Jap. J. Phycol. 26: 151-155, 1978

The function of siphonein in a siphonous green alga *Dichotomosiphon tuberosus*

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KAGEYAMA, A. and Y. YOKOHAMA 1978. The function of siphonein in a siphonous green alga *Dichotomosiphon tuberosus*. Jap. J. Phycol. 26: 151-155.

Although the function of siphonaxanthin had been determined, that of its ester siphonein had not been done owing to that the siphonous green algae with siphonein we had ever collected had also contained siphonaxanthin without exception. In this study the function of siphonein could be determined with *Dichotomosiphon tuberosus* which contained siphonein without containing siphonaxanthin.

The *in vivo* absorption spectrum of this alga has a characteristic peak at about 540 nm, which indicates that the absorption peak of siphonein is located at about 540 nm *in vivo*. The 540 nm peak also occurs in the excitation spectrum for the *in vivo* chlorophyll *a* fluorescence at room temperature, indicating that siphonein can efficiently transfer its excitation energy to chlorophyll *a* of pigment system II. Siphonein is, therefore, regarded as an efficient photosynthetic pigment specifically capturing green light. The function of this pigment is considered to be fundamentally the same as that of siphonaxanthin, a green light-absorbing pigment which is important for deep-water green algae living under green illumination in coastal deep waters.

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In our previous papers it was reported that siphonaxanthin was regarded as an efficient photosynthetic pigment which was important for the deep-water green algae growing under green illumination in coastal deep waters because this pigment had a characteristic absorption peak at about 540 nm in the green part *in vivo* and efficiently transferred its excitation energy to chlorophyll *a* (YOKOHAMA *et al.* 1977, KAGEYAMA *et al.* 1977, KAGEYAMA & YOKOHAMA 1977).

KLEINIG (1969) reported that most of siphonous green algae contained siphonein, the ester of siphonaxanthin besides siphonaxanthin. Although the function of siphonaxanthin could be determined with deep-water green algae containing siphonaxanthin without containing siphonein, we had not been able to determine that of siphonein owing to that all the algae with sipphonein ever collected had also contained siphonaxanthin without exception. Recently we could collect the exceptional alga *Dichotomosiphon tuberosus* which had been reported by KLEINIG (1969) to contain siphonein without containing siphonaxanthin. The *in vivo* absorption spectrum and the excitation spectrum for *in vivo* chlorophyll *a* fluorescence of this alga were determined in this study. The results we obtained will indicate the function of siphonein in this paper.

Contributions from the Shimoda Marine Research Center, No. 335.

Materials and Methods

Dichotomosiphon tuberosus was collected form a puddle of freshwater at Okinawa Island in the southernmost part of Japan. *Ulva amamiensis* used for control experiments was collected from a depth of 5 m on the coast of Amami Island in the vicinity of Okinawa. *Monostroma nitidum* and

Codium latum used for the same purpose were from higher intertidal zone and lower intertidal zone on the coast of Shimoda in central Japan, respectively.

The thalli (ca. 1 g fresh weight) were ground with a small volume of cold methanol in a glass homogenizer. Homogenates were filtered through a glassfiber filter, and the extraction was repeated several times until the residue became colorless. The combined methanol extract (10–15 ml) was mixed with a nearly equal volume of diethylether in a separating funnel. The pigments were transferred to the ether layer by shaking with a 10% NaCl solution, the ether layer was dried up under reduced pressure, and the residue was redissolved in a small volume of ether.

The pigments were separated at room temperature by cellulose thin-layer chromatography. The mixture of n-hexane, diethylether and n-propanol (50 : 50 : 0.5, v/v/v) was used as the developing solvent. All analysis procedures for pigments were carried out under dim light or in the dark below 2°C unless otherwise indicated.

Absorption spectra of the intact thalli of the algae were determined with a Shimadzu UV-200 Spectrophotometer. The opal glass method of SHIBATA *et al.* (1954) was used for the spectroscopy. Fluorescence spectra and fluorescence excitation spectra were determined with a Shimadzu RF-502 Spectrofluorophotometer at the Ocean Research Institute, University of Tokyo, by courtesy of Prof. Y. FUJITA. Thallus of *Monostroma nitidum* or *Ulva amamiensis* was held between a pair of lucite plastic frames and placed in a foursided transparent cell (10×10×40 mm) so as to face the excitation beam at an angle of 45°. In the case of the filamentous alga *Dichotomosiphon tuberosus* a few filaments of it were placed in the cell. The cell was filled with sea water to avoid drying of the thallus. To prevent photosynthetic electron transfer, 3-(3, 4-dichlorophenyl)-1, 1-dimethylurea (DCMU, 10^{-4} M) was added. Measurements were carried out at room temperature (about 20°C).

