Red Light Induced Change in the Saponin Content of Prolamellar Bodies in *Avena sativa*

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Dedicated to Professor W. Menke, Köln *on the Occasion of His 70th Birthday.*

Etioplast, Protochlorophyllide, Prolamellar Body, Saponin, Red Light

Exposure to red light of high intensities leads to saponin and prolamellar body degradation in etiolated *Avena sativa* seedlings. Exposure to red light of low intensities increases the saponin percentage found in prolamellar bodies without changing the overall saponin content. Protochlorophyllide is assumed to act a decisive part in the phototransformation of the PLB as expressed by their saponin content. This is indicated by the action spectrum of this stimulation as well as by experiments with long-wave red (729 nm) and ALA/LA treatment. In weak light the pchlide phototransformation is decelerated-probably due to lack of energy. The PLB are interpreted to be modified by the increased pchlide concentration so that more saponin can be incorporated.

In further experiments a barley mutant (*albina* f^{17}) lacking the capacity to esterify phytol as well as the capacity to degrade the PLB during the greening process was investigated. The results substantiate our hypothesis, as PLB transformation – indicated by PLB saponin concentration – is decelerated with increasing pchlide concentration in greening etioplasts.

Introduction

Mesophyll cells of primary leaves of dark-grown oat (*Avena sativa* L.) contain etioplasts, a type of plastid typical of etiolated plants. The dominant feature of etioplasts are prolamellar bodies, in the following referred to as PLB. Their structure is highly regular and therefore gives a paracrystalline impression. The PLB consist of branched and interconnected tubules. Analysis of isolated PLB showed two steroid saponins, called avenacosid A and B, to belong to the main components of these tubules [1].

Reaggregation experiments on PLB components indicate a special role of these saponins in the differentiation of tubule structure [2]. Light exposure of etiolated plants causes a structural change of the PLB to flat, intact vesicles, the thylakoids. Assuming that saponins are shape-determining structural components of the PLB, it can be expected that the light induced PLB transformation is connected with changes in their saponin content. Two possibilities are evident: either saponins are metabolized and cannot be identified in the plastids any longer, or they lose their place as integrating part of the inner plastid structure and become dissolved in the stroma.

As expected, PLB from white light treated oat etioplasts do show drastic changes in the saponin content [3].

Experiments on the influence of light of different wavelenghts on the saponin content of oat etioplasts and their PLB are reported herein. Special emphasis is given to the fate of saponins during the actual light induced transformation of etioplast structure.

Abbreviations: ALA, aminolevulinic acid; CHLIDE, chlorophyllide; CHL, chlorophyll; EDTA, ethylene diamintetraacetate tetrasodiumsalt; EP, etioplast; IPP, isopentenylpyrophosphate; LA, levulinic acid; $NADPH_2$, nicotinamide adenosindinucleotidephosphate; PCHLIDE, protochlorophyllide; PCHL, protochlorophyll; PLB, prolamellar body; TRIS, tris(hydroxymethyl)aminomethane.

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Materials and Methods

Avena sativa L. germinated at 25 °C and 75% humidity in complete darkness. Six-day old leaves were harvested and all consecutive preparation steps in order to isolate plastids and PLB were effected under safety light (green, 508 nm, DIL-filter, Schott & Co.). Wild-type *bonus* and mutants *xantha* h^{56} and *albina* f^{17} of *Hordeum vulgare* L. were treated accordingly.

Plants were illuminated either by a cold light source (KL 150 B, Schott & Co.) or by a xenon light (XBO 150/WI, Osram; in lamp housing 250, Leitz)

which held one of the following filters: IL 397 nm (417, 454, 488, 613, 628, 661), DIL 508 nm, Al 729 nm or RG 630 nm (cutt-off).

To investigate the effects of aminolevulinic acid (ALA), several drops of 0.05 M ALA in 0.05 M phosphate buffer (pH 7.0) were placed on the leaves of 5-day old etiolated seedlings which were incubated for 21 h in the dark afterwards. For according experiments regarding levulinic acid (LA), leaf pieces (2 cm long) of 6-day old etiolated seedlings were incubated for 30 min in 0.1 M LA (in 0.05 M phosphate buffer, pH 7.0) in the dark and illuminated afterwards. For according experiment regarding [1-^{14}C]isopentenyl pyrophosphate (IPP, ammonium salt, 56 mCi/mmol), IPP was diluted 1:25 with 0.05 M phosphate buffer (pH 7.0) containing 0.01 M $NADPH_2$ and 0.053 M ALA. The equivalent of 1.5 μCi of this solution was applied onto 20 leaves of 5-day old, dark-grown seedlings, and after incubation for 12 h in the dark plants were illuminated.

