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Electron Microscopic Observation of Lutein Aggregates[a)]

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Summary

Lutein, one of major carotenoids in green leaves, disperses in aqueous solutions to form chiral helical aggregates which acquire an optical activity in visible region. We confirmed that this aggregate had enormous size with ultrafiltration and sedimentation equilibrium analyses. In this paper, lutein aggregates dispersed in dilute surfactant solution, SDS or DTAB, are observed with electron microscope to estimate the size and the shape of the aggregate. Lutein aggregate showed images of rod-like shape, and their sizes are from 0.3 to 1.5 μm in length and from 0.05 to 0.2 μm in width. Possible conformation of the aggregate with helical structure was illustrated, and a working hypothesis for physiological function of lutein in thylakoid was postulated.

Introduction

We have reported that lutein, one of the major carotenoids of green leaves, forms an aggregate which is optically active in the visible wavelength where monomolecularly dissolved lutein is optically inactive[1−4]. The aggregate was formed when lutein dissolved in an organic solvent was diluted with an aqueous solvent in which lutein is sparingly soluble. Its size was large enough to make it difficult to be filtered through an ultrafilter of 0.45 μm pore[5]. The present study was carried out to make it clear whether the aggregate can be observed by the electron microscopy.

Materials and Methods

Lutein was isolated from spinach leaves[6]. Ethanol solution of lutein containing 200 μg to 300 μg in 0.6 ml was added dropwise under stirring to 16 ml of solution containing desired amount of sodium dodecyl sulfate (SDS) or dodecyltrimethylammonium bromide (DTAB). An apparently transparent solution colored pale-yellow was centrifuged at 220,000 g for 2 hr to give an orange-colored pellet of lutein aggregates and the pellet was suspended in about 0.2 ml of the supernatant. A drop of the suspension was placed on a carbon coated grid and air-dried in a dark place. Aggregates on the grid were further dried under high vacuum and shadowed with Pt-Pa with a vacuum evaporator model JEE-4 X. An electron microscope, JEOL model JEM 100 C, was used at an accelerating voltage of 80 KV.

Absorption and CD spectra were measured with a spectrophotometer (Shimadzu Multipurpose Model 3,000) and a spectropolarimeter (JASCO J-500 A), respectively, at room temperature.

Results and Discussion

Absorption and CD spectra of lutein dispersed in SDS and DTAB were shown in Fig. 1-A

a) Biochemical Studies on Carotenoids. Part XVI. For Part XV, see reference(7).
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and 1-B, respectively. Recoveries of lutein aggregates into precipitates at ultracentrifugation were shown in Table 1. These precipitates containing somewhat large aggregates were observed with electron microscope.

