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In-vivo Fluorescence Characteristics of Pteridine for Identification of Phytoplankton

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The steady state and decay characteristics of primary fluorescence of twelve clones of phytoplankton were investigated *in vivo*. At 380~435*nm* region, intense fluorescence emission spectra were obtained from the all phytoplankton examined. The primary fluorescence of phytoplankton in different growth states was examined. In order to find out fluorophores for the observed fluorescence, eight different pteridine derivatives in phosphate buffer solution were examined for their fluorescence characteristics and compared with those of phytoplankton. Fluorescence lifetimes(τ) and decay curves were compared with standard solution of candidate organic compounds. Decay kinetics of observed fluorescence were shown as bi-and tri-exponential decay curves with 430*nm* cut-off filter for phytoplankton. Comparison between fluorescence characteristics of bacteria and phytoplankton showed distinct differences for their steady state fluorecence spectra and decay kinetics.

Introduction

As a sensitive, selective and non-destructive analysis technique, fluorescence spectroscopic method has been widely used for biodetection of microorganisms such as bacteria and phytoplankton(Oldham *et al.*, 1985, Dalterio *et al.*, 1986; Baek *et al.*, 1988).

Especially, in the case of phytoplankton, fluorescence is strong between 320 and 680 *nm* when excited by UV or visible light. Protein tryptophan in phytoplankton(Baek *et al.*, 1988) and bacteria(Dalterio *et al.*, 1986) is recognized as the primary source of the fluorescence emission between 320 and 340 *nm* when excited with 290 *nm* radiation. Other known fluorophores present in phytoplankton are chlorophylls and phycobiliproteins(Gantt *et al.*, 1976; Goodwin, 1976).

Recently many efforts have been made to characterize phytoplankton, by their pigments which absorb lights and fluoresce in specific regions(Yentsch *et al.*, 1979; Oldham *et al.*, 1985). These studies are based on the unique pigment composition of different kinds of marine phytoplankton. The proper marker compound(or fluorophore) for differentiation of phytoplankton is the one which all phytoplankton possesses and which has different regions for light absorption and emission depending upon kinds of phytoplankton. In case of chlorophyll *a*, it is presented in all kinds of phytoplankton, but chlorophyll *a* shows similar emission and excitation maxima disregarding kinds of phytoplnakton. And phycobiliproteins have characteristic and non-overlapping emission and excitation maxima but presents only in three different classes of phytoplankton. If there is a fluorophore which has non-overlapping emission and excitation maxima with chlorophyll *a* and phycobiliproteins, and if it is presented in all kinds of phytoplankton, then it can be used as an additional parameter for identification of phytoplankton and other microorganisms. One of this kinds of fluorophores is pteridines which are highly fluorescent. The presence of a remarkably high concentration of pteridines in blue-green alga, *Ancystis nidulans*, was demonstrated by Forrest *et al.*(1957). Since then, a number of different pteridines have been isolated and identified. Extensive reviews of the properties of folic acid and related pteridines are available(Blakely, 1969; Pfeiderer, 1975).

In this study, twelve clones of phytoplankton

from four different classes are investigated for their fluorophores as candidates for the kind of marker compound. Attempts have been made to attribute the algal fluorescence to molecular components known to exist in phytoplankton. In addition, the extent to which fluorescence lifetimes can be used in combination with fluorescence excitation and emission data to identify phytoplankton will be reported.

Materials and Methods

All phytoplankton were grown at 15℃, in 12:12 light:dark cycle, with a light intensity of 80 $\mu mol/m^2/sec$ in a Sherer incubator. These culture stocks were obtained from the Graduate School of Oceanography, University of Rhode Island, Kingston, RI. The sources for these algae were listed in Table 1. The culture medium was of 30 ppt salinity with 1/2 strength nutrients of Guilliard's 'f' medium (Guillard *et al.,* 1962). Bacterized cultures were made axenic using a mixture of penicillin and streptomycin.

In order to get concentrated algae, algal samples were either sedimented or centrifuged. After the concentration of the samples, the culture medium was decanted. The algal suspensions used for experimental measurements were prepared by diluting the concentrated algal samples with 0.05 M phosphate buffer(pH 6.6). The phosphate buffer solution was made by adjusting a solution containing 0.025 M Na_2HPO_4 and 0.025 M NaH_2PO_4 to pH 6.6 with additional NaH_2PO_4, while monitoring the pH with a metter. Algal suspensions were always stored at 4℃ and were equilibrated to room temperature(between 20℃ and 23℃) just before the fluorescence measurements were made.

