Inhibition of Photosystem II-Reactions in Blue-Green Algae by the Antisera to Lutein and Neoxanthin

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An antiserum to lutein agglutinates thylakoids of *Nostoc muscorum* and *Oscillatoria chalybea*. From this it follows that lutein is located in the outer surface of the thylakoid membrane of these blue-green algae. The same result is obtained for an antiserum to neoxanthin. As neoxanthin is supposed not to occur in blue-green algae it follows that in this case the antibody action should be directed towards a carotenoid with allenic structure. The antisera to lutein and neoxanthin inhibit in both investigated algal species photosynthetic electron transport on the oxygen-evolving side of photosystem II. Moreover, the inhibition sites of both antisera are identical in *Nostoc muscorum* and are located between the sites of electron donation of the artificial electron donors tetramethyl benzidene and diphenylcarbazide. In the case of the blue-green alga *Oscillatoria chalybea* the inhibition sites of both antisera differ. Whereas the inhibition site of the antiserum to neoxanthin lies again between the sites of electron donation of tetramethyl benzidine and diphenylcarbazide, the inhibition site of the antiserum to lutein appears to be situated at least partially beyond the site of electron donation of tetramethyl benzidine.

The degree of inhibition of electron transport reactions with *Nostoc muscorum* is for both antisera 50–60 per cent and is pH-dependent. The pH-optimum lies at pH 7.2 for the antiserum to neoxanthin and at 7.8 for the antiserum to lutein.

In comparison to this data the same antisera inhibit electron transport in chloroplasts from higher plants only by 20%. This low degree of inhibition in higher plants is apparently due to the fact that the surfaces of the thylakoids are not accessible to antibodies within the grana. In contrast to this the thylakoid surfaces of blue-green algae are fully accessible because the thylakoids are unstacked.

The thylakoids of *Oscillatoria chalybea* have the tendency towards aggregation. Therefore, the results concerning the accessibility of the carotenoids to antibodies are not so clear cut as with *Nostoc muscorum*.

In two previous publications we have reported on the effects of antisera to lutein and neoxanthin on photosynthetic electron transport in stroma-free swellable chloroplasts from tobacco [1, 2]. The inhibitory action of these antisera was directed towards the oxygen-evolving side of photosystem II which followed from the fact that Hill reactions were inhibited whereas the photosystem II-mediated photooxidations of diphenylcarbazide were unaffected by either antiserum [1, 2]. However, it appeared that the relative degree of inhibition inflicted upon electron transport by these antisera was generally only around 10–20%. This led us to the assumption that these carotenoids, involved somehow in the functioning of photosystem II, were located in the partition regions of the lamellar system of higher plants, that is in a location which was not accessible to antibodies [1–3]. As the lamellar system of these blue-green algae is reported to consist of single unstacked thylakoids [4] it appeared worthwhile to investigate the effect of the antisera to lutein and neoxanthin on the electron transport in blue-green algae.

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Abbreviations: DCMU, dichlorophenyl dimethyl urea; DCPIP, 2,6-dichlorophenol indophenol; TMB, tetramethyl benzidine; DPC, diphenylcarbazide; PMS, phenazine methosulfate; A-2-Sulf, anthraquinone-2-sulfonate.

Material and Methods

Algal material: Nostoc muscorum (B 1453/12 a) and *Oscillatoria chalybea* (B 1459/2) were obtained from the algal collection of the Pflanzenphysiologisches Institut Göttingen.

The culture medium is described as medium D by Kratz and Myers [5].

Nostoc muscorum was cultivated at 30±1 °C as liquid culture in glass tubes which were gassed with air supplemented with 2% CO_2. The CO_2 supply was stopped 14 h before harvesting the cells. The tubes were constantly illuminated with 110 lx

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white light provided by Sylvania Daylight tubes (F15TR-D).

Oscillatoria chalybea was grown on clay plates which were just immersed in the above culture medium in large petri dishes. The algal cultures in the petri dishes were illuminated in a 14 h light/10 h dark cycle with Sylvania Daylight tubes providing an averaged light intensity of 4484 erg·sec^{-1} cm^{-2}.

