J. Fac. Appl. Biol. Sci.,
Hiroshima Univ. (1985), 24: 33 – 42

Dissolved Organic Phosphorus in Sea Water, its Molecular Weight Fractionation and Availability to Phytoplankton

Osamu MATSUDA, Ken-ichi OHMI and Ken SASADA

Faculty of Applied Biological Science, Hiroshima University, Fukuyama

Received: August 29, 1985

Although dissolved organic phosphorus (DOP) is one of the major fractions of phosphorus in sea water[1),2)] and plays a significant role in the phosphorus cycling especially in eutrophic coastal regions,[3)] principal constituents or chemical species of DOP are little known.[4),5)] This is also suggested by the usual analytical method of DOP because DOP in sea water is determined only by the difference between dissolved total phosphorus (DTP) and dissolved inorganic phosphorus (DIP).[6)] Therefore, DOP hitherto determined is only identified with "dissolved" and "unreactive" fraction of phosphorus.

In relation to the detailed characteristics of DOP, molecular weight of dissolved organic matter in some many fresh water samples were studied,[7),8),9)] but few studies have been conducted so far on the molecular weight fractionation of DOP in sea water. However, it has been experimentally demonstrated that some axenic culture of phytoplankton could utilize organic phosphorus compounds[10)] and that DOP was excreted into environmental media[11), 12)] through algal lysis, and also through grazing activity of zooplankton.[13)] Hence, it is very important to make clear the basic constituents and degradation processes of DOP in sea water, and also utilization of DOP by phytoplankton as phosphorus source.

This paper deals with firstly the molecular size fractionation of DOP in red tide sea water, secondly changes of molecular size distribution during its decomposition and thirdly the availability of DOP to the phytoplankton species isolated from red tide sea water. This study will present basic informations to the biological processes in eutrophic coastal environments especially in relation to the actual cycling of phosphorus in red tide water.

MATERIALS AND METHODS

Molecular weight fractionation of DOP

Molecular weight fractionation by ultrafiltration was employed to characterize DOP in sea water. As ultrafilter, Diaflo membrane (Amicon Co.) UM-05, UM-10 and XM-100 were used, sustaining molecular weight of which was approximately 500, 10000 and 100000 respectively. For filtering device, the ultrafiltration cell system (Amicon Co. type 202; 200 ml) was used under nitrogen gass pressure in the laboratory controlled at 10°C to avoid the deterioration of DOP during filtration.

To determine the DOP concentration of each molecular size fraction, DOP concentration of ultraconcentrate in the cell unit was firstly determined as the phosphorus concentration of the ultrafiltrate was liable to be influenced by the effects of ultrafilters. And then DOP concentration of each fraction was calculated by successive subtraction (Table 1). Exceptionally, as to the only one fraction, molecular weight of which was less than 500, DOP in the ultrafiltrate was directly analyzed and corrected by blank value. Thus DOP was finally classified into four fractions, Fr.1 to Fr.4, according to molecular weight (Table 1). In these series of analysis, organic phosphorus was converted to orthophosphate by potassium peroxodisulfate digestion based on the procedure of MENZEL and CORWIN[14] and the resulting phosphate was determined by the method of MURPHY and RILEY.[15]

Table 1. Scheme for the molecular weight fractionation of DOP by ultrafiltration and the approximate molecular weight range of the individual fraction (Fr.1 – Fr.4).

DOP in ultraconcentrate by XM-100	:	Fr.1	(100000 < MW)
difference	:	Fr.2	(10000 < MW < 100000)
DOP in ultraconcentrate by UM-10	:		
difference	:	Fr.3	(500 < MW < 10000)
DOP in ultraconcentrate by UM-05			
DOP in ultrafiltrate by UM-05	:	Fr.4	(MW < 500)

Although to reveal the molecular weight fractionation of DOP in every type of sea water is very important, usually DOP concentration in sea water is not so high as to avoid the effects of relatively high blank value by ultrafilter. For this reason, DOP in the red tide sea water in which DOP showed the highest concentration in the region was studied in the present work.