Steady-state fluorescence spectra were obtained with a Perkin-Elmer MPF-2A spectrofluorometer. Spectra were uncorrected. Quartz cells(1 *cm*) were used for all spectroscopic measurements. Anthracene as external standard was used to monitor instrumental changes for wavelength by its known three emission maxima at 377, 398 and 420 *nm*. The anthracene solution used as standard was 10^{-5} M in MeOH. A typical excitation band width was 10 *nm*. The emission spectra of marine phytoplankton have been obtained at about 380～435 *nm* when selectively excited at about 360～380 *nm*. The emi-

Table 1. Sources for algae.

Name	Clone	Source
1. *Alexandrium tamarensis*	GT429	CCMP
2. *Alexandrium tamarensis*	Gport	Provasoli
3. *Olisthodiscus luteus*	Olisth	CCMP
4. *Chroomonas sp.*	8C	Hargraves-N. Y.
5. *Chroomonas sp.*	J6F	Hargraves-Narragansett Bay
6. *Gloeotrichia sp.*	86W-2010	Ward's*
7. *Gloeocapsa sp.*	86W-2000	Ward's*
8. cf. *Synechococcus sp.*	228	Steele, EPA
9. cf. *Synechococcus sp.*	PJ/SYN	Johnson, URI
10. *Synechococcus sp.*	DC-2	CCMP
11. *Synechococcus sp.*	SYN	CCMP
12. *Spirulina sp.*	PJ/SPIR	Johnson, URI

(*): Purchased from Ward's Natural Science Establishment, Inc.

Class of algae: Dinophyceae: 1, 2
Chloromonadophyceae: 3
Cryptophyceae: 4, 5
Cyanophyceae: 6～11

ssion and excitation spectra of marine phytoplankton at two different physiological states were also investigated in order to see any changes in emission maxima depending upon physiological states. With excitation wavelength of 430 *nm*, the fluorescence lifetimes have been measured. The primary fluorescence observed from phytoplankton has been compared to the known fluorescence properties including lifetimes of molecular components which are known for their wide-spread occurrence in phytoplankton.

Fluorescence decay measurements were obtained with a Photochemical Research Associates System 3000. Excitation was provided by a hydrogen flash lamp with a pulse time of 2.5 *ns*, full width at half maximum. Interference filters with 8 *nm* bandpass were used for wavelength discrimination in the excitation and emission beams. The emission wavelengths for the fluorescence decay measurements of the folic acid derivatives were chosen after inspection of the excitation and emission spectra. The fluorescence emissions with excitation at 430 *nm* were collected by a single photon counting instrument with a 470 *nm* cut-off bandpass filter in order to get all emissions which have a longer wavelength than 470 *nm*. For DC2, the fluorescence emission was collected with bandpass filters, 340 and 430 *nm* for excitation and emission respectively. To optimize the signal-to-noise ratio, 5000 counts at the peak of the sample decay profile were collected. The excitation-time-intensity profile and the sample fluorescence decay profile were collected in 256 channels in a multichannel analyzer(Tracor-Northern Model TN-7200).

The channel width was 0.352 *ns* per channel. Deconvolutions were performed on the decay data from the channel containing the maximum number of counts to a channel where the counts were less than 1% of the maximum with a PDP 11/03 computer utilizing PRA softward with Decay V 3.0 deconvolution routines(Dalterio *et al.,* 1987).

Results

A. Emission and Excitation Spectra

The fluorescence characteristics of twelve different clones of marine plankton algae have been examined. The emission and excitation maxima for the characteristic primary fluorescence are listed in Table 2. A characteristic fluorescence band were observed from all algal cultures examined. Broad fluorescence emission bands centered between 380 *nm* and 435 *nm* were observed for all phytoplankton. The corresponding excitation bands which are located with maxima between 345 and 370 *nm* are quite broad and intense, although less intense than the emission bands originating from protein tryptophan residues of phytoplankton(Baek *et al.,* 1988). Depending upon the species of phytoplankton, the maxima for emission and excitation bands are different.

