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SHORT COMMUNICATION

CHLOROPHYLLIDES IN GREEN AND ETIOLATED LEAVES

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Abstract—Chlorophyllides, extracted from lyophylized fresh leaves of different species by absolute methanol, were separated from the chlorophylls by thin-layer chromatography. They amounted only to 0·04 to 0·1 per cent of the total amount of chlorophyll *a* on a molar basis. On account of their low concentration it was concluded—in contrast to an assumption made recently—that the 683 nm absorption band of mature leaves *in vivo* cannot be due to a chlorophyllide-holochrome.

USING thin-layer chromatography[1] (cellulose MN 300† and a mixture of methanol, dichloromethane and water (100:18:20, v/v/v) as developing solvent), chlorophyllides can easily be separated from chlorophylls. Chlorophyllides prepared in aqueous acetone by chlorophyllase action are found just above neoxanthin (Fig. 1), and methyl and ethyl chlorophyllides of the *a* series between neoxanthin and violaxanthin. Chlorophyllides which are esterified with alcohols of greater chain length have lower R_f-values.

Recently relatively large amounts of a chlorophyllide-like pigment were detected in extracts of *Chlorella*.[2] The possible role of chlorophyllide *a* in photosynthesis has been discussed[3] and the 683 nm absorption band *in vivo* ascribed to a chlorophyllide holochrome.

In the present paper TLC was used for quantitative detection of free, native chlorophyllide in green leaves. The plastid pigments were extracted from the finely ground powder prepared from lyophylized fresh leaves by precooled, absolute methanol. 99·5 to 99·8 per cent of the chlorophyllous pigments were removed as shown by measurement of the spectra of the glycerine-soaked residue. Further grinding and extraction released the rest of the pigments. No chlorophyllide could be detected in this second extract. The first extract was concentrated under reduced pressure in the cold and was applied directly to the plates without further purification. On account of the low concentration of chlorophyllides present, the plates were overloaded and developed until the xanthophylls separated from the chlorophylls (the chlorophyllides were not visible). Usually a run of 5–6 cm was sufficient. The streaks were then scraped off and eluted. Rapid extraction in the cold in non-aqueous medium greatly reduced the yield of chlorophyllide, in contrast to extraction with 80 per cent aqueous acetone, which can only be used after drying. For estimation, the red peak of chlorophyll *a* was used. The values were corrected for adsorption at 700 nm.

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The results are summarized in Table 1. Only traces of free chlorophyllides were present in the "neoxanthin streak". They proved to be only about one 2500th to one 1000th part of the quantity of chlorophyll *a* present. Similar results were obtained with leaves of *Spinacia*, *Lolium*, *Datura*, *Pisum* and *Phaseolus*. The region of the plate containing methyl and ethyl chlorophyllides—the possible product of enzymatic and non-enzymatic esterification during extraction and chromatography—contained further amounts. Larger amounts of chlorophyllide were detected in leaves possessing high chlorophyllase activity, and it is probable that these cases are an artefact. It should be noted that traces of a pigment with an adsorption peak similar to the red peak of chlorophyll *a* was also found in the lutein region of the TLC plate. In contrast to green leaves most of the chlorophyllous pigments are not esterified with phytol in etiolated leaves[3–5] (Table 1). In extracts from etiolated leaves which had not been exposed to light a pigment (protochlorophyllide) with red peak at 624 nm was detected just above lutein—much lower than the chlorophyllides—and another (protochlorophyll) with an R_f value slightly lower than that of chlorophyll *a*. The absolute amount of non-phytylated chlorophyllides in green and in etiolated leaves seems to be about the same.

TABLE 1. THE AMOUNT OF (PROTO)CHLOROPHYLLIDE IN GREEN AND ETIOLATED (8-DAY-OLD) LEAVES IN RELATION TO THE CONTENT OF (PROTO)CHLOROPHYLL. I: FREE CHLOROPHYLLIDE; II: I AND CHLOROPHYLLIDE ESTERIFIED WITH SIMPLE ALCOHOLS. THE VALUES ARE THE MEAN OF THREE TO FOUR EXPERIMENTS WITH FOUR REPLICATES EACH

	Ratio of nonphytylated/phytylated chlorophyllide			Chlorophyllide (ppm/ppm dryweight)		
	I	II	Etiol.	I	II	Etiol.
Hordeum vulgaris	1:2000	1:1600	7:1	17	21	34
Zea mays	1:2500	1:1300	5:1	38	73	24
Beta vulgaris var. Cicla	1:1450	1:680	—	25	56	—
Atriplex hort.	1:1000	1:490	—	41	83	—

The amount of chlorophyllide in green leaves does not exceed the amount of P 700.[6,7] It is far too low to support the theory[3] that the 683 nm absorption band occurring in mature leaves[8–11] and which is ascribed to photosynthetic system I[12] is due to a chlorophyllide holochrome. Until now there is no evidence that chlorophyllide may play an active role in photosynthetic processes.

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