Isolation and Characterization of 3 Protochlorophyllides from Pigment Mutant C-2A′ of *Scenedesmus obliquus*

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Z. Naturforsch. **41c,** 1001–1003 (1986); received July 1, 1986

Scenedesmus obliquus, Protochlorophyllide, Monovinyl Protochlorophyllide, Divinyl Protochlorophyllide

Three Protrochlorophyllides (protochlorophyllide) were separated from mutant C-2A′ of the green alga *Scenedesmus obliquus*. They show distinct differences in polarity, absorption and fluorescence characteristics. Until further characteristics are established we have to assume that we found monovinyl protochlorophyllide (MV-protochlorophyllide), divinyl protochlorophyllide (DV-protochlorophyllide) and a third protochlorophyllide with different side groups. – The question whether these protochlorophyllides are intermediates in the same pathway or precursors of chlorophylls *a*, *b* and RC I in separate pathways remains open at this stage of investigation.

Introduction

Seedlings of angiosperms and some pigment mutants of algae grown heterotrophically in darkness and form etioplasts which contain protochlorophyllide (PChlide) but only traces or no chlorophyll (Chl). PChlide, an important intermediate of Chl biosynthesis, was isolated from many organisms. With a few exceptions [1–3] it is generally assumed that only one PChlide exists and that all Chls derive from this PChlide.

The pigment mutant C-2A′ of *Scenedesmus obliquus* forms only traces of Chl in the dark but accumulates considerable amounts of PChlide (20 µg/ml packed cell volume) during the initial growth phase [4, 5]. This mutant is an excellent tool to extract and accumulate enough PChlide for separation and identification.

Experimental

Cells of mutant C-2A′ were harvested after 12–20 h of heterotrophic growth at 30 °C (for further culture conditions see Senger and Bishop [6]). After centrifugation the pellet was repeatedly extracted with hot methanol until it was colorless. The extract was evaporated, the pigments redissolved in acetone and separated on a DEAE-Sepharose column [7] or by thin layer chromatography (TLC) with the same result. For TLC separation a solvent of medium polarity (petrol ether:propanol-2:water/100:10:0.25/v:v:v) was applied. Fluorescence measurements were carried out with a Shidmadzu RF 540 spectrofluorometer with emission band width of 5 nm and excitation band width of 10 nm.

Results and Discussion

Extracts of the cells were prepared and chromatographed as described in Experimental. The band close to the start was PChlide. It was extracted and re-chromatographed in a solvent system with the same components but stronger polarity (100:40:0.75/v:v:v). This chromatography resulted in 3 different bands (Fig. 1). These bands contained PChlide in a molar ratio of about 5% (1st band), 60% (2nd band) and 35% (3rd band). From the chromatographic behavior there is no question that the separated pigments are PChlides of different nature. The PChlides of the three bands are characterized by their absorption spectra (Fig. 2A, 2B, 2C, Table I).

There is little difference in the absorption of the red bands but a significant difference in the blue Soret absorption band. Very similar data were reported by Belanger and Rebeiz [1] for pigments in

Abbreviations: PChlide, protochlorophyllide; MV-PChlide, monovinyl protochlorophyllide; DV-PChlide, divinyl protochlorophyllide; Chl, chlorophyll; TLC, thin layer chromatography.

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0341–0382/86/1100–1001 $ 01.30/0

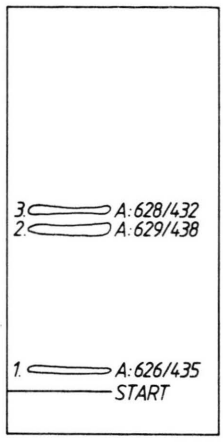


Fig. 1. Thin layer chromatogram of total PChlide. Developed with a polar solvent (petrol ether:propanol-2:water/100:40:0.75). This separation yielded three different PChlides with different absorption maxima and polarity (No. 1, 2, 3). A = absorption maxima in the red and blue region in methanol.

Table I. Absorption maxima of the three PChlides after TLC-separation (Fig. 1) in methanol. B/R = peak ratio in blue to red region. The number in brackets give the corresponding absorption maxima of monovinyl and divinyl-PChlide in ether, which were isolated from cucumber [1].