Preparation of the thylakoids of Nostoc muscorum and Oscillatoria chalybea

The cells were broken by passing them twice through a 'French Pressure Cell' (Matra David, Typ 4601 551) at a pressure of 4 – 5 tons/cm^2. The obtained sap was diluted to 100 ml with buffer containing the following components: 0.05 M Tris, 0.01 M NaCl, 0.4 M sucrose, 0.2% pectinase, 0.2% serum albumin. The pH of the suspension buffer was 7.8. The suspension was spun during 1 min at 3000 rpm at 4 °C in order to remove large cell fragments. The supernatant was subsequently spun during 1 hour at 10000 × *g*. The sediment was suspended in 10 ml suspension buffer as above and its chlorophyll content was determined. This preparation was directly used for the investigations. The preparation contained besides relative small amounts of cell fragments the thylakoids which, however, showed a tendency towards aggregation. Such preparations showed all types of partial reactions of photosynthetic electron transport. However, it appeared that especially the water splitting reaction had suffered by the French Press treatment. A further purification of the preparation resulted in a considerable and continuous loss of all electron transport activities.

Electron transport reactions and photophosphorylation reactions were carried out with the same assays described in our previous publication [6].

Results

1. Effect of the antiserum to neoxanthin on photosynthetic electron transport in thylakoids of Nostoc muscorum

The antiserum agglutinates the thylakoids of *Nostoc muscorum*. Consequently, antigenic determinants towards which the antiserum is directed are located in the outer surface of the thylakoid membrane.

The occurrence of neoxanthin is not demonstrated for blue-green algae. This allenic carotenoid, however, is one of the main carotenoids in the lamellar system of higher plant chloroplasts [7, 8]. In blue-green algae Stranski and Hager have demonstrated the presence of other xantophylls with allenic structure, namely that of caloxanthin (3,3-dihydroxy-5-hydro-7-dehydro-β-carotene) and of nostoxanthin (3,3-dihydro-5,5-dihydro-7,7-didehydro-β-carotene) [9, 10]. The comparison of the structural formula of the neoxanthin with that of nostoxanthin and caloxanthin shows that the antibody action of the antiserum to neoxanthin can only be directed towards the cumulated double bond that is towards the allenic structure. This holds under the condition that neoxanthin does not occur in blue-green algae.

The antiserum to neoxanthin inhibits photosynthetic electron transport in *Nostoc muscorum* in the region of light reaction II as described by Radunz and Schmid for higher plants [2]. The optimum of the inhibitory action on the Hill reaction with 2,6-dichlorophenol indophenol as the electron acceptor lies at pH 7.4. At this pH the maximal degree of inhibition is 60 per cent (Fig. 1). This high degree of inhibition shows that the carotenoid which reacts with the antiserum is fully or at least to a large extent accessible to antibodies. The accessibility of the carotenoid in the thylakoids of *Nostoc*

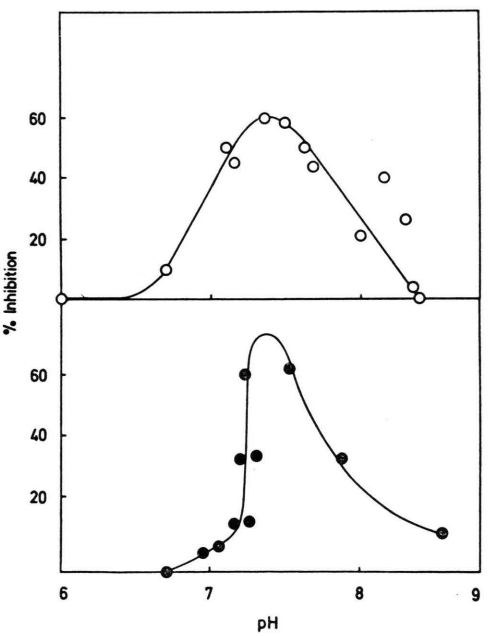

Fig. 1. pH-Dependence of the degree of inhibition of photosynthetic electron transport in the lamellar system of *Nostoc muscorum* by the antiserum to neoxanthin. (○) DCPiP-Hill reaction; (●) reaction in the system tetramethyl benzidine/ascorbate→ anthraquinone-2-sulfonate.

muscorum is several fold higher than in the lamellar system of higher plant chloroplasts [2] and could be due to the fact that the thylakoids of blue-green algae are distributed in an unstacked state in the cytoplasm. In turn, it means that neoxanthin, which somehow plays a role in the functioning of photosystem II in higher plants is preponderantly located in the partitions.

