PROTOCHLOROPHYLL(IDE) HOLOCHROME SUBUNITS FROM A MUTANT DEFECTIVE IN THE REGULATION OF PROTOCHLOROPHYLL(IDE) SYNTHESIS

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1. Introduction

Etiolated seedlings of angiosperms accumulate protochlorophyll(ide) (Pchl), which upon illumination, is reduced to chlorophyll(ide) *a* (Chl). The photoconvertible portion of the Pchl exists in a pigment-protein complex [1] called protochlorophyll holochrome [2]. With the aid of a mixture of detergents, saponin, protochlorophyll holochrome subunits (Pchl–H) with an apparent mol. wt. of 63 000 and giving no evidence of more than one Pchl molecule per unit have been obtained from the primary leaves of etiolated barley (*Hordeum vulgare* L.) seedlings [3–5].

When δ-aminolevulinic acid (ALA) is fed to etiolated leaves in darkness, Pchl accumulates in much greater quantities than in untreated leaves [6]. ALA-fed leaves show an absorption maximum near 633 nm rather than at 650 nm [7]. The amount of Pchl that is photoconvertible to Chl in such leaves by a brief, but saturating flash of light is not increased by the treatment with ALA [8]. The newly formed Chl has its maximum near 675 nm, shifting to 672 nm with time [7]. After conversion of the active Pchl, with time in darkness, a portion of the inactive Pchl becomes photoactive [9, 10].

Several *tigrina* mutants, considered as constitutive mutants in regulatory genes for Pchl synthesis [11], when grown in darkness, resemble ALA-fed, wild type seedlings with respect to the accumulation and absorption properties of the photoinactive Pchl, the absorption properties of Pchl and Chl after photoconversion of the initially active Pchl, and the regeneration of active Pchl from its inactive form [12].

This paper compares the absorption characteristics of Pchl–H isolated from seedling leaves with the genotypes ±/±, ±/*tig-o*^{34}, and *tig-o*^{34}/*tig-o*^{34}. Also, the kinetics of the photoconversion of Pchl to Chl in Pchl–H from three sources are compared.

2. Materials and methods

The plant material used was wild type barley (*Hordeum vulgare* L., cultivar Svalöf's Bonus) and the nuclear gene mutant *tig-o*^{34} from the same variety. Seedlings were grown at 23°C in darkness. Dim green safelight was used for operations requiring vision. Shoots were harvested and classified as will be described elsewhere. The segregation of *tig-o*^{34} seed material fitted a 1 ±/*tig-o*^{34} to *tig-o*^{34}/*tig-o*^{34} segregation of *tig-o*^{34} seed material fitted a 1 ±/*tig-o*^{34} to 1 *tig-o*^{34}/*tig-o*^{34} segregation [13].

Pchl–H was extracted as described earlier [3], except that the last centrifugation was omitted. The final pellet was resuspended with 0.5% (w/v) saponin and 17% (v/v) glycerol in 0.1 M tricine buffer adjusted to pH 8.5 with NaOH. The Pchl–H from *tig-o*^{34}/*tig-o*^{34} was further diluted by a factor of 1.7 with resuspending medium before use.

The light source used for partial photoconversions was a xenon lamp (Osram XBO 450 W/P) supplied with stabilized direct current. The light was filtered through a 640 nm Depil interference filter (No. 282244) and a red glass cut-off filter (RG 1), both from Jenaer Glaswerk, Schott und Gen., Mainz, Germany. A 12.5 cm layer of water was introduced into the light path. Energy measurements have been described elsewhere [4].

Samples of 1.5 ml of the preparations of Pchl–H in 1.0 cm cuvettes were irradiated at temperatures ranging from 6–10°C for periods of 7–100 sec with