Even though there are individual differences in emission and excitation maxima among several phytoplankton, they can be classified into two groups based on emission and excitation maxima. One is the group with emission maximum at about 386 *nm* and excitation maximum at about 349~366 *nm*. The phytoplanktons which belong to this group are Dinophyceae(GT429 and Gport) and Cyanophyceae (PJ/SYN, PJ/SPIR, SYN and DC2). Among them, a pair of phytoplankton of the same species originating in different places have shown similar values for emission maxima within experimental error. They are GT429 and Gport which have shown emission maxima at 386 and 384 *nm*, respectively. Their excitation maxima are identical within experimental error. The other pair which shown similar emission maxima is PJ/SYN and PJ/SPIR, which are all cyanobacteria but belong to different genus. Their emission maxima values were 386 *nm* and 384 *nm*, respectively. However, the excitation spectra of PJ/SYN has a additional small peak centered at 308 *nm*, which is absent from excitation spectra of PJ/SPIR. For DC2 and SYN, which are all cyanobacteria and possibly different species, their emission maxima were shown at 480 *nm* and 390 *nm* and their excitation maxima values were quite different(12 *nm* differences).

The other group of phytoplankton, including Olisth, 86W-2000, and 86W-2010 have shown their emission and excitation maxima at 435 and 340~

366 *nm*, respectively. These are Chloromonadophyceae and Cyanophyceae. Even though these phytoplanktons showed their emission maxima at about 436 *nm*, their excitation maxima were distinctively different. For Olisth(Chloromonadophyceae), light absorption at 366 *nm* was observed with less intense absorption at 391 *nm*. On the other hand, 86W-2010 and 86W-2000 absorb lights at 345 *nm* and 338 *nm*, respectively. About 8 *nm* differences were observed. These are all cyanobacteria but belong to different genus. For 8C(Cryptophyceae), emission spectra were different since their peak shape is a kind of shoulder, rather than a prominent peak. However, the emission maximum at 431 *nm* of 8C shows it can be include in the latter group.

The primary fluorescence of three phytoplankton was examined to note any changes associated with their different growth states. The results are listed in Table 3. Note that(L) represents the logarithmic state of growth and (S) stands for the senescent growth state of phytoplankton. All the emission maxima of old phytoplankton (S) have shifted ever so slightly to shorter wavelengths. However, the shifts of the emission maxima are very small, within the experimental error. In fact, only a 1 *nm* shift has been observed for emission maxima associated with the physiological changes for all three phytoplankton. There was, however, a substantial change for a shoulder at 415 *nm* in the emission spectra of Olisth(L), i.e., a shift to 410 *nm* for an

Table 2. Fluorescence excitation and emission maxima for algae[a]

Sample	Emission(*nm*)	Excitation(*nm*)
GT429	386*(s)[b], 410(sh)	349*(s), 390(sh)[c]
Gport	384*(s), 410(sh)	350*(s), 390(sh)
Olisth	395(sm), 415(sh), 436*(s)	366*(s), 391(sh)
86W-2010	436*(s)	345*(s)
86W-2000	435*(s)	338*(s)
PJ/SYN	386*(m)	308(sm), 340(sm), 360*(s)
PJ/SPIR	384*(m)	340(sh), 360*(s)
8C	410(sh), 431(sh)	345*(m), 389(sm)
SYN	408*(s), 435(sh)	353*(s)
DC2	390*(s), 435(sh)	365*(s)

a: Excitation and emission slits were 10*nm* for scan. Average two scans
b: (*) indicates the most intense band.
c: (s) = strong, (m) = moderate, (sm) = small, (sh) = shoulder.

Table 3. Fluorescence excitation and emission maxima for algae[a] in different physiological states

Sample	Emission(*nm*)	Excitation(*nm*)
GT429(L)[b]	386*(s)[d], 410(sh)	3498(s), 390(sh[e]
GT429(S)[c]	385*(s)	350*(s), 390(sh)
Gport(L)	384*(s), 410(sh)	350*(s), 390(sh)
Gport(S)	383*(s), 411(sh)	350*(s), 391(sh)
Olisth(L)	395(sm), 415(sh), 436*(s)	366*(s), 391(sh)
Olisth(S)	410(sh), 435*(s)	369*(s)

a: Excitation and emission slits were 10*nm* for scan. Average two scans
b: (L) = Logarithmic growth state of algae.
c: (S) = Senescent growth state of algae.
d: (*) indicates the most intense band.
e: (s) = strong, (m) = moderate, (sm) = small, (sh) = shoulder.

older Olisth(S) culture.

