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Myxol 2'-dimethyl-fucoside, (3*R*,2'*S*)-myxol 2'-(2,4-di-*O*-methyl-a -L-fucoside), in *Synechocystis* sp. PCC 6803 and nomenclature of myxoxanthophyll

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Introduction:

Myxoxanthophyll is a major carotenoid glycoside, widely distributed in cyanobacteria, and some species also contain oscillaxanthin. These carotenoids have a very unique glycoside linkage: a hydroxyl group at C-2 of the ψ end group of carotenoids is bonded to glycoside. This structure is only found in these carotenoids, and the presence is limited to cyanobacteria. Hertzberg and Liaaen-Jensen (1969) ultimately determined the structures of myxoxanthophyll and oscillaxanthin from *Arthrospira* sp. as myxol 2'-rhamnoside and oscillol 2,2'-di(L-rhamnoside), respectively. The determination of the sugar moieties of these carotenoids, including the L- or D-type and the α- or β-linkage, has been done only for a few species of cyanobacteria. The chemical structure of myxoxanthophyll in *Synechocystis* sp. PCC 6803 has not been determined. In the present study, we identified myxoxanthophyll, and proposed a new, more specific nomenclature of the carotenoid and the sugar moieties of such carotenoid glycosides.

Materials and Methods:

Synechocystis sp. PCC 6803 and the *crtR* (β-carotene hydroxylase) mutant were used. For purification of each carotenoid, columns of silica gel and DEAE-Toyopearl and ODS-TLC were used. For identification, absorption spectra by a photodiode array detector attached to the HPLC apparatus, CD spectra, relative molecular masses by FD-MS, and ^{1}H- and ^{13}C-NMR spectra were used.

Results:

Known pigments, Chl *a*, β-carotene, (3*R*,3'*R*)-zeaxanthin, echinenone, and (3'*R*)-3'-hydroxyechinenone, were identified by spectroscopic methods.

The absorption maxima of the polar carotenoid were 294, 365, 448, 473, 503 nm in methanol, and the spectral fine structure of %III/II was 58. These results indicated that the carotenoid was a derivative of γ-carotene with 12 conjugated double bonds. The molecular mass was 758. The formation of a diacetyl and a tri-trimethylsilan derivatives indicated the presence of two primary and/or secondary and one tertiary hydroxyl groups. The CD

spectrum was the same as that of (3*R*,2'*S*)-myxol 2'-rhamnoside. The ^{1}H- and ^{13}C-NMR spectra indicated that the carotenoid moiety was myxol. The ^{1}H doublet signal at 5.10 ppm indicated the presence of an α-glycoside linkage. The sugar moiety agreed with those of α-L-fucose. The NOESY and HMBC correlations indicated that the two hydroxyl groups at C-2'' and C-4'' of the sugar moiety were methylated. Thus, the structure was identified to be myxol 2'-dimethyl-fucoside, (3*R*,2'*S*)-myxol 2'-(2,4-di-*O*-methyl-α-L-fucoside) (Fig. 1). IUPAC-IUB semi-systematic name of this carotenoid glycoside is (3*R*,2'*S*)-2'-(2,4-di-*O*-methyl-α-L-fucopyranosyloxy)-3',4'-didehydro-1', 2'-dihydro-β,ψ-carotene-3, 1'-diol.

One minor carotenoid in the wild type was a major one in the *crtR* mutant. The absorption spectrum of this carotenoid was the same as that of myxol 2'-dimethyl-fucoside. The molecular mass was 742. The CD spectrum was the same as that of (2'*S*)-phleixanthophyll. These results and the NMR spectra indicated that the structure was deoxymyxol 2'-dimethyl fucoside, (2'*S*)-deoxymyxol 2'-(2,4-di-*O*-methyl-α-L-fucoside), (2'*S*)-2'-(2,4-di-*O*-methyl-α-L-fucopyranosyloxy)-3',4'-didehydro-1',2' dihydro-β,ψ-caroten-1'-ol.