To isolate PLB, 20–200 primary leaves (depending on the experiment) were homogenized in 125–500 ml 0.05 M Tris/HCl, 0.25 M sucrose, pH 8.0. After filtration through eightfold cheesecloth, the homogenate was centrifuged for 20 min at $500 \times g_{av}$. The pellet containing intact etioplast was resuspended in a mixture of Percoll and isolation medium ($\varrho = 1.109$ g/ml, 0.12 M sucrose), put on a layer of Percoll ($\varrho = 1.130$ g/ml) and centrifuged for 15 min at $9000 \times g_{av}$. In the developing gradient, the etioplast appeared in a band at $\varrho = 1.090$ g/ml. A part of the etioplast fraction was fixed immediately by adding glutaraldehyde (final concentration 1%), and the number of etioplast per ml was counted on a hemocytometer (type Thoma).

The remaining etioplast fraction was cleaned from Percoll on a millipore filter (pore size 0.45 μm ∅), etioplasts were osmotically ruptured with 0.05 M Tris-0.01 M EDTA (pH 8.0) and free PLB then rinsed in the same buffer. PLB were extracted with 80% aqueous acetone first, followed by 99% aqueous acetone.

For quantification of the saponin content by means of thin-layer chromatography, the acetone extract was put on SILGUR 25 with chloroform/methanol/water (70/30/4) as solvent system. Saponin spots fluoresced in UV-light after spraying with 0.1% ANS (8-anilino-naphthalene-1-sulfonic acid), were scraped off the plates after co-chromatography with reference saponins (avenacosid A and B) which were made visible by orcin/H_2SO_4 [4], and their actual sugar content was determined using the anthrone method [5], a mixture of rhamnose/glucose (1:4) used as standard.

To investigate the radioactive labelled material, leaves from 20 seedlings were homogenized and extracted in a mortar with 80% aqueous acetone. The homogenate was then centrifuged for 5 min at $5000 \times g_{av}$ and the supernatant was treated chromatographically as described above. Autoradiography of the thin-layer plates was effected on Kodak Industrex 620 for 4 days. After exposure of the film, the spots were scraped off the plates, saponins were dissolved in methanol, and their activity was determined on a packard scintillator.

Determination of protochlorophyllide was carried out according to Klein and Schiff [6].

Results and Discussion

When dark-grown plants are exposed to white light of medium or high intensity, etioplasts will develop into typical chloroplasts, *i.e.* crystalline PLB will disappear and the grana- and stroma-thylakoids become synthesized. Parallel to the transformation of the crystalline structure of the PLB the saponine content of the latter decreases (Fig. 1). Comparable results were found for plants exposed to red light of high intensities ($37\,000$ erg $\times$ cm^{-2} $\times$ s^{-1}, Fig. 1). When plants are exposed to red light of low intensity (3000 erg $\times$ cm^{-2} $\times$ s^{-1}, Fig. 1), additional PLB develop [7, 8] and the saponin content of the PLB fraction increases for a short time (Fig. 1).

This increase in PLB saponin content presumably is not due to *de novo* synthesis of saponins. As shown in Fig. 2, etioplasts contain more saponins than exists in the PLB in crystalline form. When plants are exposed to weak red light, the saponin content of the whole etioplasts increases only slightly; however, the percentage of overall etioplast saponins located in the PLB fraction increases from 27% in the dark to 85% after exposure to red light for 15 min. Therefore, a balance between structurally combined and free saponins in the etioplast is assumed.

Apparently, the initial light energy is not sufficient to catalyse enzymatic degradation of saponins, but is sufficient to alter the metabolic state of the