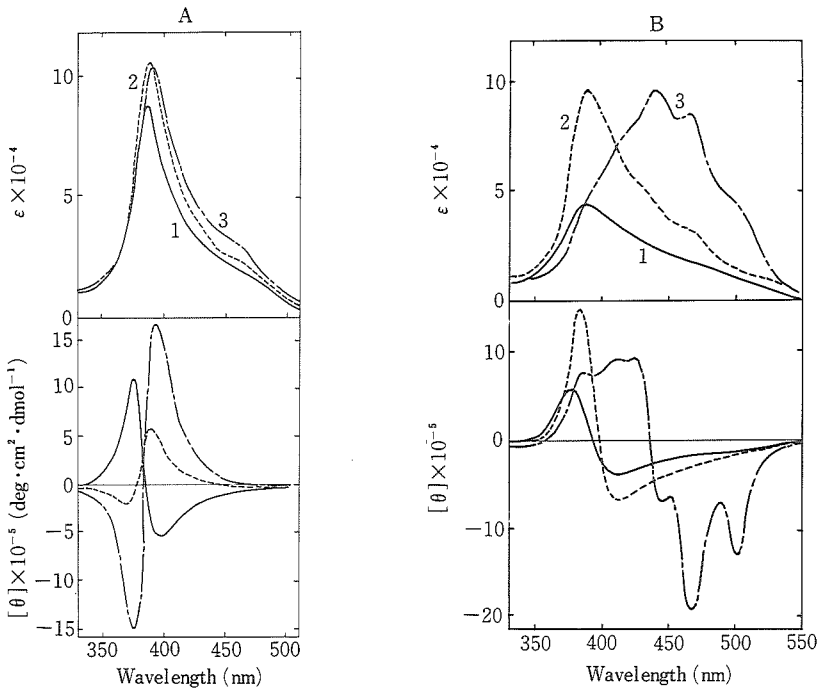

Fig. 1. Absorption and CD spectra of lutein aggregate dispersed in SDS (A) and DTAB (B) solution of various concentrations, respectively, in 1 mM phosphate buffer, pH 7.0.
(A) SDS concentration ; 1) 0.3 mM, 2) 1 mM and 3) 3 mM.
(B) DTAB concentration ; 1) 3 mM, 2) 7 mM and 3) 10 mM.

Table 1. Precipitation of lutein aggregates dispersed in SDS or DTAB solution with various concentrations

Conc. of SDS	Recoveries of lutein aggregate*	Conc. of DTAB	Recoveries of lutein aggregate*
0.1 mM	79.1%	1.0 mM	98.2%
0.3	61.0	3.0	98.4
0.5	47.9	5.0	62.6
0.7	38.9	7.0	54.8
1.0	41.4	10.0	23.5
3.0	56.3	15.0	11.9

* Precipitates subjected for electron microscopic observation.

Electron micrograph of lutein aggregate in SDS solution

Figure 2-A, 2-B and 2-C show typical electron micrographs of the suspensions obtained when lutein was dispersed in the presence of 0.3, 1.0 and 3.0 mM SDS, respectively. Below 0.3 mM SDS, lutein aggregate gives an image with spindle-like form which coagulates mutually to make an apparent enomous aggregates (Fig. 2-A). Above critical micelle concentration (1 mM) of SDS, images of aggregates were long rod like one (Fig. 2-B and 2-C). When the supernatant at ultracentrifugation was used as a specimen, no such image was observed, and these facts were the same as in this case throughout various concentration of SDS. The apparent width of

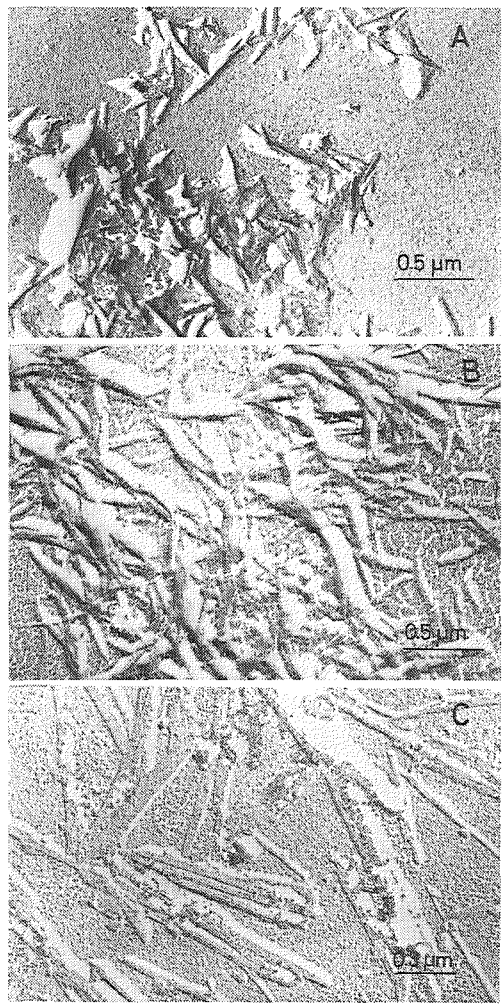

Fig. 2. Electron micrograph of lutein aggregate in SDS.
SDS concentration, A) 0.3 mM, B) 1 mM and C) 3 mM.

the aggregates, which was estimated from the angle and length of shadow, was about 0.1 μm in average. On the other hand, their length increased steadily (Fig. 3). It is on the way of the elongation that the marked change in CD spectrum was observed for lutein dispersion[4] as is also shown in Fig. 3 using CD intensity at 374 nm as a measure. It should be noted that the shape becomes most regular and the aggregates are dispersed independently in the presence of 3 mM SDS. Above this concentration, for example 10 mM, lutein was completely solubilized giving no precipitate when centrifuged.

