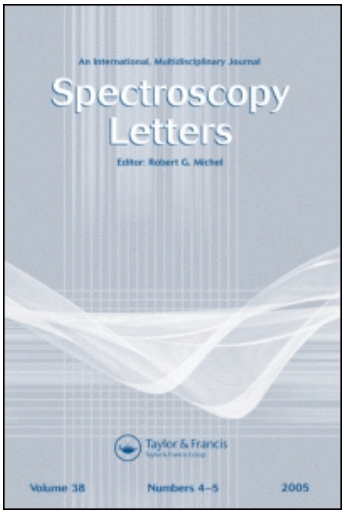


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# Linear Dichroism of Chloroplasts and Subchloroplast Fractions Oriented by Flow

F. Tjerneld[a]; B. Norden[a]; H. E. Akerlund[a]; B. Andersson[a]; P. -A. Albertsson[a]
[a] Departments of Biochemistry 1 and Inorganic Chemistry 1, University of Lund, Chemical Center, Lund, Sweden

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# LINEAR DICHROISM OF CHLOROPLASTS AND SUBCHLOROPLAST FRACTIONS ORIENTED BY FLOW

F. Tjerneld, B. Nordén*, H.E. Åkerlund, B. Andersson and P.-Å. Albertsson.

Departments of Biochemistry 1 and Inorganic Chemistry 1 (*), University of Lund, Chemical Center, P.B. 740, S-220 07 Lund, Sweden.



Chloroplasts as well as subchloroplast vesicles show linear dichroism (LD) when subjected to a hydrodynamic gradient. LD is positive for the $\pi\pi^*$ in-plane polarized bands indicating that the chlorophyll porphyrin ring is in average oriented parallell to the elongated membrane surfaces. The vesicle fraction enriched in photosystem II shows lower LD/A at 660 nm indicating lower average orientation of chlorophyll b and the fraction enriched in photosystem I shows higher LD/A at 700 nm indicating that P700 is more oriented than the bulk chlorophyll.

The thylakoid membrane system in chloroplasts contains the components responsible for the capture and transformation of light energy into chemical energy. The central light-absorbing pigments of photosynthesis in higher plants are chlorophyll a and b. These pigments are arranged in two types of photosystems (I and II). Each photosystem is thought to be made up of one or two "reaction center" chlorophyll molecules called P680 and P700, and surrounded by a large number of "antenna" chlorophylls and some accessory pigments transfering exitation energy to the reaction centers. Very litle is known about the orientation of the various chlorophyll molecules in the complex thylakoid membrane system.

By disintegration of the chloroplast lamellae followed by partition in an aqueous dextran-poly(ethylene)glycol two-

phase system, fractions containing mainly photosystem I or photosystem II can be obtained. [1] These subchloroplast fractions which still are membrane vesicles provide an interesting material for LD studies on the chlorophyll orientation of the two different photosystems.

The subchloroplast vesicles as well as the unbroken chloroplast lamellae (class II chloroplasts) are of elongated shape and can be expected to be oriented in a hydrodynamic gradient. FIG 1 shows the LD spectrum of class II chloroplasts studied in a Couette cell with a method described earlier, [2] ($Ld = A_{||} - A_{\perp}$, || = flow direction, A = random absorption, LD baseline is taken at zero gradient). LD shows that the chlorophylls are not randomly oriented in the elongated chloroplast. Dips in LD/A (arrows) show the presence of negative LD contributions. The spectrum resembles that of unfractionated broken chloroplast material obtained by the method of spreading and magnetic field aligning. [3] Both positive and negative components can in a first approach be assigned to $\pi \rightarrow \pi^*$ transitions in the chlorophyll plane. The positive band at 680 nm is generally assigned to the $Q_y$ transition in chlorophyll a, which indicates the preferential orientation of one of the in-plane symmetry axes (y) parallell to the long-axis of the thylakoid membranes. The negative band

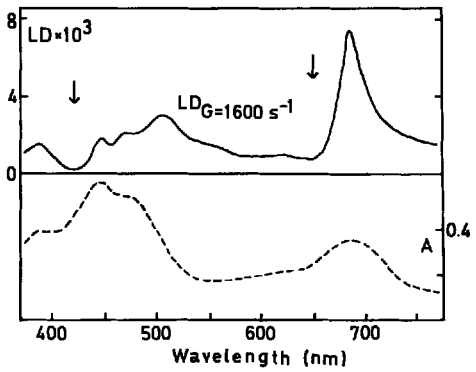


FIG 1.
LD and A of class II chloroplasts (d = 0.10 cm).