An electron transport reaction between tetramethylbenzidine/ascorbate as the electron donor and anthraquinone-2-sulfonate as the electron acceptor is also inhibited by the antiserum (Fig. 1).

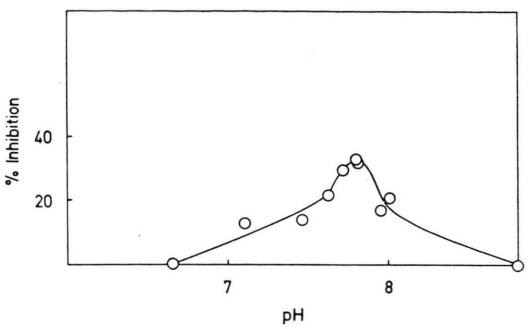

Fig. 2. pH-Dependence of the degree of inhibition of photo synthetic electron transport in the lamellar system of *Nostoc muscorum* by the antiserum to lutein. (○) DCPiP-Hill-reaction.

According to Harth, Oettmeier and Trebst tetramethylbenzidine feeds in its electrons on the oxygen-evolving side of photosystem II [11]. The donor system tetramethylbenzidine/ascorbate substitutes in intact chloroplasts for water as the electron donor and suppresses oxygen evolution [12]. The maximal relative degree of inhibition is just as for the DCPiP-Hill reaction 60% and the pH-optimum at pH 7.4 (Fig. 1). As the electron transport distances of both reactions differ it follows from the identical degree of inhibition for both reactions, that only one inhibition site is touched by the antiserum to neoxanthin.

The dependence of the inhibition of the amount of added antiserum is depicted in Fig. 3. The curve

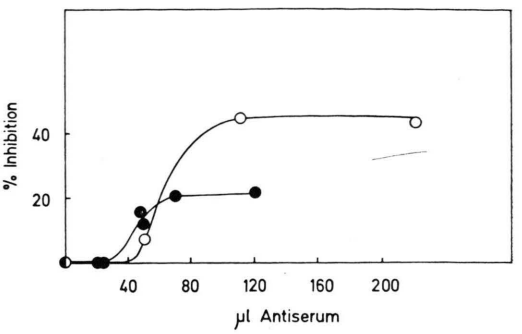

Fig. 3. Dependence of the degree of inhibition of the DCPiP-Hill reaction on the amount of added antiserum in *Nostoc muscorum*. (○) Antiserum to neoxanthin; (●) antiserum to lutein.

was established at pH 7.15 which is not exactly the optimum and shows a sigmoidal shape which hints at a cooperative effect of the antibody action. At the optimal pH 7.4 and with saturating amounts of antiserum we attempted to localize the inhibition site in the electron transport scheme. Table I shows the influence of the antiserum to neoxanthin on different electron transport reactions. It is seen that the electron transport reactions between water and anthraquinone-2-sulfonate, between tetramethylbenzidine and anthraquinone-2-sulfonate and between water and 2,6-dichlorophenol indophenol are affected to the same extent. This means that all 3 electron transport pieces are hit by the antiserum. From Table I it is also seen that photooxidations of diphenylcarbazide are not affected or affected to a lesser extent by the antiserum. It follows that the

Table I. Influence of the antiserum to neoxanthin and lutein on photosynthetic electron transport reactions of the lamellar system of *Nostoc muscorum*.

Electron transport system	Antiserum to neoxanthin		Antiserum to lutein	
	Control rate µmol acceptor reduced/mg chlorophyl/h	% Inhibition	Control rate µmol acceptor reduced/mg chlorophyl/h	% Inhibition
$H_2O \rightarrow$ A-2-Sulf	12	60	16	50
TMB/asc → A-2-Sulf	30	65	40	40
DPC → A-2-Sulf	50	0	40	0
$H_2O \rightarrow$ DCPiP	35	60	6	35
DPC → DCPiP	20	10	30	10
DCPiP/asc → A-2-Sulf	190	0	254	0

inhibition site is located on the oxygen-evolving side of photosystem II and located in the electron transport scheme formally between the site of electron donation of tetramethylbenzidine and diphenylcarbazide. In agreement with these observations typical photosystem I reactions such as the photoreduction of anthraquinone-2-sulfonate with DCPiP/ascorbate as the electron donor in the presence of DCMU are not affected by the antiserum (Table I).

