CYBER-73 computer using the RESOLV program developed by Dr. D. D. Tunnicliff of the Shell Development Laboratory, Houston, Texas, and revised as well as kindly provided by Dr. C. S. French. For the scope of the program cf. FRENCH et al. (1969), FRENCH & LAWRENCE (1972), and OUDSHOORN & THOMAS (1975). The studied spectral region ranged from the C_a absorption band around 640 nm up to that of the C_a 695 form. The following chlorophyll bands were studied: C_b , C_a 640, C_a 662, C_a 670, C_a 680, C_a 865, and C_a 695. The exact peak location of these bands varied between 653.0–648.6, 644.1–637.8, 663.3–660.6, 672.3–669.2, 681.3–676.5, 691.8–682.4, and 698.5–692.6 nm, respectively. The relative amounts of these components are expressed as their areas in percents of the total area of the pertaining complex red absorption band.

3. RESULTS

Since the spectra refer to diverse plant species with more or less diverging shapes of the absorption bands of the various pigment complexes, it is to be expected that the data show a relatively high scattering. This phenomenon is obvious from *fig. 1*. It should be emphasized that the drawn lines in this figure, instead of representing the correct shapes, only serve to show the relation between the plotted values for C_b -containing and C_b -free plant species. It then can be concluded from *fig. 1A* that the total amount of antenna chlorophylls a C_a670 and C_a680 is highest in the absence of C_b . It declines with increasing amounts of C_b . According to *figs. 1B* and *1C* this holds for the antenna chlorophylls a of both photosystems.

As shown in *fig. 1A*, two C_a bands, C_a685 and C_a640 , are absent in both C_b -free species. This simultaneous absence possibly may be due to the fact that these bands represent the main red absorption band and its first vibrational band of only a single C_a form. Therefore, the halfwidth values of both bands

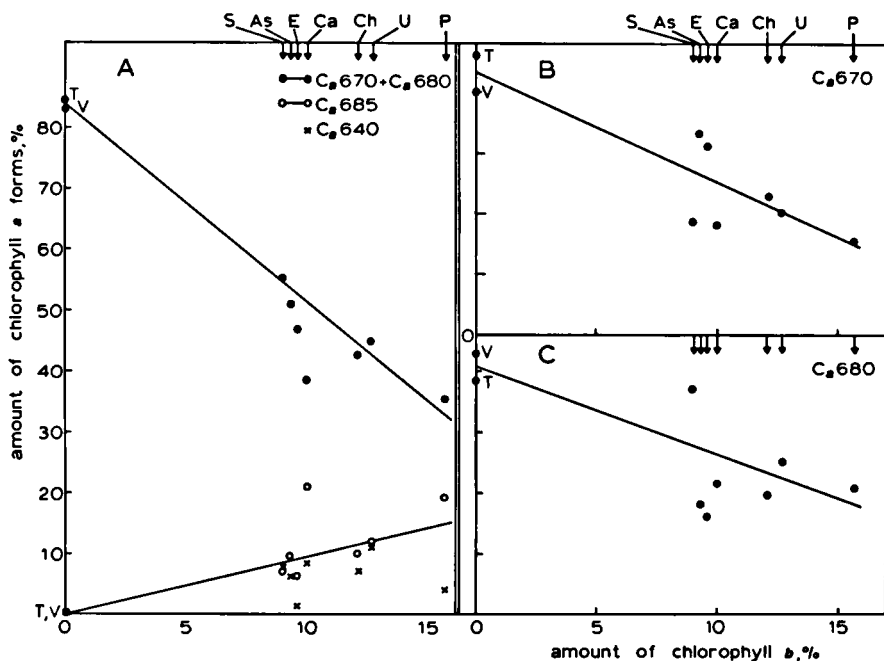


Fig. 1. Relation between the amount of chlorophyll b and that of the chlorophyll a forms C_a685 , C_a680 , C_a670 , and C_a640 in nine plant species. Amounts are expressed in band areas given as percentages of that of the total red absorption band of each plant species. T, *Tribonema*; V, *Vischeria*; S, *Spinacia*; As, *Aspidistra*; E, *Euglena*; Ca, *Callitriche*; Ch, *Chlorella*; U, *Ulva*; P, *Phaseolus*. A: relation between the content of C_b and those of $C_a670 + C_a680$, C_a685 , and C_a640 respectively. B: relation between the contents of C_b and C_a670 . C: relation between the contents of C_b and C_a680 .

Table 1. Additional spectral data concerning the species mentioned in the legends of *fig. 1*.

Species	Ratio areas C _a 680/C _a 670	Halfwidth nm		Ratio halfwidth C _a 685/C _a 640	Area, %		
		C _a 640	C _a 685		C _b	C _a 662	C _a 695
<i>Vischeria stellata</i>	1.08	0.00	0.00	—	0.00	12.13	4.53
<i>Tribonema aequale</i>	0.84	0.00	0.00	—	0.00	14.68	0.89
<i>Spinacia oleracea</i>	1.99	16.07	10.44	0.65	9.02	15.50	4.98
<i>Aspidistra elatior</i>	0.53	13.06	14.77	1.13	9.27	19.62	0.00
<i>Euglena gracilis</i>	0.51	9.69	11.38	1.17	9.55	16.56	18.86
<i>Callitriche stagnalis</i>	1.20	19.07	17.32	0.91	10.03	9.35	11.89
<i>Chlorella pyrenoidosa</i>	0.86	21.60	12.24	0.57	12.06	21.73	6.88
<i>Ulva lactuca</i>	1.24	13.19	9.83	0.75	12.66	17.68	0.98
<i>Phaseolus vulgaris</i>	1.36	14.06	14.23	1.01	15.75	15.99	9.23

as well as their ratios are given in *table 1*. These ratios show a mutual divergence. In this respect it is interesting to know whether such ratios differ for dilute C_a solutions in various organic solvents as well. To this purpose a C_a concentration yielding an optical density of about 0.4 was chosen. The results, together with the pertaining peak locations, are given in *table 2*. It shows that mutual differences between the ratios in question, be it to a smaller degree, also occur in different solvents. In the discussion these data will be considered in some more detail.