Sample sea water was taken at Tajiri port in Fukuyama, Japan on June 13, 1975 when the sea was strongly discolored by red tide. The dominant species of phytoplankton in the red tide was found to be *Heterosigma* sp. Then sea water sample was filtered through membrane filter (Millipore, HA) and filtrate was served for molecular weight fractionation.

For measuring the change in molecular size distribution of DOP during storage, the filtrate contained in glass bottle was kept in dark at 20°C and was served for successive subsampling during 18 days. In this experiment, as only dissolved fraction of phosphorus was analyzed, change in molecular weight of DOP and mineralization of DOP to DIP could be exclusively observed.

DOP availability test

As a test organism *Gymnodinium* sp. (F-4 strain) was employed in the DOP availability test. This dinoflagellate was found as dominant species of the red tide occurred off Fukuyama in September, 1973. The strain was isolated as an axenic culture from the red tide sea water and usually chain forming. Physiolosical characteristics of the strain

examined are presented in Table 2, which shows that this organism is relatively euryhaline and require vitamin B_{12} and the growth is stimulated by thiamine and biotin. The reaction of the organism to vitamin B_{12} derivertives was classified in the *Escherichia coli* type.

Table 2. Physiological characteristics of the test organism *Gymnodinium* F-4 strain.

Chlorinity	optimum range	8.0 – 12.0 o/oo
	favorable range[1)]	6.5 – 18.0 o/oo
pH	optimum point	8.5
	favorable range	7.1 – 8.9
Nitrogen[2)]	optimum source	NH_4Cl
	optimum concentration	0.3 mg N/l[3)]
	favorable concentration[1)]	0.1 – 1.5 mg N/l[3)]
Phosphorus[4)]	optimum source	K_2HPO_4
	optimum concentration	0.3 mg P/l[5)]
	favorable concentration	0.1 – 5.0 mg P/l[5)]
Vitamin B_{12}		essential
	optimum concentration	3 – 100 mμg/l
	response to derivertives	*Escherichia coil* type
thiamine		not essential but growth promoting
biotin		not essential but growth promoting

1) Range attainable for half of the maximum growth.
2) $NaNO_3$, NH_4Cl and urea were tested.
3) NH_4Cl was used as nitrogen source.
4) K_2HPO_4, Na_2-glycerophosphate, adenylic acid and guanylic acid were tested.
5) K_2HPO_4 was used as phosphorus source.

In the culture experiment of the *Gymnodinium* strain, growth was compared in three different types of media which stand for; (a) media containing individual molecular weight fraction of DOP as a sole source of phosphorus, (b) media containing K_2HPO_4 as phosphorus, (c) media containing glycerophosphate as phosphorus. In case of (b) and (c), phosphorus was set up in ten different concentrations. Growth of the test organism was compared at the stationary phase after 21 days of incubation at 20°C under 3000 lux of illumination.

Among those culture media preliminary tested which were ASP_2, ASP_2NTA, ASP_7, ASP_7M, ASP_{12}, $ASP_{12}NTA$, *Gymnodinium* F-4 strain showed the most satisfactory growth in the ASP_7M medium. For this reason ASP_7M was selected as a main base medium of the examination together with ASP_2NTA which was also served as auxiliary base medium. Compositions of both media employed are presented in Table 3.

Table 3. Composition of artificial sea water media ASP_7M and ASP_2NTA.

ASP_7M			ASP_2NTA		
DW	100	ml	DW	100	ml
NaCl	2.5	g	NaCl	1.8	g
$MgSO_4 \cdot 7H_2O$	0.9	g	$MgSO_4 \cdot 7H_2O$	0.5	g
KCl	70	mg	KCl	60	mg
Ca (as $CaCl_2$)	30	mg	Ca (as $CaCl_2$)	10	mg
$NaNO_3$	5	mg	$NaNO_3$	5	mg
Na_2-glycerophosphate	2	mg	K_2HPO_4	0.5	mg
$Na_2SiO_3 \cdot 9H_2O$	7	mg	$Na_2SiO_3 \cdot 9H_2O$	15	mg
P II metals [1]	1	ml	P II metals	3	ml
S II metals [2]	1	ml	Na_2CO_3	3	mg
Fe (as Fe·EDTA)	30	μg	Fe (as $FeCl_3$)	50	μg
Vitamin mixture I [3]	0.1	ml	Vitamin B_{12}	0.2	μg
Tris	0.1	g	Vitamin mixture S3 [4]	1	μg
NTA	7	mg	Tris	0.1	g
pH	7.8 – 8.0		pH	7.8	