2. *Effect of the antiserum to lutein on photosynthetic electron transport in thylakoids of Nostoc muscorum*

The antiserum to lutein agglutinates the lamellar system of *Nostoc muscorum*. Consequently, just as in the case of the antiserum to neoxanthin, antigenic determinants are situated in an accessible location in the outer surface of the thylakoid membrane. According to the literature the xantophyll lutein is present in the thylakoid membrane of blue-green algae [7].

The antiserum inhibits the DCPiP-Hill reaction (Fig. 2). The optimum of the inhibition of electron transport by the antiserum lies at pH 7.8 – 7.9 which is distinctly different from the antiserum to neoxanthin. Also, the maximally observed inhibition with 40 per cent is much lower than in the case of the antiserum to neoxanthin. The dependence of the antiserum action on the amount of antiserum used yields again a sigmoidal curve (Fig. 3). In contrast to higher plants the inhibition sites of the antiserum to neoxanthin and lutein cannot be distinguished from each other (Table I). The inhibition site of the antiserum to lutein is located in the electron transport scheme with *Nostoc muscorum* at the same site as that of the antiserum to neoxanthin.

3. *Effect of the antiserum to neoxanthin on photosynthetic electron transport in Oscillatoria chalybea*

Thylakoids of *Oscillatoria chalybea* have been prepared as described for Nostoc but gave 20 – 30 per cent better electron transport rates than the *Nostoc* preparations. However, with the *Oscillatoria* preparations we observed under the light microscope that the isolated thylakoids tended to aggregate in the investigated pH-region, forming equally sized clumps. This property pointed at the possibility that the accessibility of antigenic determinants to antibodies in the thylakoid membrane would be reduced.

The antiserum to neoxanthin agglutinates the *Oscillatoria* preparations which again means that antigenic determinants are located in the outer surface of the thylakoid membrane. Inhibition of the DCPiP-Hill reaction and of electron transport in the system tetramethyl benzidine/ascorbate → anthraquinone-2-sulfonate are generally lower than described for *Nostoc muscorum* which might be explained by the above mentioned aggregation of the lamellar system. The pH-optimum for the inhibition lies for both reactions with *Oscillatoria* at pH 8.2 (Fig. 4) which is almost one pH-unit more alcaline

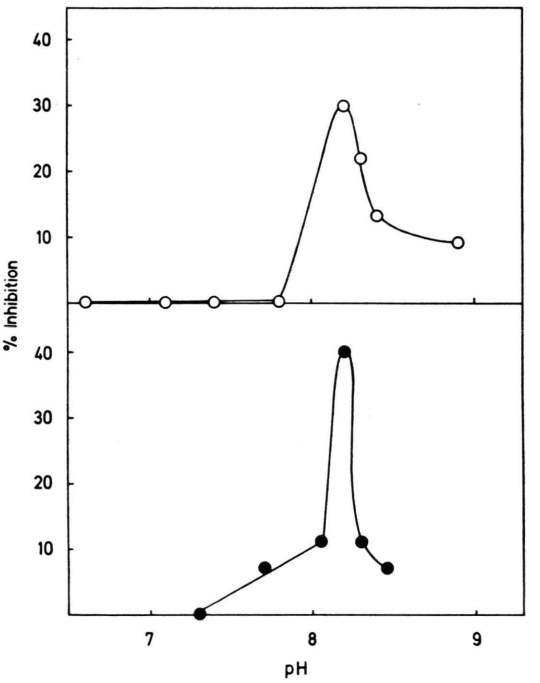

Fig. 4. pH-Dependence of the degree of inhibition of photosynthetic electron transport in the lamellar system of *Oscillatoria chalybea* by the antiserum to neoxanthin. (○) DCPiP-Hill reaction; (●) reaction in the system tetramethyl benzidine/ascorbate → anthraquinone-2-sulfonate.

than with *Nostoc muscorum*. As the pH-optimum of the inhibition is the same for both electron transport reactions shown in Fig. 4 and as the relative degree of inhibition at the pH-optimum is also the same for both reactions it may be assumed that only one site in the electron transport scheme is affected

Table II. Influence of the antiserum to neoxanthin and lutein on photosynthetic electron transport reactions of the lamellar system of *Oscillatoria chalybae*.