Results

The chromatograms of pigments from the four chlorophycean algae are shown in **Fig. 1.** As is seen, siphonein which is contained by *Codium latum* can be detected in *Dichotomosiphon tuberosus*, while siphonaxanthin contained by *Codium latum* and *Ulva amamiensis* can not be detected in this alga.

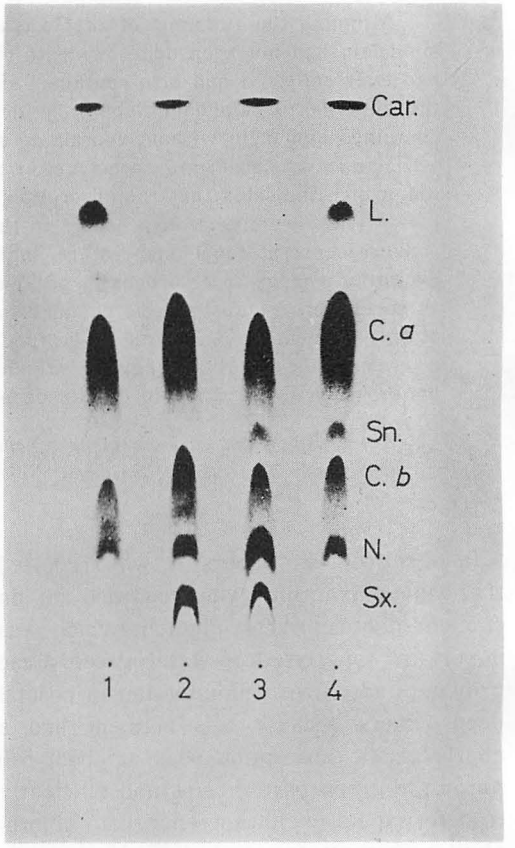

Fig. 1. Cellulose thin-layer chromatograms of pigments from *Monostroma nitidum*(1), *Ulva amamiensis*(2), *Codium latum*(3) and *Dichotomosiphon tuberosus*(4). The developing solvent: n-hexane, diethylether and n-propanol (50 : 50 : 0.5, v/v/v). Car.=carotene(s); L. lutein; C. *a*=chlorophyll *a*; Sn.=siphonein; C. *b*=chlorophyll *b*; N.=neoxanthin; Sx.=siphonaxanthin. Violaxanthin is detected with the other developing solvent (n-hexane: methyl ethyl ketone=4 : 1) in *M. nitidum*, *U. amamiensis* and *D. tuberosus* although the fraction of this carotenoid is not distinguishable from that of chlorophyll *a* on the chromatograms shown in this figure.

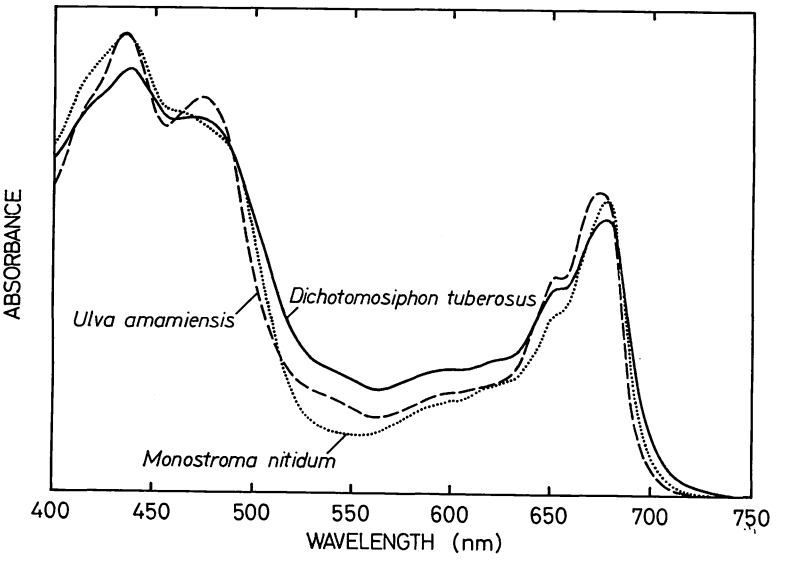

Fig. 2. *In vivo* absorption spectra of *Monostroma nitidum* lacking siphonaxanthin and siphonein, *Ulva amamiensis* containing siphonaxanthin without containing siphonein and *Dichotomosiphon tuberosus* containing siphonein without cotaining siphonaxanthin.