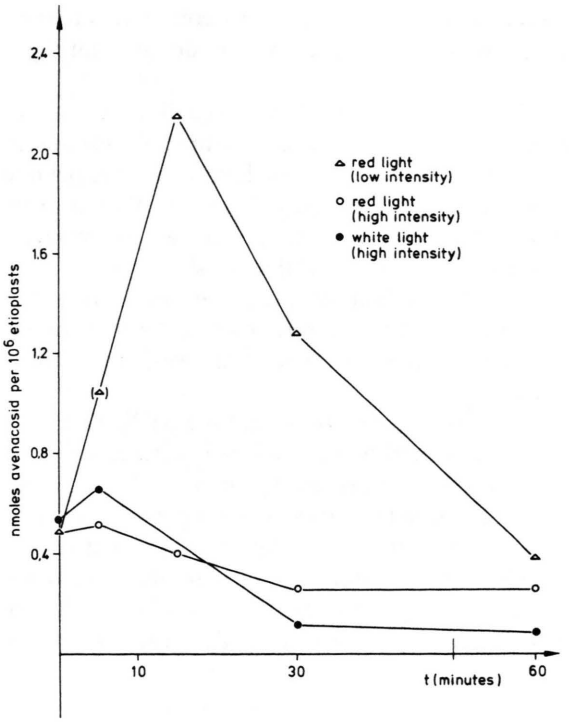

Fig. 1. Saponin content (total of avenacosid A and B) of prolamellar bodies isolated from 6-day old etiolated *Avena* plants after illumination with white light (cold-light source, 40000 erg × cm^{-2} × s^{-1}). The value for 5′ weak red light was taken from a parallel experiment.

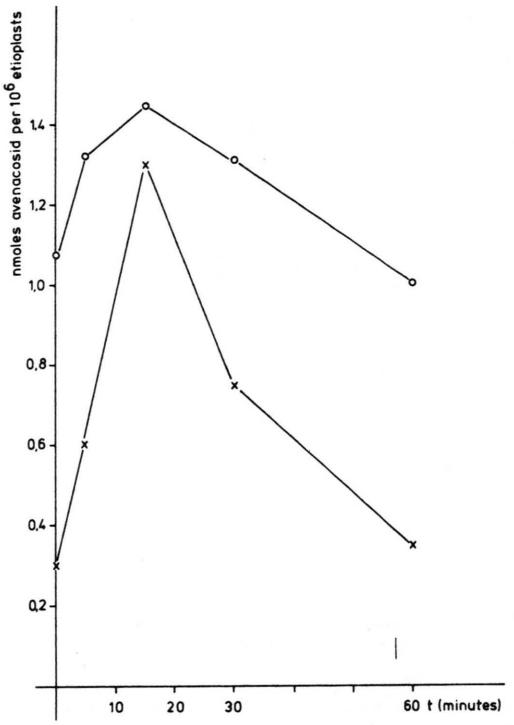

Fig. 2. Comparison of the etioplast (○) and the PLB (×) saponin content (total of avenacosid A and B) isolated from 6-day old etiolated *Avena* plants after irradiation with weak red light (4000 erg × cm^{-2} × s^{-1}).

cytoplasm so that PLB bound saponin increases. Experiments to label the steroid part of the saponine by [1-^{14}C]isopentenylpyrophosphate (Table I) failed to show *de novo* synthesis of saponin or its precursors, as only 4% of the total IPP uptake was incorporated into the avenacosids, independent of the duration of light exposure. As the fast increase in PLB saponin is not due to *de novo* synthesis, a different cause for the changes in the etioplasts leading to altered saponin percentages has to be looked for.

The action spectrum (Fig. 3) for the short-term increase in PLB saponin in weak light shows a maximum in the red (661 nm). It resembles the action spectrum for chlorophyll synthesis and might indicate that the phytochrome system is involved in the transformation of the PLB. Phytochrome stimulates the formation of chlorophyll at the ALA synthesis level by interfering with the negative feedback from pchlide or its precursors to the ALA-producing system [9]. In weak light, pchlide is not changed into chlide and esterified with phytol to chlorophyll as at high intensities, but the products of the phytochrome-controlled ALA synthesis accumulate, and the saponin content of the PLB increases accordingly (Fig. 1).

Table I. Incorporation of [1-^{14}C]Isopentenylpyrophosphate into avenacosid of 6-day old dark grown *Avena sativa* L. leaves irradiated with 3000 erg × cm^{-2} × s^{-1} (RG 630).

	Light exposure [min]				
	0	5	15	30	60
*p*mol [^{14}C]IPP/20 leaves incorporated into avenacosid	8.4 ± 4.4	10.2 ± 2.6	4.8 ± 1.0	5.2 ± 1.0	7.0 ± 3.2