Electron transport system	Antiserum to neoxanthin		Antiserum to lutein	
	Control rate µmol acceptor reduced/mg chlorophyl/h	% Inhibition	Control rate µmol acceptor reduced/mg chlorophyl/h	% Inhibition
$H_2O \rightarrow$A-2-Sulf	20	30	40	48
TMB/asc → A-2-Sulf	70	40	60	20
DPC → A-2-Sulf	60	0	80	0
$H_2O \rightarrow$ DCPiP	15	25	20	37
DPC → DCPiP	25	10	20	13
DCPiP/asc → A-2-Sulf	240	0	200	0

by the antiserum. Not only the pH-optimum is different in comparison to *Nostoc* but also the curve shape of the dependency of the inhibitory action on the amount of antiserum added. Whereas the curve shape is sigmoidal with *Nostoc* (Fig. 3) it is hyperbolic with *Oscillatoria* (Fig. 6). At the optimal pH 8.2 and an optimal antiserum concentrations we determined the inhibition site in the electron transport scheme (Table II). The Table clearly shows as described for *Nostoc* and earlier for higher plants [2] that the inhibitory action of the antiserum to neoxanthin lies between the sites of electron donation of the artificial electron donors tetramethyl benzidine and diphenylcarbazide on the oxygen-evolving side of photosystem II.

4. *Effect of the antiserum to lutein on photosynthetic electron transport in Oscillatoria chalybea*

The antiserum to lutein also agglutinates the lamellar system of *Oscillatoria chalybea*. The antiserum to lutein inhibits electron transport reactions in the system $H_2O \rightarrow$ DCPiP and tetramethyl benzidine/ascorbate → anthraquinone-2-sulfonate. The pH-optimum of the inhibitory actions is at pH 7.1 (Fig. 5), a clear difference to the antiserum to neoxanthin (Fig. 4). The maximal inhibition at pH 7.1 is for the Hill reaction 40 per cent (Fig. 5).

The localization of the inhibition site in the electron transport scheme is seen in Table II. It appears that the inhibitory action of the antiserum is lower for the system tetramethyl benzidine/ascorbate → anthraquinone-2-sulfonate than for the Hill reaction (Table II) (see also Fig. 5). From this data it follows that in *Oscillatoria* the inhibition site of the antiserum to lutein is different from that of the antiserum to neoxanthin. The situation in *Oscillatoria* is similar to that in tobacco chloroplasts described earlier by Radunz and Schmid [1].

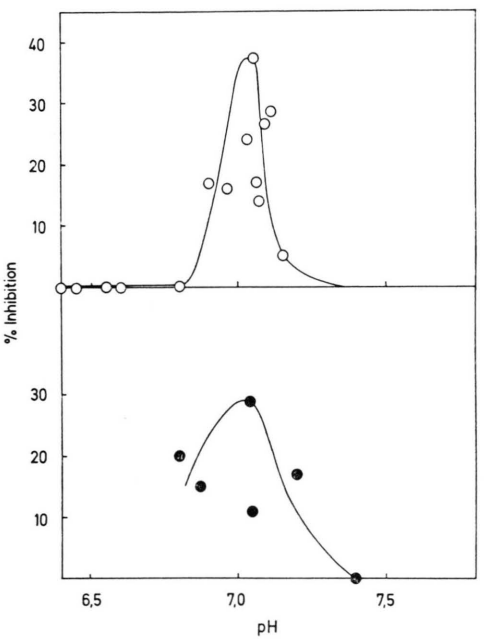

Fig. 5. pH-Dependence of the degree of inhibition of photosynthetic electron transport in the lamellar system of *Oscillatoria chalybea* by the antiserum to lutein. (○ DCPiP-Hill reaction; (●) reaction in the system tetramethyl benidine/ascorbate → anthraquinone-2-sulfonate.