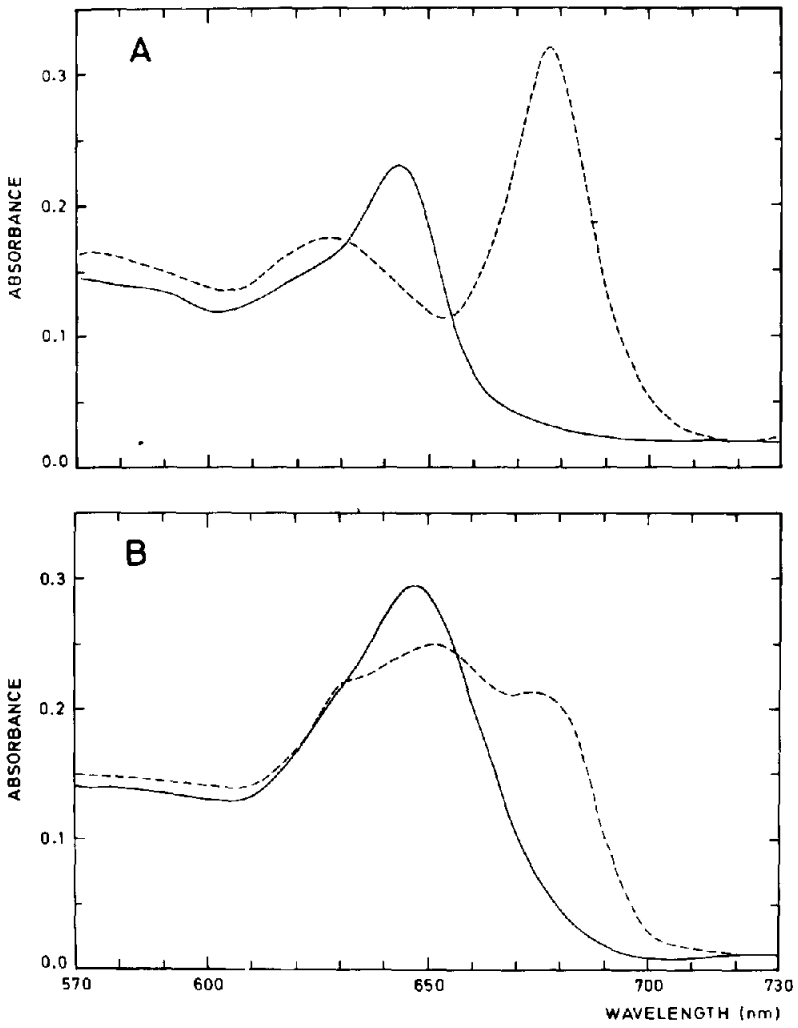

Fig. 1. Absorption spectra of saponin-containing Pchl–H (A) from ±/*tig-o*^{34} leaves and (B) from *tig-o*^{34} leaves: (———) before illumination; (- - - - -) after maximal photoconversion.

near-monochromatic light of the intensities $1.7 \cdot 10^3$ erg·cm^{-2}·sec^{-1} (Pchl–H from ±/± leaves) and $1.3 \cdot 10^3$ erg·cm^{-2}·sec^{-1} (Pchl–H from ±/*tig-o*^{34} and *tig-o*^{34}/*tig-o*^{34} leaves). Maximal photoconversion was obtained by exposure to red light for 300 sec or by a 2 min irradiation with a tungsten filament lamp.

Absorption spectra were recorded with a Zeiss RPQ-20 A recording spectrophotometer equipped with an IP 22 phototube, and those presented here have been transformed to a linear wavelenght scale.

3. Results and discussion

Pchl–H preparations from etiolated ±/± leaves have an absorption maximum, due to Pchl, in the red region near 644 nm (3–5). After maximal photoconversion, one maximum near 678 nm, due to Chl, and another near 630 nm due to the remaining, inactive Pchl and the secondary absorption of Chl, occur (3–5). Since the absorption spectra of Pchl–H extracted from etiolated ±/± barley leaves in this study

differed in no significant way from those published earlier, they are not reproduced here.

The absorption properties of Pchl–H from ±/*tig-o*^{34} leaves (fig. 1A) were identical with those of Pchl–H from ±/± leaves before illumination and after maximal photoconversion. Thus, the presence of one wild type allele and one *tig-o*^{34} allele does not affect the absorption of Pchl–H extracted from the leaf material. On the other hand the maximum of Pchl–H from *tig-o*^{34}/*tig-o*^{34} leaves, with about 20% of the Pchl photoconvertible to Chl, lies near 648 nm (fig. 1B). After maximal photoconversion, the main absorption maximum of the remaining Pchl occurs near 652 nm; Chl is evidenced by an absorption band near 678 nm (fig. 1B) as determined from a difference spectrum. The absorption properties in the red region of Pchl–H from *tig-o*^{34}/*tig-o*^{34} are similar to those of Pch–H from ALA-fed, wild type leaves [5] and resemble those of Pchl aggregates in benzene [14, 15].