PChlide N.	Absorbance max.			B/R
PChlide 1	626;	575;	435	8.0
PChlide 2 (DV-PChlide)	629; (625)	578; (574)	438 (436)	6.85
PChlide 3 (MV-PChlide)	628; (623)	575 (571)	432 (431)	6.0

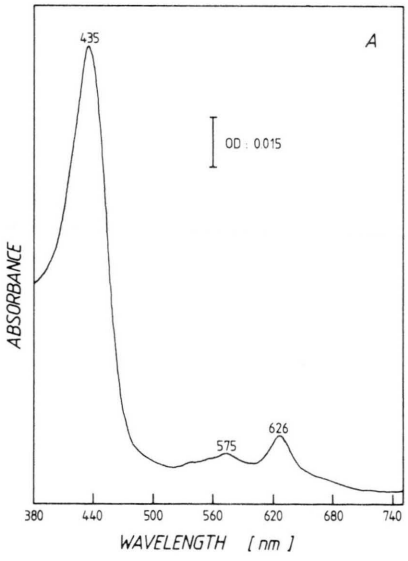
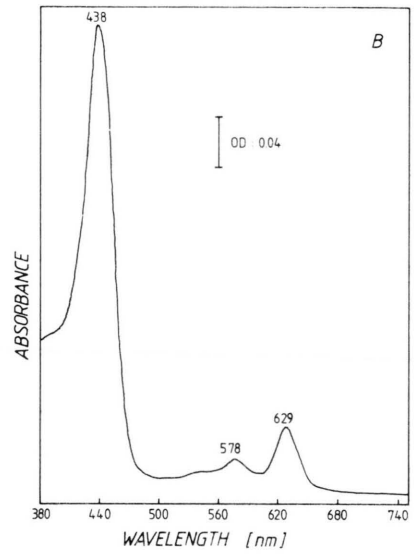
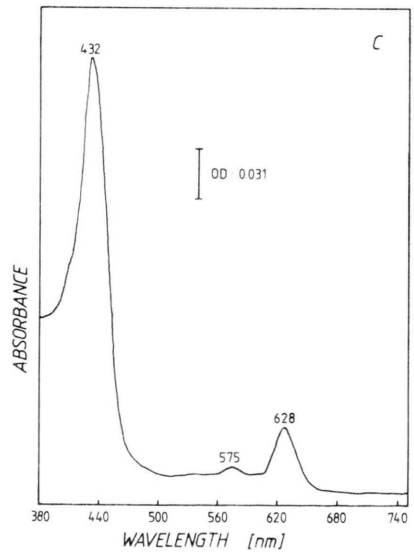


Fig. 2. Absorption spectra of the three isolated PChlides in methanol at room temperature. Spectra were recorded with a Kontron spectrophotometer (Uvikon 820) at 1 cm light path.
A = absorption spectra of PChlide 1 (see Fig. 1).
B = absorption spectra of PChlide 2 (see Fig. 1).
C = absorption spectra of PChlide 3 (see Fig. 1).





ether (Table I). Small differences derive from the different solvents. Following the interpretation of Belanger and Rebeiz [1], we have to assume that the main band (No. 2) is the divinyl and band No. 3 is the monovinyl form of PChlide. The nature of band 1 is uncertain. From its absorption characteristics it looks like a mixture of the divinyl and monovinyl forms, but the chromatographic behavior contradicts this interpretation.

Fluorescence excitation and emission spectra in acetone (Fig. 3) confirm the results obtained with absorption spectroscopy: There is a significant difference in the excitation of the Soret bands but little difference in the wavelengths of the emitted fluorescence. The symmetry of the fluorescence peaks proves the purity of the samples. Acidification of the samples caused for all 3 PChlides the formation of their phaeophorbides with the according shifts in wavelengths. This proves that the PChlides isolated, still contained Mg as their central atoms and were no phaeophorbides.

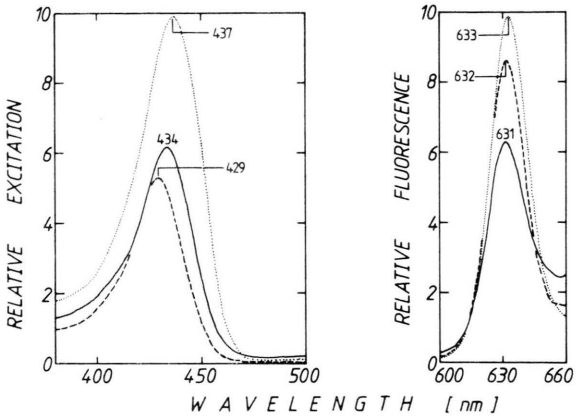


Fig. 3. Fluorescence excitation and emission spectra of the three PChlides (——— first band, ····· second band and ------ third band of Fig. 1) in acetone at room temperature. Spectra were recorded with a Shimadzu spectrofluorophotometer RF-540. Excitation spectra (slit width 10 nm) were measured for emission at 632 nm. For emission spectra (emission band width: 5 nm) fluorescence was excited with a wavelengths identical to the maxima in the excitation spectra.

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