Table 4 lists fluorescence characteristics of pteridine derivatives that were obtained in this study and partly reprinted from Dalterio *et al.*(1987). The fluorescence of these model compounds which exist in many microorganisms such as bacteria and phytoplankton was measured in order to give a comparison between the fluorescence properties of the model compounds and phytoplankton. When possible, the assignment of the primary fluorescence of phytoplankton to the specific pteridine derivative was attempted from the comparison of these fluorescence data. All eight fluorescence emission and excitation maxima of pteridine derivatives including pterin are given in Table 4. Although there is few exact matches between the fluorescence emission and excitation maxima of algae and candidate organic compounds, there are similiarities in the band shape and relative intensities between them. Especially the second group of phytoplankton, those which have emission and excitation maxima respectively at about 435 and 340~366 *nm* showed excitation and emission maxima values similar to those of Pterin, Biopterin, and Neopterin. For FMN and FAD, the emission and excitation maxima of these compound are quite different. According to the fluorescence data, the observed emissions at 380~435 *nm* are not seem to be originated from these compound. It is well known that the pH of the solution has a marked effect on the fluorescence of pteridine(Blakely, 1969). Many buffers also exert a marked quenching effect. One of the possible reason for few exact matches for emission and excitation maxima between phytoplankton and pteridine is existence of variable oxdized form in the whole cells as pteridine metabolites.

B. Lifetime Data

Fluorescence lifetimes measured for eight different phytoplankton with excitaiton at 430 *nm* are listed in Table 5. For phytoplankton, the fluorescence decays with excitation at 430 *nm* were descri-

Table 5. Fluorescence decay times(ns) for algae[a]

Sample	τ_1	τ_2	τ_3	χ^2 (CHISQ)
8C	1.12	3.64	*	0.90
J6F	0.65	5.20	*	1.23
DC2[b]	2.39	7.35	*	0.87
SYN	0.55	1.82	7.89	1.22
SYN/MIL	0.48	2.78	8.75	0.95
SYN/SPIR	0.94	3.70	8.93	0.85
862-2000	0.75	3.24	9.34	1.03
228	0.61	2.98	10.48	1.05

a: The wavelength of the excitation filter used was 430*nm* and the 470*nm* cut-off bandpass filter was used.

b: The decay time for DC2 were measured with excitaton at 340*nm* and emission at 430*nm*.

Table 4. Fluorescence excitation and emission maxima and fluorescence decay times of folic acid derivatives

Compound	Excitation(*nm*)		Emission(*nm*)		Lifetime(*ns*)
	Buffer[b]	MeOH	Buffer[b]	MeOH	Buffer[b]
FAD	379, 451, 467*	468*	520*	514*	3.39
FMN	379, 451, 467*	467*	520*	514*	4.66
Pterin-6-Carboxylic acid	356*	377*	438*	453*	4.47
Lumazine	331*	373*	458*	457*	6.57
Pterin	353*	356*	440*	435*	4.79
Biopterin	358*	360*	442*	437*	5.45
Neopterin	356*	360*	443*	437*	5.40
Folic acid	361*	442*	442*		1.60(1), 4.78(2)

a: Excitation and emission slits were 6*nm* for scans. Average two scans.

b: Reprinted from Dalterio et al.(1987).

bed as either bi- or triexponential decay functions. The shorter lifetimes for biexponential decays ranged from 0.65 to 1.12 *ns*. These are substantially shorter than those of bacteria(Dalterio *et al.*, 1987). The longer lifetimes are in the range of 3.64 and 5.20 *ns*, which are also considerably shorter and with greater variation than those of bacteria.