The lack of siphonaxanthin in *D. tuberosus* was further confirmed with a quantitative analysis. **Fig. 2** shows the *in vivo* absorption spectra of *D. tuberosus*, *U. amamiensis* and *M. nitidum*. As can be seen, the characteristic peak around 540 nm occurs not only in the spectrum of *U. amamiensis* containing siphonaxanthin but also in that of *D. tuberosus* containing siphonein without containing siphonaxanthin. These results indicate that the *in vivo* absorption maximum of siphonein is located at about 540 nm as well as is that of siphonaxanthin because any pigment other than siphonein detectable in *D. tuberosus* can not be responsible for the 540 nm peak in its *in vivo* absorption spectrum. The carotenoids other than siphonein detectable in this alga were carotenes, lutein, violaxanthin and neoxanthin which are not regarded to be responsible for the 540 nm peak since all of them are abundantly contained by *Monostroma nitidum* which lacks the 540 nm peak in its *in vivo* absorption spectrum.

Fluorescence spectra of *M. nitidum* and *D. tuberosus* are shown in **Fig. 3.** The

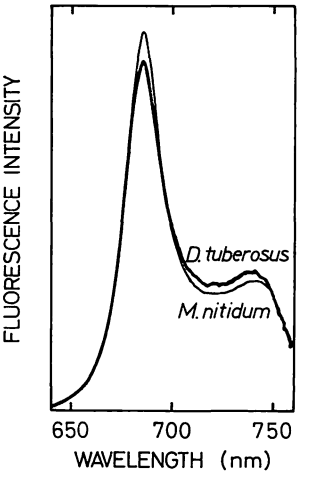

Fig. 3. Fluorescence spectra (half-bandwidth, 5 nm) at room temperature (about 20°C) of the thalli of *Monostroma nitidum* and *Dichotomosiphon tuberosus*.

emission peak was observed at about 685 nm in both algae. The location of the emission peak is similar to those reported for other green algae (GOEDHEER 1964, KAGEYAMA *et al.*, 1977). It is shown in **Fig. 4** that the excitation spectrum for the 685 nm emission

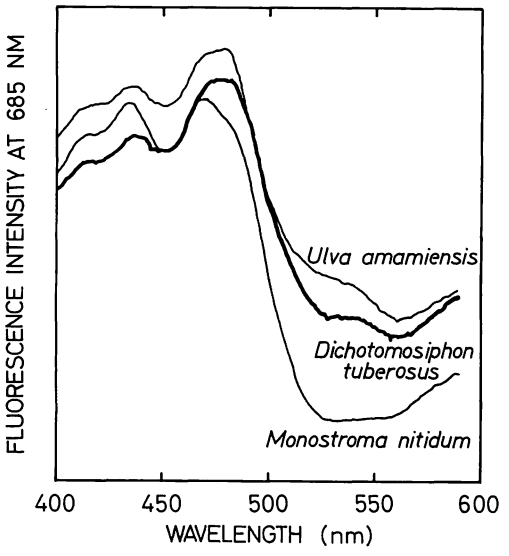

Fig. 4. Excitation spectra of 685 nm fluorescence of the thalli of *Monostroma nitidum*, *Ulva amamiensis* and *Dichotomosiphon tuberosus* mesured at room temperature. Fluorescence intensity at 685 nm (half-bandwidth, 7 nm) per incident energy was recorded against wavelength of excitation light. Half-bandwidth for the excitation, 5 nm.

of *D. tuberosus* has again a characteristic peak at around 540 nm, which the spectrum of *M. nitidum* lacks. The 540 nm peak in the excitation spectrum indicates that siphonein in *D. tuberosus* can efficiently transfer its excitation energy to chlorophyll *a*.

Discussion

The existence of the 540 nm peak in both the *in vivo* absorption spectrum and the 685 nm fluorescence excitation spectrum of *D. tuberosus* indicates that the function of siphonein may be the same as that of siphonaxanthin, specifically absorbing green light and efficiently transferring its excitation energy to chlorophyll *a*. These pigments may function as components of pigment system II since fluorescence is known to be emitted from chlorophyll *a* of pigment system II at room temperature (MURATA *et al.*, 1966). Although siphonaxanthin is regarded as the photosynthetic pigment important for deep-water green algae living under green illumination in coastal deep waters (YOKOHAMA *et al.* 1977, KAGEYAMA *et al.* 1977, KAGEYAMA & YOKOHAMA 1977), siphonein does not seem to be important for *D. tuberosus* living at a sunny site. However, this pigment is also contained by most siphonous green algae besides siphonaxanthin (KLEINIG 1969). As many species of them are growing in deep waters, siphonein is regarded as an important pigment for them as well as is siphonaxanthin. In some lakes *D. tuberosus* is growing at a depth of 40 feet or more (PRESCOTT 1969 p. 113). This alga may be able to grow in such a deep water owing to siphonein specifically capturing green light dominant in the illumination of its habitat.