Table 1 furthermore demonstrates that no correlation whatsoever occurs between the amounts of C_a662 or C_a695 and that of C_b. Moreover it is shown in the latter table that the mutual proportions of the amounts of the antenna chlorophylls *a* from both photosystems are not correlated with the amount of C_b.

Table 2. Location and halfwidth values of the main red absorption band and the first vibrational band of chlorophyll *a* dissolved in organic solvents.

Solvent	Location of absorption maximum		Halfwidth		Ratio a/b
			main band (a)	vibrational band (b)	
	nm	nm	nm	nm	
Ethanol	665.44	617.45	21.20	33.06	0.64
Methanol	666.52	620.24	22.93	35.00	0.66
Ether	662.25	615.13	17.04	30.76	0.55
Acetone	664.33	617.26	20.50	33.58	0.61

4. DISCUSSION

It is shown in *fig. 1* that the amount of C_b in diverse types of plants is inversely proportional to that of the antenna chlorophylls a . Apparently C_b is capable of taking over the antenna function of C_a . When considering the fact that C_b is not required for the proceeding of photosynthesis, the direct involvement of C_b in this process may well be restricted to its antenna function.

The relation between the amounts of C_b and C_a in diverse wild-type plants is in disagreement with the results obtained in experiments with mutants, mentioned in the introduction, cf. SAGROMSKY (1974) and THORNER & HIGHKIN (1974).

The C_{a640} and C_{a675} bands are observed only in the presence of C_b . In this respect it is mentioned that KLEIN & VERNON (1974) isolated a light-harvesting C_b complex containing C_a as well.

The fact that the C_{a640} and C_{a685} bands are absent in both studied C_b -free species may indicate that these bands are due to C_a bound to the mentioned light-harvesting component. This suggestion is supported by experiments on *Phaseolus vulgaris* by Kleinen Hammans (unpublished) showing that both C_b and the C_{a685} form are less soluble in dilute, 0.1 %, sodium dodecyl sulphate (SDS) than the major C_a types.

OGAWA et al. (1966) obtained two chlorophyll-protein complexes, complex I and complex II, by electrophoresis of SDS-solubilized spinach chloroplast lamellae. According to THORNER & HIGHKIN (1974) complex II represents the light-harvesting component carrying C_a and C_b in a 1/1 ratio. A survey of the data about the light-harvesting C_a/C_b protein complex II, and complex I, is presented by THORNER (1975).

BROWN et al. (1974) analyzed absorption spectra of such chlorophyllprotein complexes from diverse plants. Some of these analyses, in particular those for *Tribonema* and *Phormidium* complexes seem to be contradictory to our conclusion that the C_{a640} , C_{a685} , and C_b bands occur only concomitantly. As to this it should be remarked that the present data refer to samples non-treated with detergents instead of isolated chlorophyll-protein complexes from SDS-solubilized lamellae. It may be that in some cases minor artificial absorption bands, due to the isolation procedure for these complexes, are observed. More information about this possibility is needed. Therefore, only the results obtained with 'non-treated' chloroplast fragments will be considered further in the present discussion.

One may wonder whether the C_{a640} and C_{a685} bands belong to a single form of C_a . DÖRING et al. (1969) and LEPPINK & THOMAS (1973) observed that the C_a band around 640 nm is correlated with a C_a band at 682 nm in the sense that the former one represents the first vibrational band of C_{a682} . According to the first-mentioned authors this C_a form acts as the reaction center pigment of photosystem 2. From *table 1* it is obvious that the ratios of the halfwidth values of both kinds of bands are diverging considerably. This fact renders it questionable whether these bands are related to each other. However, cf.

table 2, the ratios of the halfwidth values of the main red band and its first vibrational one in dilute C_a solutions in organic solvents also show variations, be it to a lower degree. Since the *in vivo* situation, in particular the complex formation with proteins, as well as, possibly, the occurrence of chlorophyll aggregates, cf. BROWN (1972), differs widely from that in dilute solutions, a considerably smaller divergence of these ratios is to be expected in the latter case.

The ratio of the areas of the main and the vibrational bands, cf. *fig. 1*, also differs from the usual ratio of about 1/5 in most cases. It is possible that this fact is to be ascribed to the approximative character of the computer analyses.

Summarizing the discussion about a possible relation between the C_a640 and C_a685 bands it can be said that two facts suggest the occurrence of such a relation, namely (a) the simultaneous absence of both bands in the studied C_b -free organisms, and (b) the earlier observed correlation of the C_a640 and C_a682 bands by DÖRING et al. (1969) and LEPPINK & THOMAS (1973), whereas possible reasons for the considered, anomalous features can be indicated. Therefore, it is tentatively suggested that the C_a640 and C_a685 bands are due to one and the same C_a form. If so, however, this C_a form cannot represent the reaction center pigment of system 2. For, its concentration of about 10% of the total chlorophyll *a* is much too high for that of the reaction center C_a concentrations, which is in the order of 0.5%. One then may think of the possibility that the C_a form in question functions as an energy collector pigment for the system 2 reaction center pigment in a way as proposed by KOK (1966) with regard to a collector pigment, originally termed C700, in the direct environment of the reaction center pigment of system 1. Future research is needed to study this possibility.

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