Three out of eight phytoplankton examined have shown biexponential decay characteristics(Figure 2) and distinctively different lifetimes for each species. The shorter lifetimes ranged from 0.65 to 2.39 *ns* and the longer life times from 3.64 to 7.35 *ns*. For the other five phytoplanktons, the fluorescence decays were best described by three-exponential decay functions(Figure 3). The shortest lifetimes ranged from 0.48 to 0.94 *ns*. The middle lifetimes ranged from 1.82 to 3.70 *ns* and the longest lifetimes ranged from 7.89 to 10.48 *ns*. The longest lifetimes have shown the greatest variation, 2.59 *ns*, between different species of phytoplankton. The data seem to suggest that there is greater variability in the types and/or relative amounts of the fluorescent components with longer lifetimes.

From Table 5, the lifetimes of phytoplankton are noted to be in the same region as those from molecular compounds(Table 4) pH 6.8 phosphate buffer solution. For example, phytoplankton 8C has shown the shorter lifetime as 1.12 *ns* and longer lifetime as 3.64 *ns*. These lifetimes are close to

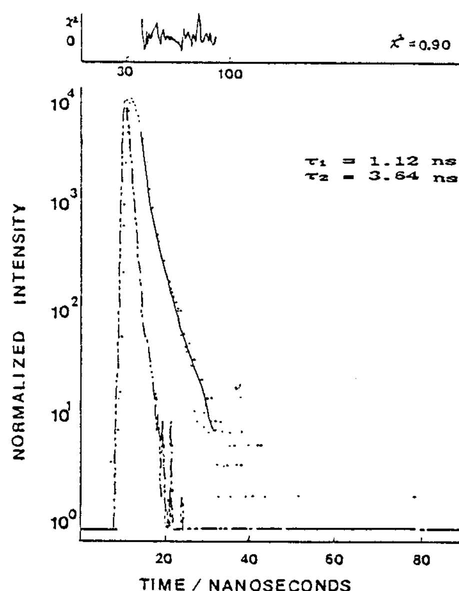

Fig. 2. Flashlamp excitation-time profile(----) and fluorescence decay from 8C (points and solid line "best fit"). Upper plot is weighted residuals for the calculated emission decay profile after deconvolution of the emission decay from channels 40~90.

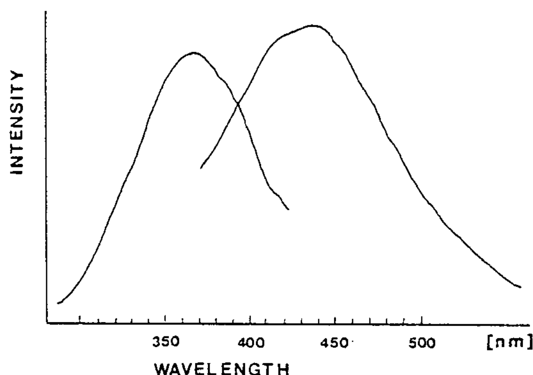

Fig. 1. Fluorescence excitation and emission profiles from Olisth. Excitation scans were obtained with $\lambda_{em}=450nm$ and emission scans with $\lambda_{ex}=340nm$. The fluorescence intensities were arbitrarily adjusted.

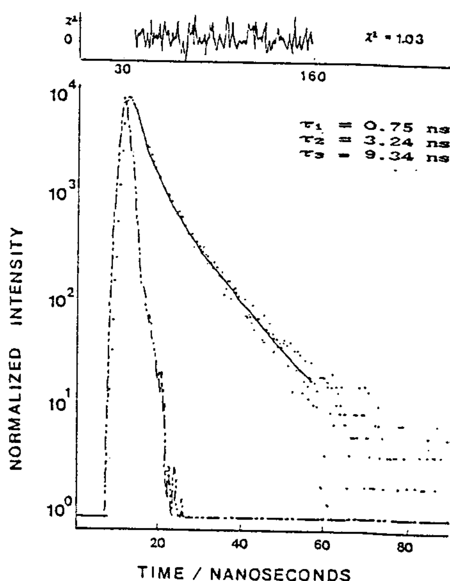

Fig. 3. Flashlamp excitation-time profile(----) and fluorescence decay from 86W-2000. Upper plot is weighted residuals for the calculated emission decay profile after deconvolution of the emission decay from channels 38~160.

those of folic acid which has $\tau_1=1.60$ *ns* and $\tau_2=4.78$ *ns*.