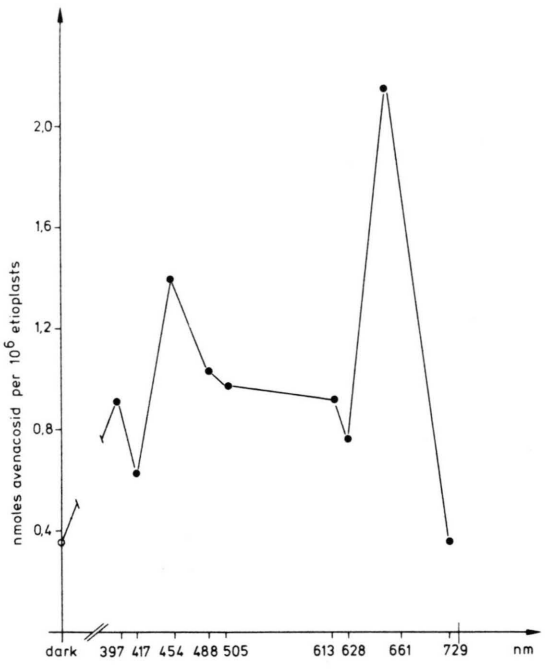

Fig. 3. Action spectrum: saponin content of PLB isolated of 6-day old etiolated *Avena* plants after 10 min low intensity illumination at different wave-length: light of equal quanta, approx. 1500 erg × cm^{-2} × s^{-1}.

As expected in general from phytochrome-related reactions [10], plants exposed to long-wave red (729 nm) neither show ALA synthesis [9] nor a change in PLB saponin (Fig. 4). If ALA is added or its production stimulated by simultaneous exposure to long- and short-wave red, the saponin content of the PLB fraction increases as described above for low light intensity treatment (Fig. 4). The amount of ALA available apparently is related to the PLB saponin content.

To find out whether ALA functions as regular itself, *i.e.* whether an artificial increase in ALA stimulates PLB-synthesis, pchlide synthesis was blocked by adding levulinic acid [11], the competitive inhibitor for the ALA-dehydratase. As a result, ALA accumulated without recrystallization of saponins at the PLB nor PLB *de novo* synthesis [12]. When plants were exposed to weak red (3000 erg × cm^{-2} × s^{-1}) after preincubation with LA, the usual increase in PLB saponins could not be detected. This indicates that ALA is not the regulator itself.

However, the light induced changes observed in saponin and chlorophyll synthesis could also be connected through pchlide, whereas the intermediate steps from ALA to pchlide are not light dependent.

While the pool of photoconvertible pchlide in etioplasts is small [10] and completely exhausted after 5 min of high intensity light exposure, pchlide increases in low intensity light (in 15 min from 15 µg/10^3 etioplasts to the tenfold) as photoconversion from pchlide to chlide is slowed down, apperently due to lack of energy. Accordingly, saponine and PLB disappear after strong light exposure, but PLB remain unaltered after weak light treatment.

The apparent hypothesis that the pchlide increase influences the PLB metabolism is further strengthened by results given by Klein *et al.* [13], showing that PLB recrystallization is combined with ALA-induced pchlide accumulation. Apparently, the pchlide increase alters the PLB so that increasing amounts of saponins can be bound. After 30 min (Fig. 1), PLB saponin content decreases as pchlide

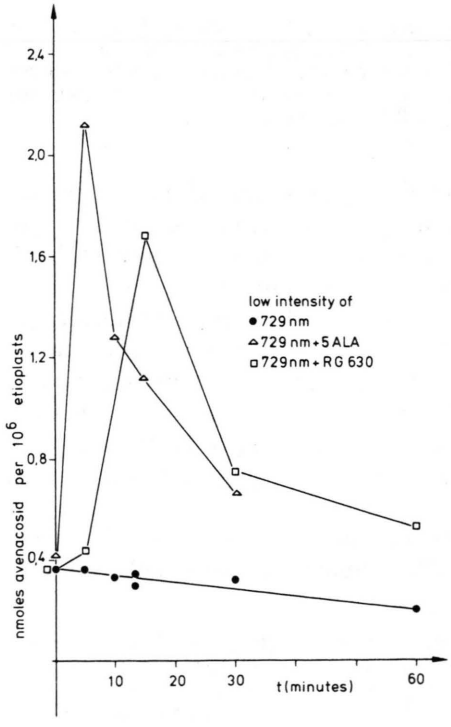

Fig. 4. Saponin content of PLB isolated of 6-day old etiolated *Avena* plants after illumination with 729 nm (3000 erg × cm^{-2} × s^{-1}), in some cases preincubated with ALA (see materials and methods) or irradiated at the same time with red light (RG 630, 3000 erg × cm^{-2} × s^{-1}).

