Short Communication

Light-induced De-epoxidation in Lettuce Chloroplasts

VI. DE-EPOXIDATION IN GRANA AND IN STROMA LAMELLAE

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ABSTRACT

Grana and stroma lamellae fractions prepared from illuminated chloroplasts (Lactuca sativa L. var. Manoa) by French press treatment contained less violaxanthin and more zeaxanthin than the corresponding fractions from dark controls. In both fractions, only part of the total violaxanthin was de-epoxidized under illumination, and the ratio of de-epoxidized and unchanged violaxanthin was similar. This not only shows that the de-epoxidation system is present in both grana and stroma thylakoids but also that violaxanthin is heterogeneous in both membranes. The presence and similarity of the de-epoxidation system in grana and stroma lamellae suggest that the function of the violaxanthin cycle is linked to photosynthetic activities which are common to both types of membranes.

Violaxanthin in thylakoid membranes is heterogeneous in its biochemical activity in that only two-thirds of the total pigment are available for de-epoxidation (11, 12). The reason for this heterogeneity is not clear. Recently, we suggested that the active two-thirds might be located near the internal membrane surface where they are available to the de-epoxidase, whereas the inactive one-third might be located near the external membrane surface where it remains unavailable for de-epoxidation (12). This hypothesis assumes that violaxanthin heterogeneity is uniform throughout the thylakoid membrane system. An alternative would be that active and inactive violaxanthin fractions are located in different membrane regions; in this case, de-epoxidation would be limited to membrane regions with specific biochemical activities. Since grana and stroma lamellae differ significantly in their photosynthetic activities, de-epoxidation in these lamellae was compared. It is shown that de-epoxidation takes place in grana as well as in stroma lamellae and that violaxanthin is heterogeneous in both membranes.

MATERIALS AND METHODS

Washed whole chloroplasts were isolated from Lactuca sativa L. var. Manoa as described previously (11). De-epoxidation was monitored spectrophotometrically (11, 14). Grana and stroma lamellae fractions were prepared from naked chloroplasts according to Sane et al. (10). Pellets sedimented at 1,000g, 10,000g, 40,000g, and 160,000g were quantitatively extracted with acetone, and the extracts were analyzed for pigment composition.

Total Chl was determined according to Arnon (1). Chlorophylls and carotenoids of the concentrated acetone extract were separated by reverse phase TLC according to Egger (3) using Kieselguhr G (Merck) layers impregnated with hydrogenated coconut oil. Carotene was determined in hexane, the Chl in acetone, and the xanthophylls in ethanol using the extinction coefficients of Hager and Meyer-Berthennath (6).

RESULTS AND DISCUSSION

French press treatment of lettuce chloroplasts yielded fractions with Chl content (59% and 6% of the total recovered Chl in the 10K and 160K fractions, respectively) and Chl a/b ratios (Table I) similar to those reported by Sane et al. (10) for spinach chloroplasts. The carotenoid distribution in the 10K and 160K fractions (Table I, columns 1 and 3) was also similar to that of grana and stroma lamellae of spinach chloroplasts (13).

Light- and dark-treated chloroplasts yielded fractions from equivalent membrane regions since Chl a/b ratio, carotene/Chl a ratio (Table I) and yield (data not shown) of corresponding fractions were identical. However, light treatment of the chloroplasts did decrease violaxanthin content while increasing zeaxanthin content in both grana and stroma lamellae. Thus it is evident that de-epoxidation took place in grana as well as in stroma lamellae.

Table I shows that violaxanthin in grana and in stroma lamellae was only partially de-epoxidized under illumination and that the ratio of active and inactive violaxanthin fraction was similar. Thus violaxanthin is heterogeneous in both membrane types. Based on our recent interpretation of violaxanthin heterogeneity (12), this result would indicate that violaxanthin in grana and stroma lamellae is similarly arranged, with the larger fraction near the internal and the smaller fraction near the external surface of the membrane.