Discussion

A. Emission Spectra and Excitation Spectra

As shown in the case of bacteria(Dalterio *et al.*, 1987), all marine plankton algae examined also exhibited primary fluorescence emission maxima between 380 and 435 *nm* and the intensity of this fluorescence was in general quite high. This implies that the molecular species responsible must have wide-spread occurrence in phytoplankton and have relatively high quantum yields. Naturally occurring compounds satisfying these requirements are the nitrogen-heterocyclic pteridines, the structurally related flavins, and the pyridine coenzymes.

Pterin, a 2-amino-4-oxo-pteridine which is synthesized by most microorganisms with a p-aminobenzoyl glutamic acid side chain in the 6-position, comprises folic acid. Various pterins substituted in the 6-position, and as well as dihydro forms have been isolated and identified in phytoplankton(Blakely, 1969). Since Forrest *et al.*(1957) found a remarkably high concentraton of pteridines in blue-green algae, *Anacystis nidulans,* a number of different pteridines have been isolated and identified. For example, 2-amino-4-hydroxy-pteridine, 2,6-diamino-4-hydroxy-pteridine, and yellow pteridines which were later identified as 2-amino-4-hydroxy-6-propionyl-5,8 or 7,8-dihydropteridine, have been studied(Hartfield *et al.*, 1961). Most pteridines are intensely fluorescent(Blakely, 1969).

Other possible candidates for the origin of fluorescence between 380 and 435 *nm* are riboflavin and flavin mononucleotide(FMN) and flavin adenine dinucleotide(FAD). These molecular compounds are intensely fluorescent species(Dalterio *et al.*, 1987) that are synthesized by most or all microorganisms.

Since the observed fluorescence emission maxima appeared in the characteristic region which does not overlap with any other known fluorophores in the phytoplankton with quite high quantum yield, the fluorophores for the observed emission spectra must be present in high concentration with some biological function. The experimental data for assignment of the fluorophore may not be sufficient to pinpoint out specific compound. However, with all the information about pteridine and the observed fluorescence characteristics, pteridines seems to be the most possibile origin for the observed algal fluorescence at 380~435 *nm*. The fluorescence emission bands of phytoplankton between 360 and 435 *nm* are broader and smoother than those of bacteria. The broad emission bands of phytoplankton may due to the emission from a wide range of modified forms of fluorophore(s) at the region of the emission maximum wavelength.

B. Lifetime Data

The fluorescence decays of the several phytoplankton obtained with 430 *nm* excitation can be described as either bi- or tri exponential functions. None of the fluorescence decays of the phytoplankton examined can be fit with a monoexponential decay function. The biexponential decays of the three phytoplankton(8C, J6F and DC2) showed substantial differences in lifetimes. Greater differences were observed between the longer lifetimes than the shorter ones.

The heterogeneity of the primary fluorescence observed likely originates from either the many different fluorophores or a particular fluorophore in a variety of microenvironments. There is a reason to believe that many different fluorophores are present. Forrest *et al.*(1957) isolated and identified many different types of pteridine derivatives present in phytoplankton in high concentration. Several oxidized and reduced forms of the pteridine are possibly present also. A comparison between the fluorescence lifetime characteristics of phytoplankton and those of molecular compounds including folic acid and pteridine derivtives allows some assignments to be made for algal fluorescence.

As in the case of bacteria(Dalterio *et al.*, 1987), few exact matches are found in the comparison between the fluorescence decay characteristics of phytoplankton and those of candidate molecular compounds. The reason for this observation may be due to the many possible changes in fluorophores

and their microenvironments within the phytoplankton which are different from buffer solution environments. Increased quenching will reduce lifetimes. Several oxidized and reduced forms of model compounds in phytoplankton give rise to different lifetimes. Varying polarities of media cause changes. Binding to various macromolecules such as proteins, close proximity to membranes and to molecules to which energy can be transferred with subsequent fluorescence, and proximity to fluorescence quenchers can produce major lifetime changes(Stewart, 1974). All these various factors can affect energies of absorption and emission, the quantum yields, and the decay times.

Other possible cnandidates for the origin of the fluorescence between 380 and 435 *nm* are several reduced and oxidized flavoproteins. Wide-spread occurrence of the flavins in most or all microorganisms are well known(Dalterio *et al.,* 1987). Visser *et al.*(1979) have reported fluorescence lifetimes of several reduced and oxidized flavoproteins. Most showed biexponential decay characteristics. In most cases, greater than 50% of the fluorescence decayed between 0.8 and 3 *ns* and the longer lifetime component decayed between 4.7 and 10.8 *ns*. Based on lifetime values of flavoproteins reported and known for wide-spread occurrence in phytoplankton, the fluorescence observed with excitation at 430 *nm* and emission at 470 *nm* cut-off bandpass filters might be assigned to many of these compounds.