Acknowledgment

We wish to thank Dr. S. Enomoto of Iwaya Marine Station, Kobe University for his kind offer of the specimen of *Ulva amamiensis* collected at Amami Island. We also wish to express out thanks to Dr. S. Kamura of Sesoko Marine Science Laboratory, University of the Ryukus, who kindly accorded us a convenience of collecting *Dichotomosiphon tuberosus* at Okinawa Island.

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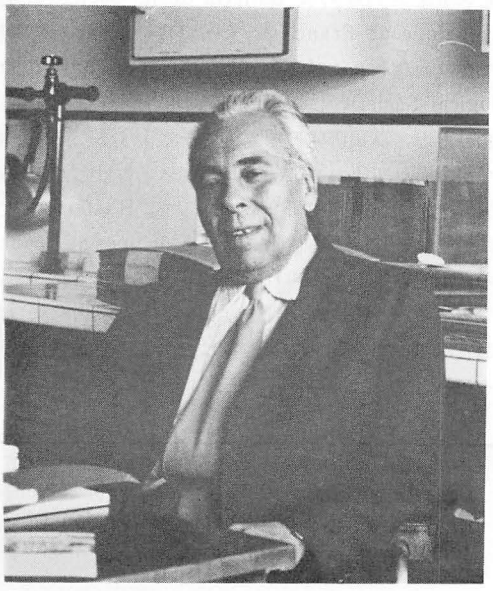
影山明美*・横浜康継*：チョウチンミドロにおける Siphonein の機能

深所性の緑藻に含まれる Siphonaxanthin が深所に優占する緑色光を捕捉する光合成色素とみなしうることはすでに報告したが，この色素のエステルでクダモの多くに含まれる Siphonein については，従来入手できた Siphonein 含有種がすべて Siphonaxanthin をも含んでいたため，その機能を明らかにできなかった。本研究においては，例外的に Siphonaxanthin を含有せずに Siphonein を含有するチョウチンミドロを採集し得たため，これを用いて，生体吸収スペクトルおよび生体クロロフィル *a* 螢光に対する励起スペクトルを調べたところ，Siphonaxanthin 含有種にみられたと同様な 540 nm（緑色部）附近のピークが明らかにみられた。このため，Siphonein も緑色光を特異的に吸収して，その励起エネルギーを効率よくクロロフィル *a* に伝達する光合成色素とみなすことができる。(*415 静岡県下田市 5-10-1，筑波大学下田臨海実験センター)

吉田忠生：Jean FELDMANN 先生をしのぶ

Tadao YOSHIDA: Professor FELDMANN (1905-198), in memoriam



France の Jean FELDMANN 先生が1978年9月18日亡くなられた。1905年生れで享年73才あった。France のみならず，世界の藻学界にとっても大きな損失である。

先生は Paris で高等教育を受けられ，その頃から生物学の各方面に関心を持たれて，種子植物・菌類を広く勉強された。1925年 Bretagne 地方の Roscoff における海藻学臨海実習を通じて学問的興味の中心を海藻に向けられることになったという。その当時 France では BORNET, THURET の亡きあと，SAUVAGEAU が Bordeaux において活潑な研究を進めていた他には CHEMIN, HAMEL, OLLIVIER 等がいたけれども，Paris で勉強を進めておられた先生にとって特に師と呼べる人はいなかったようである。SAUVAGEAU の助言によって開始された地中海沿岸 Banyuls 地方の海藻の研究は，先生のその後の幅広い研究活動の基礎になったものである。それまであまり手のつけられていなかった海藻の生態学の分野に進むことを目指しながら，そのために必要不可欠な分類学的・分布的な知見の不足を自らの手で満たすために，同時に France では最も知られていなかったその地方の海藻相を明らかにすることにも努力された。その結果として "Les algues marines de la côte des Albères I-IV" (1937, 1942) が発表された。この大著の示唆に富む記述にはその後の研究の萌芽が多く含まれており，後年の成果と考え合わせて観察の鋭さにただ感心させられるのみである。生態学的な面をまとめた "Recherches sur la végétation marine de la Méditerranée. La côte des Albères" (1937) はこの分