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Lactucaxanthin Accumulation and Stacking in the Thylakoid Membrane of a Chlorella Mutant Strain

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Lactucaxanthin, instead of violaxanthin which is an essential carotenoid for violaxanthin cycle, and zeaxanthin were identified in the extract from a mutant strain of green algae, Chlorella ellipsoidia, by proton NMR spectroscopy. The positive exiton chirality was observed by the CD measurements, suggesting that the stacking with these xanthophylls was of the right handed helical mode in the chlorophyll-deficient thylakoid membrane.

Recent investigations on the thylakoid membrane structure revealed that the xanthophylls were essential for assembly of protein-pigment complexes in thylakoid membrane,[1,2] energy transfer in light-harvesting complexes[2] and protection of the membrane from oxygen radicals generated by the photosynthetic activity.[3] These xanthophylls, comprising zeaxanthin and violaxanthin in the thylakoid membrane of green algae, were thought to be non-covalently associated with chlorophyll-protein complexes or each other. However, it is difficult to analyze these interactions between xanthophylls and other components of thylakoid membrane because of the optical interference of chlorophylls which are the main pigments of thylakoid membrane from wild algae or plants. We prepared a chlorophyll-deficient and xanthophyll-rich thylakoid membrane from a mutant strain of Chlorella ellipsoidia and characterized the supramolecular conformation of xanthophylls in the lipid bilayer of this naturally modified thylakoid membrane which appeared to contain lactucaxanthin, a carotenoid closely related to the structural formula of zeaxanthin and violaxanthin.

The pigment, which was accumulated in the cells of this mutant strain of Chlorella ellipsoidia, was extracted with hot aqueous acetone and separated on 2 mm-thick silica gel plates (Merck).[4] The main yellow band on the plates was extracted and used for 400 MHz proton-NMR (JEOL product) measurement. The mutant thylakoid membrane corresponding to the LHC (light-harvesting chlorophyll a/b protein complex) membrane of wild type Chlorella cells was prepared by Sepharose CL-4B column chromatography of thylakoid membrane which was collected from the yellow band in stepwise sucrose density gradient centrifugation of membrane fragments of Chlorella cells disrupted by French Pressure Cell.[5] The membrane vesicle which contained most yellow substances from the cell preparation was eluted at dead volume. The fraction containing the membrane vesicle was then centrifuged and the precipitate was suspended in 50 mM Tris-borate buffer, pH 9.5, and used for CD measurement (JASCO product, Type J-500A).

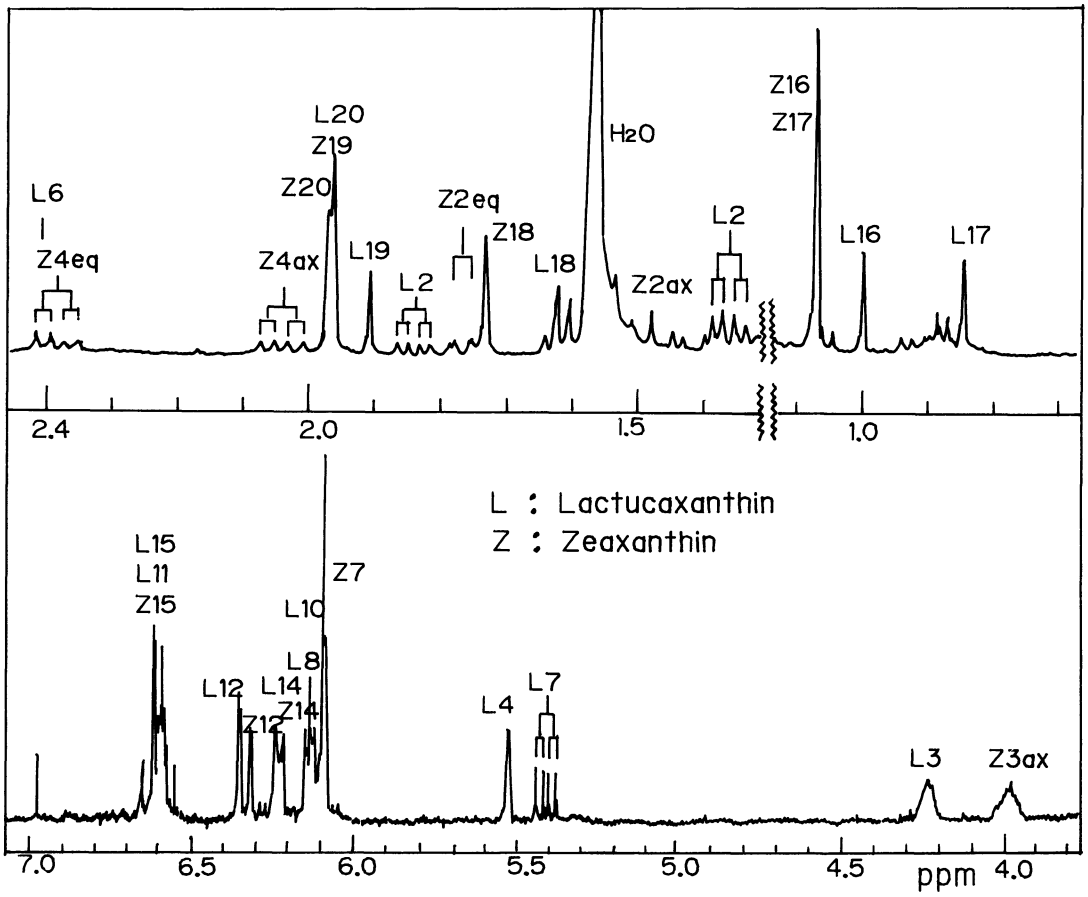

Fig. 1. 400 MHz Proton-NMR spectrum of xanthophylls purified from Chlorella mutant. ca. 1 mg xanthophyll in 1 ml $CDCl_3$ was used for the measurement.