Earlier(Dalterio *et al.,* 1987) it was found that bacterial identification on the basis of steady-state and decay fluorescence parameters was possible, at least for certain well-defined systems. Especially, in the case of *P. fluorescens,* fluorescence was characterized by a single specific marker fluorophore, a pteridine.

A comparison of phytoplankton and bacterial fluorescence is instructive. For bacteria(Dalterio *et al.,* 1987), the fluorescence decay times with excitation at 430 *nm* were best described as either mono- or biexponential decay functions. *P. fluorescens*(Dalterio *et al.,* 1987) which has shown a monoexponential decay. The other four bacteria have shown biexponential decays with the shorter life times(1.64~2.38 *ns*) and the longer lifetimes(6.12~6.78 *ns*). The lifetime of *P. fluorescens* emission was 5.54 *ns*. For phytoplankton, the fluorescence decays with excitation at 430 *nm* were described as either bi- or triexponential decay functions. None of the phytoplankton have shown monoexponential decays. The longer lifetimes are in the range of 3.64 and 5.20 *ns*, which are also considerably shorter and with greater variation than those of bacteria. For DC2, on the other hand, the lifetimes were $\tau_1=2.39$ *ns* and $\tau_2=7.35$ *ns* with excitation 340 *nm* and emission 430 *nm* bandpass filters. These are similar to bacterial lifetimes(Dalterio *et al.,* 1987) which vary between 2.13 and 2.32 *ns* and 8.02 and 8.88 *ns* for the short and long lifetimes, respectively. Thus, the lifetimes of DC2 are very close to the lifetimes of bacteria.

However, the triexponential decays found under these conditions for five out of eight phytoplankton were very different from bacterial fluorescence decays. Thus, in most instances, in comparison of the fluorescence decay characteristics of phytoplankton and bacteria, substantial differences were found.

There are substantial differences in fluorescence spectra and lifetimes at the class level. At the species level, differences seem to be much smaller. However, it was not easy to find a common fluorescence features for a specific class, Cyanophyceae. Isolation of pteridine compounds which seem to be responsible for observed fluorescence or indirect measurement which can provide additional evidence for assignment of ptreridine as fluorophore are expected in the future, before the observed fluorescence can be used as marker compound for characterization of phytoplankton.

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Pteridine계 화합물의 *in-vivo* 형광 특성을 이용한 식물 플랑크톤의 동정에 관한 연구

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와편모조류, 남조류, Chloromonadophyceae와 Cryptophyceae를 포함한 12종의 해양 식물플랑크톤의 일차적 형광특성을 *in vivo* 상태에서 조사하였다. 형광과 exciatation 스펙트럼을 측정한 결과 약 380~435 *nm* 영역에서 강한 세기의 형광 스펙트럼이, 조사된 모든 식물플랑크톤으로 부터 얻어졌다. 식물플랑크톤의 성장 상태의 변화에 따른 형광 스펙트럼의 λ_{max}에 대한 영향을 관찰하였다. 관찰된 형광 스펙트럼의 형광소를 밝히기 위하여, 식물플랑크톤의 구성성분 중에 유사한 형광 특성을 보이는 8가지 pteridine계 화합물의 phosphate 완충용액에 대한 형광 특성과 식물플랑크톤의 380~435 *nm* 영역에서 나타난 스펙트럼의 세기와 모양, λ_{max}를 비교하였다. 식물플랑크톤의 fluorescence lifetime(τ)과 fluorescence decay curve를 식물플랑크톤에 존재하는 유기화합물들의 표준용액의 τ값과 비교하였다. 430 *nm*의 들뜨기 파장을 사용하여 얻은 식물플랑크톤의 fluorescence decay는 biexponential과 triexponential decay를 보였다. 박테리아와 식물플랑크톤의 형광 특성을 비교한 결과 형광 스펙트라뿐 아니라 붕괴 양상도 현저한 차이점을 보였다.