at 650 nm may be explained by the $Q_x$ transition if chlorophyll is oriented with its x-axis perpendicular and its y-axis parallell to the membrane plane. Another possibility is unequivalent chlorophyll a molecules (possibly by different environments) at different orientation. The negative 420 nm LD component in the Soret region may also be accounted for by an x-polarized band. The possibility of a z-polarized $n \rightarrow \pi^*$ transition has, however, recently been proposed in the case of tetraphenylporphin in certain environments. [4] This should also give negative LD at 420 nm. The positive 500 nm LD peak arises from the presence of carotenoid molecules which are preferentially aligned parallell to the membrane long axis.

FIG 2 shows LD and absorption spectra of subchloroplast particles enriched in photosystem II. LD is positive for chlorophyll a (680 nm) in agreement with a mainly parallell orientation, while the peak characteristic of chlorophyll b (660 nm) corresponds to zero or negative LD (as visualized from LD/A). Chlorophyll b is mainly present in photosystem II and we conclude it to be either more randomly oriented or oriented in a different fashion within these particles than are the chlorophyll a molecules absorbing at 680 nm. Also in this case a negative contribution (though less strong) is present at 420 nm.



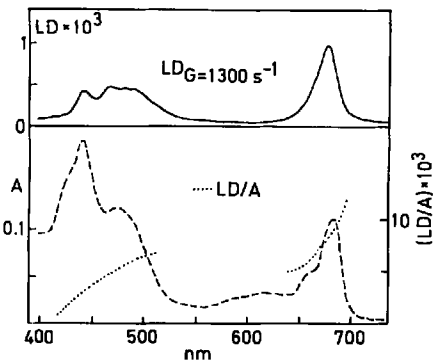


FIG 2.
PS II enriched subchloroplast vesicles.

FIG 3 corresponds to particles enriched in photosystem I. LD spectrum has the same characteristics as with PS II. The increased LD/A at 700 nm may be associated with the PS I reaction center chlorophyll (P700), indicating a higher degree of orientation parallell to the elongated membrane plane of these pigments.

In FIG 4 LD has been plotted versus the gradient for the various examined suspension types. A very strange hydrodynamic behaviour is observed in the case of class II chloroplasts, with a sign reversal of the entire LD spectrum at a characteristic

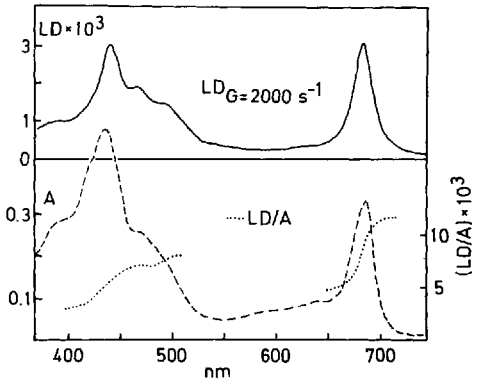


FIG 3
PS I enriched subchloroplast vesicles.

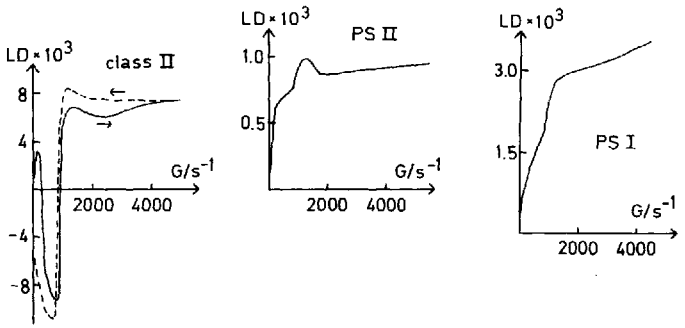


FIG 4.
LD versus G for the various systems.

gradient. This gradient position is also observed for a diluted solution. This does not exclude, however, that the phenomenon is due to ruptures of aggregates (which are differently oriented) at higher gradients. Alternatively various membrane fractions with different hydrodynamic propeties compete in orientation. This anamalous phenomenon is not observed for the subchloroplast vesicles. However, characteristic Peterlin-Stuart LD growth curves [2] are not either observed, possibly due to remaining polydispersity.

Although this investigation is at a preliminary stage it shows how chloroplasts and subchloroplast membrane particles can be oriented and studied in a hydrodynamic field. The chlorophyll orientation will be further studied especially in subchloroplast fractions enriched in photosystem I and II respectively.



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