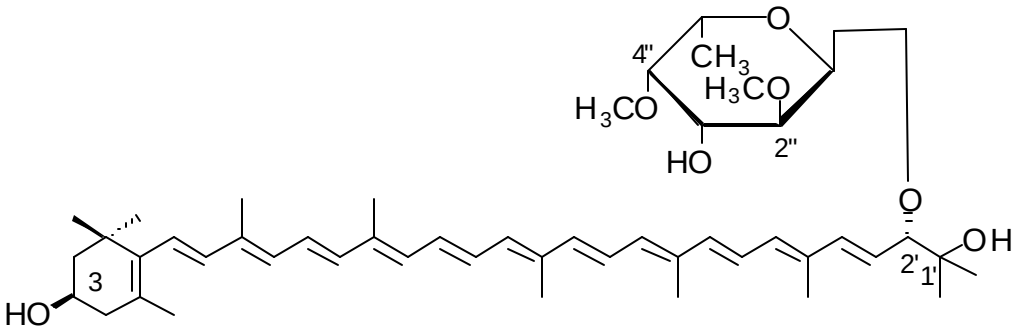

Fig. 1. Structure of myxol 2'-dimethyl-fucoside.

The carotenoid composition (mol %) of the wild type and the *crtR* mutant cultured under light for 5 - 6 days was 26, 26% β-carotene, 14, 0% zeaxanthin, 18, 32% echinenone, 4, 0% 3'-hydroxyechinenone, 1, 41% deoxymyxol 2'-dimethyl-fucoside and 36, 0% myxol 2'-dimethyl fucoside, respectively. In the mutant, zeaxanthin and myxol 2'-dimethyl-fucoside disappeared, deoxymyxol 2'-dimethyl-fucoside appeared, and echinenone increased. Although total carotenoid content decreased in the mutant cells, the growth was almost the same with the wild-type cells.

Discussion:

Figure 2 presents a proposed biosynthetic pathway of the carotenoids in *Synechocystis* sp. PCC 6803. More than 10 enzymes must be involved in this pathway. Only five genes are functionally identified: *crtB* phytoene synthase, *crtP* phytoene desaturase, *crtQ* ζ-carotene desaturase, *crtR* β-carotene hydroxylase and *crtO* β-carotene ketolase. *crtE* Geranylgeranyl pyrophosphate synthase is suggested by the sequence homology. Lycopene cyclase *crtL* is found in *Synechococcus* sp. PCC 7942, while the gene is not found in *Synechocystis* sp. PCC 6803 even by the sequence homology.

Although the biosynthetic pathway of myxol 2'-dimethyl-fucoside remains unknown, the enzymes described in Fig. 2 may be present. In the first step, γ-carotene synthesis is catalyzed by lycopene cyclase or γ-carotene synthase. Next, modification of the ψ end group

of γ-carotene may be similar to that in purple photosynthetic bacteria by CrtC- and CrtD-like