Electron micrograph of lutein aggregate in DTAB solution

Lutein aggregate dispersed in DTAB, cationic surfactant, gave similar results in electron micrograph to those in SDS, anionic surfactant, except for high concentration of DTAB, while the absorption and CD spectra are different markedly (Fig. 1-B) with that in SDS (Fig. 1-A). In electron micrograph below 3 mM DTAB (Fig. 4-A), aggregates coagulate mutually to give an obscure image and enormous size in the same manner as seen in SDS below 0.3 mM. Individual image observed is broad and short at 3 mM DTAB. The images of the aggregates become long and disperse independently with increasing DTAB concentration (Fig. 4-B and 4-C). Width of

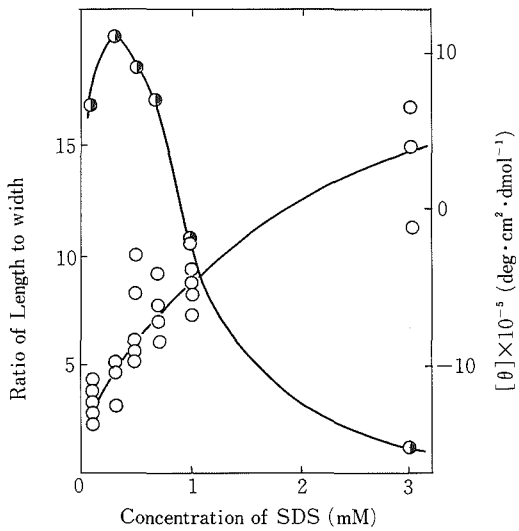

Fig. 3. Effect of SDS concentration on ratio of length to width of lutein aggregate in 1 mM phosphate buffer, pH 7.0. Ratio, ○——○ . Molar ellipticity at 374 nm in various SDS concentrations, ◑——◑ .

the aggregates ranges from 0.1 μm to 0.2 μm which is slightly broader than that in SDS. Length of the aggregates in the range from 0.25 μm to 1.5 μm is also longer than that in SDS. The ratio of length to width becomes large with an increase of DTAB concentration (Fig. 5), and this fact indicates that lutein aggregate changes in shape from the short and broad to the slim. In such transformation of aggregate, DTAB is a modulator. Molar ellipticity at peak of CD spectrum, 376 nm and 410 nm, gives the maximum at 5 mM of DTAB, which means that a chilarity of aggregate is maximum in this concentration. From the results mentioned above, it is found that lutein aggregates show the similar shape and size even if lutein is dispersed in different surfactant solutions.

Possible conformation of lutein aggregate

The electron microscopic observations have good agreement with the conclusion in previous papers[4,5,7], which elucidates that lutein associates mutually side-by-side to form right handed helical structure. The size of the images observed is incomparably larger than that expected for the aggregate. But the rod-like shape seems to suggest that the aggregate image is composed of rod-like components. Possible illustration of lutein aggregate of chiral card pack model[4], therefore, may be shown in Fig. 6. Hydroxyl groups at 3 or 3´ position of ionone rings are oriented on the surface of the aggregate in the manner surrounding helix to acquire more hydrophilic properties than lutein monomer. Inside the aggregate conjugated polyenes are continuously packed side-by-side, although slightly shifted in relation to each other. In this assembly of conjugated polyenes, dipole-dipole interaction between π-electrons of adjacent lutein molecules easily occurred, which will indicate the probability for this aggregate to act electron conductor.

As almost lutein is present in chloroplast, especially in thylakoid membrane, the cooperation with other photosynthetic pigments or the function as electron carrier is strongly expected. A working hypothesis for physiological function of lutein from results mentioned above can be proposed as the following. "If lutein aggregates are built up in thylakoid membrane in relation with photoreaction in photosynthesis, it seems likely that electron transport occurrs through helical tunnel of lutein aggregate from annthena pigment of light to photosynthetic reaction center or from photosynthetic reaction center to electron acceptor".

Effects of lutein aggregate on photosynthetic electron transport activity are now studied to obtain some interesting results suggesting the action as electron conductor.