The 652 nm absorption band of Pchl–H extracted from ALA-fed, wild type barley leaves has been attributed to aggregates of Pchl, because of the evidence for exciton interaction among these Pchl molecules in circular dichroism spectra [5]. Since it is unlikely that aggregation of holochrome particles in solution would form groups of pigment molecules with exciton interaction and that 652 nm absorbing Pchl in vitro became detached from protein and aggregated subsequently [5], the 633 nm absorbing species of Pchl in vivo were ascribed to aggregated Pchl [5]. Granick and Gassman [10] also suggested that the 633 nm absorbing Pchl in vivo might be aggregated Pchl, due to the minor decrease in 633 nm absorption during the activation of inactive 633 nm Pchl.

For the resemblance of *tigrina* mutants and ALA-fed, wild type leaves [12] and the similarity of the absorption properties, before illumination and after maximal photovonversion, of Pchl–H isolated from etiolated *tig-o*^{34} and ALA-fed, wild type leaves, the 633 nm absorbing Pchl in *tigrina* mutants is ascribed to aggregated Pchl. Thus, regeneration of active Pchl from photoinactive 633 nm absorbing Pchl [9, 10, 12] may include a step of disaggregation of Pchl molecules.

One way of examining whether the photoconversion sites in Pchl–H from the *tigrina* mutants are altered is to compare the kinetics of the photoconversion of Pchl in Pchl–H from the mutants and the wild type. The fractions of active Pchl remaining after

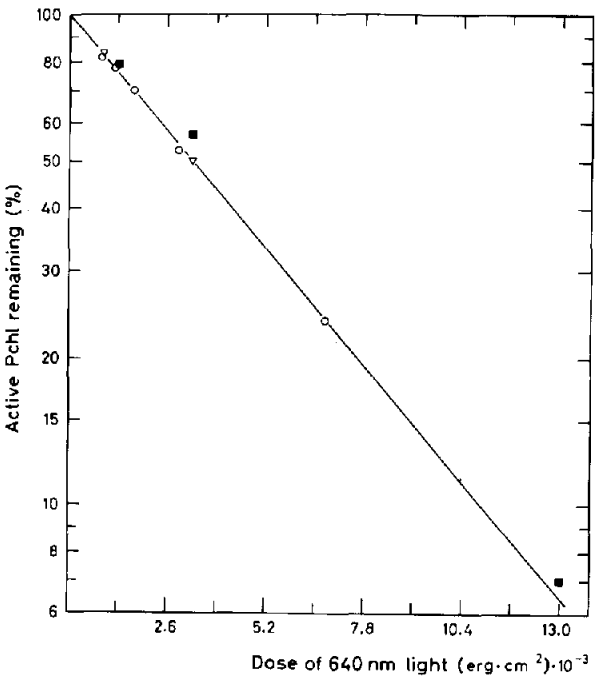

Fig. 2. Semi-logarithmic plot of the progress of photoconversion of Pchl in saponin-containing Pchl–H from leaves with the genotypes: (○)±/±,(▽)±/*tig-o*^{34},(■)*tig-o*^{34}/*tig-o*^{34} (○) and (■) represent single observations (▽) the average of two observations.

doses of light ranging from 7–100 sec in samples of Pchl–H extracted from ±/±,±/*tig-o*^{34}, and *tig-o*^{34}/*tig-o*^{34} leaves, respectively, were determined as described earlier [4]. The progress of photoconversion of Pchl from the three genotypes is similar, if not identical (fig. 2), and as found earlier for Pchl–H from the wild type [4], follows first order kinetics in all cases. The small departures of the points representing Pchl–H from *tig-o*^{34}/*tig-o*^{34} from the line (fig. 2) may not be meaningful. If the efficiency of photoconversion really is lower than in the other Pchl–H preparations, this very likely can be attributed to greater screening by inactive Pchl.

Thus, the study of Pchl–H from *tig-o*^{34} supports the conclusion [12] that the mutation of a regulatory gene for Pchl synthesis does not interfere with the formation of or affect the function of the initially photoactive Pchl-protein complex. The study also establishes a new point of similarity among etiolated *tigrina* mutants and ALA-fed, wild type seedlings,

namely the spectroscopical resemblance of the saponin-containing protochlorophyll holochromes extractable from the two sources.

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