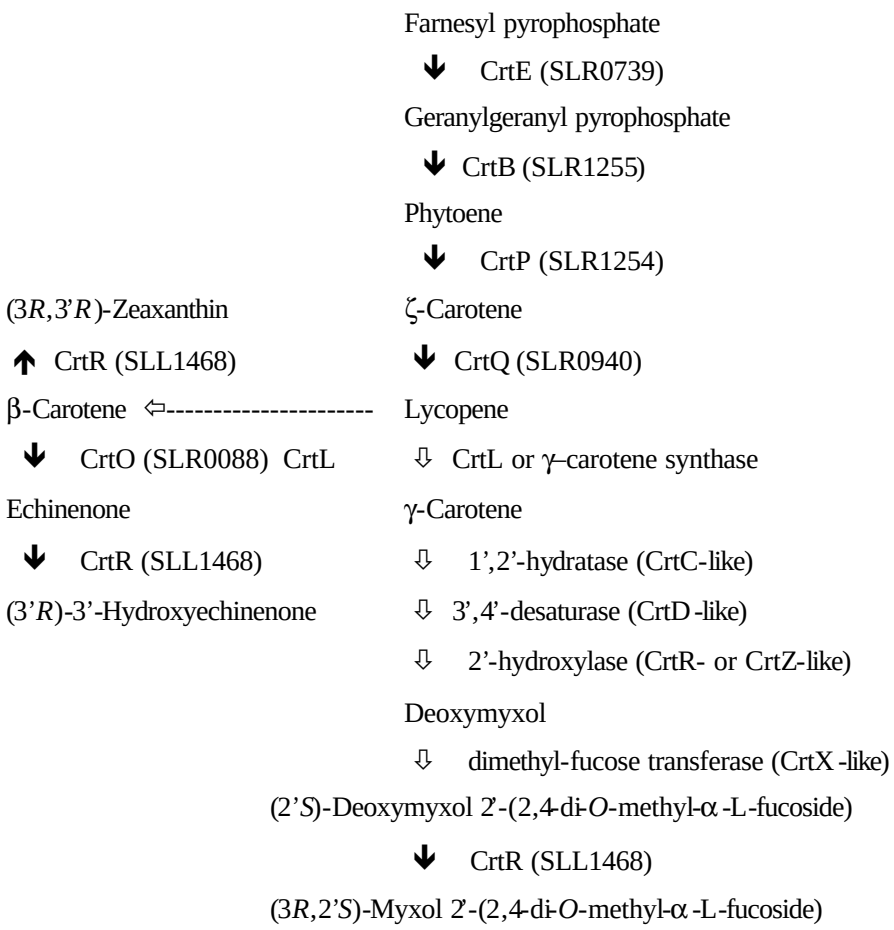

Fig. 2. Biosynthetic pathway of carotenoids and their enzymes in *Synechocystis* sp. PCC 6803. Parentheses indicate the ORF number of this cyanobacterium. The presumed enzymes (white arrows) are based on the known ones from bacteria and cyanobacteria.

enzymes. It is unknown whether methylated fucose is transferred to the carotenoid moiety or methylation occurs on the carotenoid fucoside. The open reading frame *slr1125* has the sequence similarity to zeaxanthin glucosyltransferase CrtX but did not possess glucosyltransferase activity for both myxol and diacylglycerol.

This cyanobacterium was the second one found to contain fucose in the sugar moiety of carotenoid glycosides. The monomethylated fucose, 3-*O*-methyl-α-L-fucose, was found in *O. bornetii*. All of the sugar moieties in cyanobacteria have been reported to be the α-L-type, that is, α-L-rhamnoside, α-L-fucoside, α-L-chinovoside and their methylated derivatives, while that in bacteria except for cyanobacteria is mostly β-D-glucoside. These sugars are less polar than glucose. At present, the functions of the carotenoid glycosides in both cyanobacteria and photosynthetic bacteria are unknown, and the roles of the diversity of sugar moieties in cyanobacteria are also unknown. Most species of cyanobacteria contain carotenoid glycosides, but sugar moieties have been identified in only a few species. They should be investigated in other species as well as whether they are L- or D-type and whether they are associated with an α- or β-linkage. Furthermore, the classification of cyanobacteria

has been rapidly changing and some new species have been described in recent years, thus it is appropriate to re-examine the chemical structure of the carotenoid glycosides using new techniques, as well as to identify the species.

We propose herein the following nomenclature for the trivial names of myxoxanthophyll, oscillaxanthin and related compounds: the carotenoid moieties (aglycone) of myxoxanthophyll and oscillaxanthin are myxol and oscillol, respectively; that of aphanizophyll is 4-hydroxymyxol, although 4-ketomyxol is also found. When the sugar moieties have not been determined, they should be named myxol glycoside and oscillol glycoside. If the sugar moieties have been identified, the names should be, for example, myxol 2'-rhamnoside, myxol 2'-α-L-rhamnoside and (3*R*,2'*S*)-myxol 2'-(2,4-di-*O*-methyl-α-L-fucoside). Since the names such as myxoxanthophyll and oscillaxanthin cannot specify the sugar moieties, the use of such indefinite terms should be avoided.

Reference:

S Takaichi, T Maoka, K Masamoto (2001) Myxoxanthophyll in *Synechocystis* sp. PCC 6803 is myxol 2'-dimethyl-fucoside, (3*R*,2'*S*)-myxol 2'-(2,4-di-*O*-methyl-α-L-fucoside), not rhamnoside. *Plant Cell Physiol.* **42** (7) 756-762.