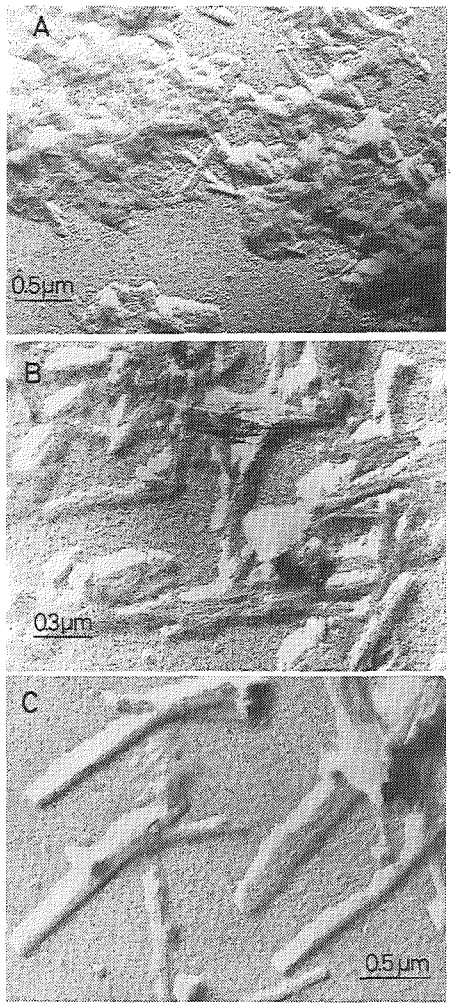

Fig. 4. Electron micrograph of lutein aggregate in DTAB. DTAB concentration ; A) 3 mM, B) 7 mM and C) 10 mM.

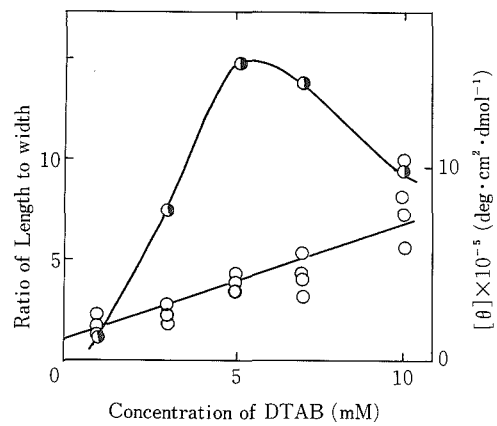

Fig. 5. Effect of DTAB concentration on ratio of length to width of lutein aggregate in 1 mM phosphate buffer, pH 7.0. Ratio, ○——○. Molar ellipticity at 370 nm (below 5 mM DTAB) and at 410 nm (above 7 mM), ◑——◑.

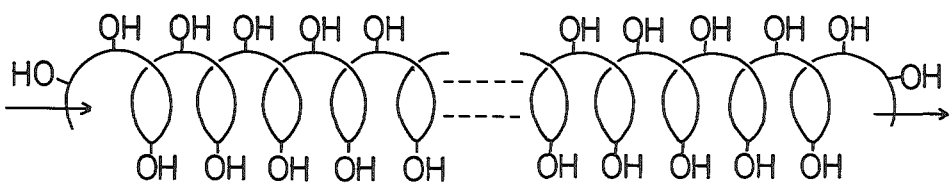

Fig. 6. Possible conformation of lutein aggregate in aqueous system. See text.

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ルテイン集合体の電子顕微鏡による観察

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緑葉の主要カロチノイドの１つであるルテインは水系溶媒に分散してキラールならせん状集合体を形成し可視領域に光学活性を示す．限外沪過，沈降平衡分析によって集合体は巨大なものであることをすでに確認している．本報告では SDS 及び DTAB のうすい水溶液に分散した集合体の電子顕微鏡像を観察し，その大きさ，形態及びらせん状の有無を調べた．超遠心沈澱によって得た集合体に少量の上澄を加えて懸濁液とし，カーボン蒸着グリッド上にのせ，風乾後 Pt-Pa にてシャドーイングし電子顕微鏡で観察した．SDS 及び DTAB 水溶液では共に長さ 0.3 から 1.5 μm，幅 0.05～0.2 μm の長い棒状を示し，界面活性剤濃度が低いと短かくなる傾向がある．予想したらせん構造はここでは認められなかったが，電子顕微鏡像にもとずきキラールなルテイン集合体の推定しうる形態を示し，その生理的機能について作業仮説を立てた．