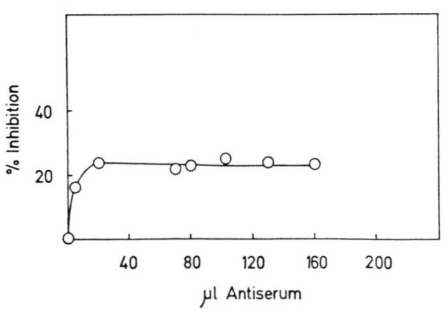

Fig. 6. Dependence of the degree of inhibition of the DCPiP-Hill reaction on the amount of added antiserum to neoxanthin in *Oscillatoria chalybea*.

Table III. Effect of the antiserum to neoxanthin and lutein on cyclic and non-cyclic photophosphorylation in lamellar system preparations of *Nostoc muscorum*.

Electron transport system	Antiserum to neoxanthin	Antiserum to lutein
	[μmol [^{32}P]ATP esterified/mg chlorophyll/h]	
PMS	141	
PMS + antiserum	168	153
PMS + control serum	159	149
$H_2O \rightarrow$ Anthraquinone-2-sulfonate (A-2-Sulf)	1.16	
$H_2O \rightarrow$ A-2-Sulf + antiserum	4.3	2.9
$H_2O \rightarrow$ A-2-Sulf-control serum	4.0	3.5
$H_2O \rightarrow$ A-2-Sulf + 5×10^{-7} M DCMU	0.36	

5. Influence of the antiserum to lutein and neoxanthin on photophosphorylation reactions in Nostoc muscorum

Photophosphorylation reactions of the cyclic and non-cyclic type showed under our conditions low rates in comparison to chloroplasts from higher plants (Table III). It looks as if the French press treatment uncouples photophosphorylation to a large extent. No effect of either antiserum on these photophosphorylation rates was observed, which could mean that the antibody action is directed towards the so called basal electron transport which is not coupled to photophosphorylation [13]. This would also explain why the maximal degree of inhibition of electron transport does not exceed 50 – 60 per cent [13]. However, in this case the interpretation is certainly limited by the low rates of photophosphorylation.

Discusion

The presented data shows, that lutein and at least one carotenoid with an allenic structure are located in the outer surface of the thylakoid membrane of the blue-green algae *Nostoc muscorum* and *Oscillatoria chalybea*. As reported earlier for higher plants [1, 2] both types of carotenoids are somehow involved in the functioning of photosystem II. As already stated for tobacco chloroplasts we feel that the inhibitory action on electron transport induced by the antibody binding might either be caused by a conformational change of a protein to which the respective carotenoid is attached or by a change of the molecular structure of the thylakoid membrane [2]. However, regardless of the inhibitory mechanism, for both antisera and both blue-green algae the localization of the inhibitory site in the electron transport scheme comes out to be on the oxygen-evolving side of photosystem II.

It should be noted that the inhibitory action of both antisera occurs in very narrow pH-ranges. These narrow pH-ranges show on the one hand how easily the antiserum effect can be overlooked and on the other hand that apparently a very defined condition or state must be realized in the thylakoid membrane which only then can lead to the inhibition by the antibodies.

The degree of inhibition of electron transport caused by the antisera to neoxanthin and lutein lies between 50 – 60% with thylakoid preparations of *Nostoc muscorum* (Table I). In higher plants the maximal degree of inhibition is only 15 – 20% [1, 2]. From this it follows, that both antigenes are better accessible to antibodies in the thylakoids of *Nostoc muscorum*, then they are in stroma-free swellable chloroplasts of higher plants. As the lamellar system of higher plants is differentiated into grana- and intergrana regions, this low accessibility with higher plant chloroplasts is due to this differentiation. This would be additional evidence for what has been suggested earlier by Menke and Schmid *et al.* [3, 14] namely that antibodies do not penetrate between the stacked thylakoids of the grana regions of the lamellar system. It follows furthermore, that although lutein and neoxanthin are located in the lamellar system of higher plants in the outer surface of the thylakoid membrane, the part of these carotenoids which is somehow involved in the photosystem II-activity is nevertheless inaccessible to antibodies because these carotenoids are preponderantly located in the partitions of the grana region. This would fit the observation by Trosper and Allen who concluded from carotenoid analyses in light and heavy particle fractions of spinach chloroplasts that lutein was

mainly located in the partition region [15]. It would also mean that in higher plants photosystem II happens to be preponderantly located in the grana regions of the lamellar system.

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