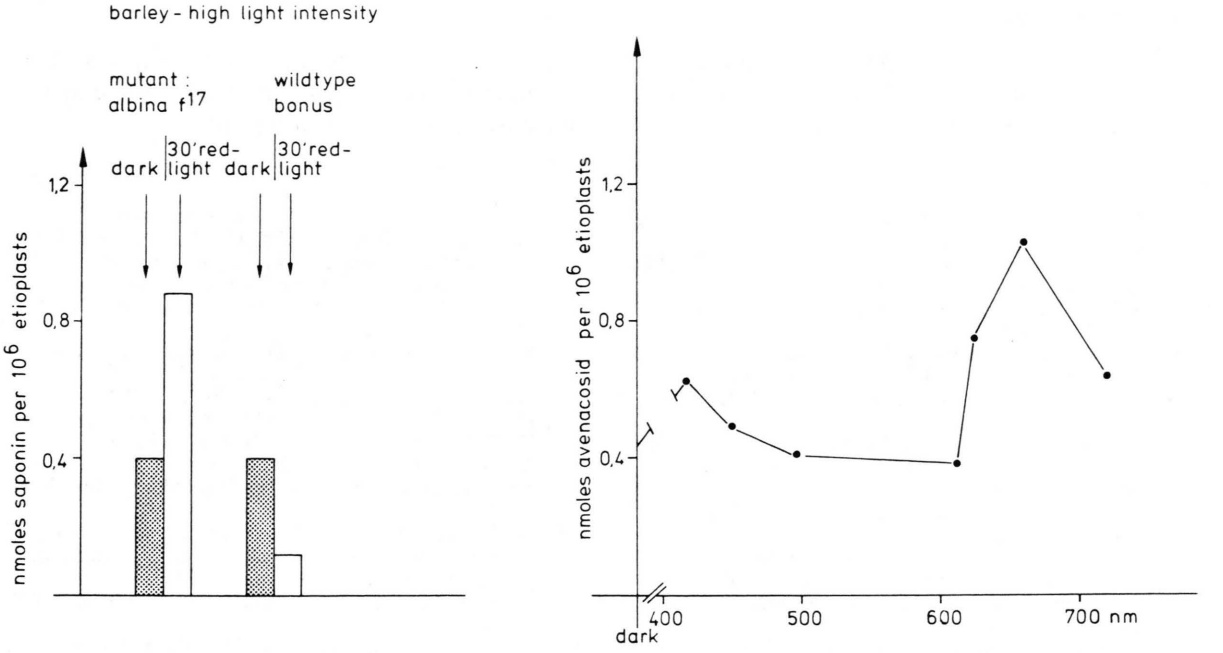

Fig. 5a. Saponin content of PLB isolated of 6-day old etiolated Barley (*Hordeum vulgare*: wildtype *bonus* or mutant *albina* f^{17}) dark grown and 30 mmn illuminated with red light (RG 630, 37000 erg × cm^{-2} × s^{-1}). b. action spectrum: saponine content of the PLB of 6-day old etiolated Barley-mutant *albina* f^{17} after 10 min illumination (9000 erg × cm^{-2} × s^{-1}) with light of equal quanta.

production is decreased by means of pchlide-ALA feedback, and the pchlide available slowly becomes photoconverted. A correlation between increased pchlide content, PLB synthesis, Shibata-shift, or chlide esterification and PLB degradation, respectively, is assumed by other authors as well [6, 14, 15].

To substantiate this hypothesis, barley mutants were used for further experiments:

– The wild-type *bonus* reacts exactly as *Avena* plants, as etioplast saponins and PLB are degraded in strong light (Fig. 5a).

– No saponin could be found in the mutant *xantha* h^{56}, the etioplasts of which do not have PLB nor pchlide [16].

– The mutant *albina* f^{17} does not show Shibata-shift from chlide, nor esterifies phytol, and is unable to degrade the PLB [17]. If this mutant is exposed to red light of high intensity, the saponins will not be degraded, but the same stimulation of the PLB saponins as in *Avena* plants exposed to weak light only is found (Fig. 5a). The action spectrum for PLB saponins of this barley mutant *albina* f^{17} (Fig. 5b) exposed to strong light is, with its maximum in the red, comparable to that of *Avena* exposed to weak light (Fig. 3).

Whether the molecular transformation of the freshly produced chlide from holochrome to other proteins as expressed in the Shibata-shift [18] is blocked in the mutant, or whether the chlide synthesis is reduced by weak light treatment of the wild-type, in both cases phytol cannot be esterified and the degradation of saponins or PLB, respectively, is prevented. These results are in accordance with statements by Boardman [19] that a minimum of esterified phytol is required to catalyse the degradation of the PLB saponins by providing a more hydrophobic surrounding.

In greening plants showing phytochrome-induced pchlide synthesis but not phytol esterification of chlide, and displaying a changed ATP-level [20] as well as a pH-shift [21] in the cytoplasm after light exposure, the PLB are modified enabling them to crystallize more saponin.

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