Since carotenoids are not limited to the thylakoid membranes but have also been detected in the chloroplast envelope (2, 9), it should be pointed out that the lamellae fractions used in this study were prepared from envelope-free chloroplasts. Also, the presence of an inactive violaxanthin fraction in chloroplasts is not an artifact due to isolation since violaxanthin availability is rather insensitive towards isolation methods, chloroplast aging, or osmotic shock (data not shown); in fact, an inactive violaxanthin fraction has been observed in intact cells of various species (4, 15).

The fact that de-epoxidation took place in grana and stroma lamellae shows that the de-epoxidase was present in the loculi of...
Plant NaCl, 50 mM HEPES-NaOH buffer (pH 7), 16 mM sodium ascorbate, and whole chloroplasts equivalent to 20 µg Chl/ml, were kept in the dark or illuminated for 20 min with saturating red light. Chloroplasts were separated from their envelopes by osmotic shock in distilled H2O (10 min) followed by centrifugation, resuspended in 20 ml of 50 mM sodium phosphate buffer (pH 7.4) plus 100 mM NaCl and fractionated by French press treatment and differential centrifugation. The isolated fractions were analyzed by reverse phase TLC. (This method does not separate isomeric carotenoids like lutein and zeaxanthin.) The chloroplasts were checked for complete de-epoxidation spectrophotometrically by the 505 nm change.

<table>
<thead>
<tr>
<th>Pigments</th>
<th>10K Fraction (Grana Lamellae)</th>
<th>160K Fraction (Stroma Lamellae)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dark (1) Light (2) Dark (3) Light (4)</td>
<td></td>
</tr>
<tr>
<td><strong>Experiment I</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chl a/b</td>
<td>3.4 3.7 6.3 6.0</td>
<td></td>
</tr>
<tr>
<td>Carotene</td>
<td>10.3 10.5 12.4 13.3</td>
<td></td>
</tr>
<tr>
<td>Lutein + zeaxanthin</td>
<td>12.5 18.0 8.3 14.0</td>
<td></td>
</tr>
<tr>
<td>Violaxanthin</td>
<td>10.1 3.9 9.6 3.9</td>
<td></td>
</tr>
<tr>
<td>Violaxanthin decrease in light²</td>
<td>61.4% 59.4%</td>
<td></td>
</tr>
<tr>
<td><strong>Experiment II</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chl a/b</td>
<td>3.4 3.3 6.1 6.0</td>
<td></td>
</tr>
<tr>
<td>Carotene</td>
<td>9.6 10.1 12.7 12.2</td>
<td></td>
</tr>
<tr>
<td>Lutein + zeaxanthin</td>
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<td></td>
</tr>
<tr>
<td>Violaxanthin</td>
<td>9.0 3.2 8.6 3.5</td>
<td></td>
</tr>
<tr>
<td>Violaxanthin decrease in light²</td>
<td>64.4% 59.3%</td>
<td></td>
</tr>
</tbody>
</table>

¹ Moles pigment/100 moles Chl a.
² Per cent of dark control.

both thylakoid types. It further suggests that both loculi of grana and stroma thylakoids were acidified under illumination since the enzyme needs acidic pH for de-epoxidation (5). Hauska and Sane (7) reported proton pumping in stroma lamellae preparations with cyclic electron flow mediated by phenazine methosul fate. The present results indicate that stroma thylakoids in whole chloroplasts can be acidified even in the absence of artificial mediators. This could be a result of proton pumping driven by an endogenous cyclic electron transport system; electrons circulating in this system might be supplied via linear electron transport of grana lamellae. On the other hand, we cannot exclude the possibility that acidification of the stroma thylakoids was caused by proton diffusion from the grana through the uncom partimented loculus space.

According to Henriques and Park (8) grana and stroma lamellae have distinctive and independently working centers of biosynthesis. It is interesting that the complex de-epoxidation system — and consequently the whole xanthophyll cycle — is developed by both centers. Its presence in both types of thylakoids suggests that the presumed function of this cycle is linked to photosynthetic activities which are common to both types of membranes, as for example cyclic electron flow through photosystem I.

**LITERATURE CITED**