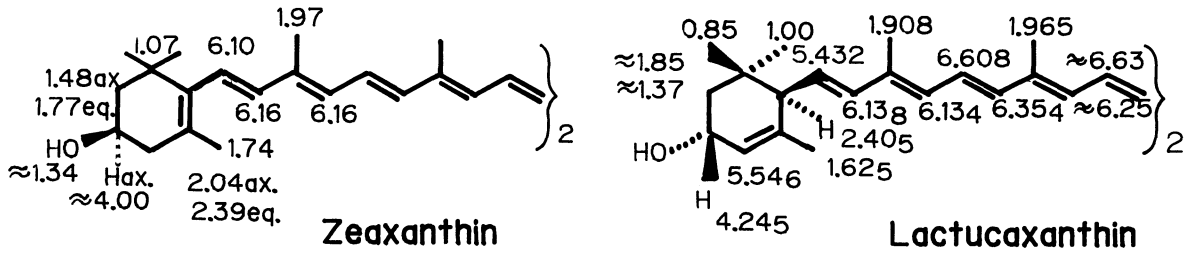

Fig. 2. Assignment of the proton NMR signals. The data were referenced from G. Englert.[6]

As shown in Fig. 1 and Fig. 2, the proton NMR spectrum obtained from the xanthophyll preparation can be fully elucidated by the signals of two xanthophylls, zeaxanthin and lactucaxanthin. The ratio of the two xanthophyll contents was estimated to be about 1:1 by the integral data of several signals. The circular dichrogram of purified membrane of etiolated thylakoid was shown in Fig. 3. The strong split type dichroism was obtained at the first peak (386 nm) of xanthophyll absorption spectra, where the positive and negative dichroisms were observed at 397 nm and 378 nm, respectively. The main absorption peaks at 410 nm to 480 nm were optically little active. No dichroism was observed with the acetone or chloroform extract of these xanthophylls.

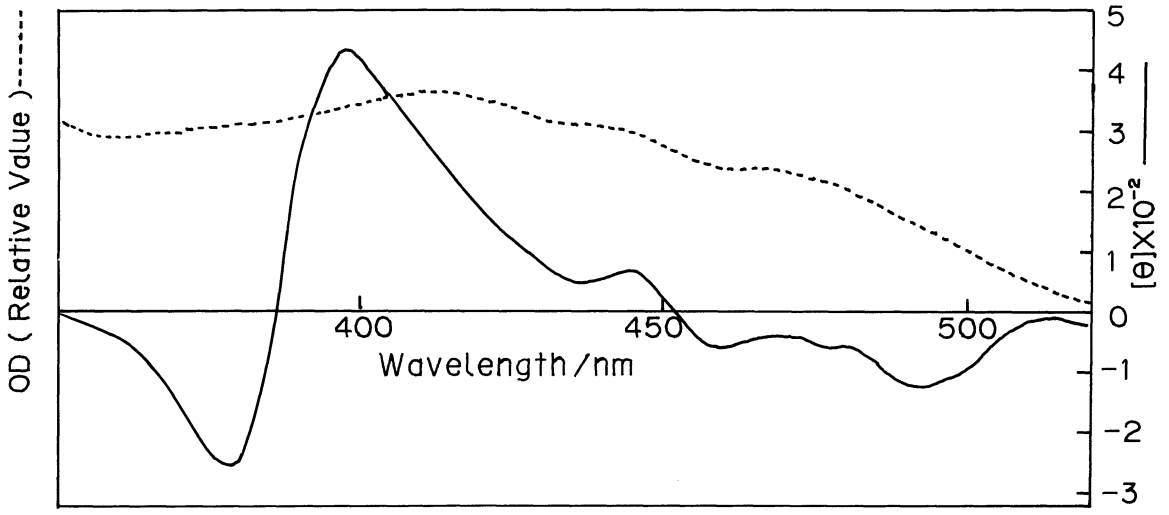

Fig. 3. Absorption and CD spectra of the thylakoid membrane vesicle from Chlorella mutant. Dichroic intensity based on the total xanthophyll concentration is smaller than that of lutein aggregate,[8] likely depending on the partial contribution of the random state. Little absorption and dichroism were observed at the wave length corresponding to those of the chlorophylls.

While zeaxanthin, violaxanthin and antheraxanthin are essential xanthophylls in violaxanthin cycle of wild type cells,[7] it was established that zeaxanthin cannot afford to produce violaxanthin in this mutant strain of Chlorella cells. As some flowers of higher plants (Lactuca sp.) are abundant with lactucaxanthin and the synthesis of this xanthophyll was stimulated by the irradiation of light, the enzymatic pathway for lactucaxanthin synthesis may be regulated by light. It was demonstrated that the lactucaxanthin, as well as zeaxanthin, in the etiolated thylakoid membrane, which corresponds to LHC membrane of wild type Chlorella cells, specifically interacted with the molecules in the membrane. Rather intense absolute dichroisms at 397 nm and 378 nm indicate specific aggregation of the xanthophylls in the membrane. Recently, Takagi et al.[8] observed the intense negative exiton chirality of CD spectra with lutein-containing synthetic liposomes, in which a left-handed helical structure of lutein aggregate was identified by electron microscopy. Therefore, the local aggregate corresponding to right-handed helical structure of lactucaxanthin and zeaxanthin in this membrane can be suggested on the basis of moderate intensity of the opposite CD spectrum observed in this experiment.[9]

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