1) One ml of P II metals contains: Na_2EDTA 1 mg, B (as H_3BO_3) 0.2 mg, Fe (as $FeCl_3$) 10 μg, Mn (as $MnCl_2$) 40 μg, Zn (as $ZnCl_2$) 5 μg, Co (as $CoCl_2$) 1 μg.
2) One ml of S II metals contains: Br (as $NaBrO_3$) 1 mg, Sr (as $SrCl_2$) 0.2 mg, Rb (as RbCl) 20 μg, Li (as LiCl) 20 μg, Mo (as Na_2MoO_4) 50 μg, I (as KI) 1 μg.
3) One ml of vitamin mixture I contains: Vitamin B_{12} 0.2 μg, biotin 1μg, thiamine 0.1 mg.
4) One ml of vitamin mixture S3 contains: thiamine 0.05 g, nicotinic acid 0.01 mg, Ca pantothenate 0.01 mg, p- aminobenzoic acid 1.0 μg, biotin 0.1 μg, inositol 0.5 mg, folic acid 0.2 μg, thymine 0.3 mg.

It has been reported that not a few species of dinoflagellate can multiply to some extent in the phosphate free media after new inoculation because the cell can reserve enough phosphate for a successive few cell divisions. To avoid the influence of this phenomenon known as excess uptake or luxury consumption of phosphate, starve culture was made in the phosphate free ASP_7M medium during 19 days to exhaust reserved phosphorus in the cell. After that, these phosphate starving culture of *Gymnodinium* was served to DOP availability test.

RESULTS

Molecular size distribution of DOP and its changes during decomposition

Molecular size distribution of DOP and its changes during the decomposition experiment are shown in Fig.1, which showed that DOP strongly dominated among dissolved fraction of phosphorus at the initial stage. Therefore it was suggested that DIP were found to be almost converted to DOP and to cell phosphorus of red tide organism in the sampled sea water. Among DOP, the lowest molecular weight fraction (MW < 500 ; Fr.4) was dominant at the initial stage. Next to Fr.4, the highest molecular weight fraction (MW > 100000; Fr.1) occupied the second largest portion.

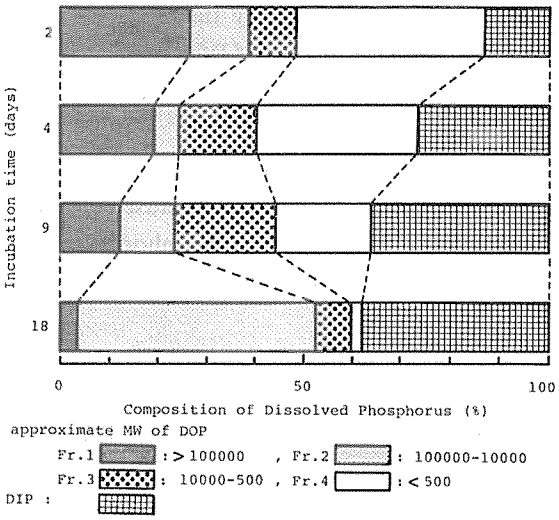

Fig. 1. Molecular size distribution of dissolved organic phosphorus (DOP) in red tide sea water and its change during decomposition with special reference to dissolved inorganic phosphorus (DIP) concentration.

During the time course of decomposition, the percentage of DOP in dissolved phosphorus gradually decreased from 86% at the initial condition to 62% on the 18th day on the contrary to the increase of DIP, which showed the obvious mineralization of DOP (Fig.1). Mean mineralization rate itself decreased with time showing 0.101 μg-at/l/day in the first stage, 0.03 μg-at/l/day in the middle stage and 0.0017 μg-at/l/day at the last stage of decomposition (Fig. 2).

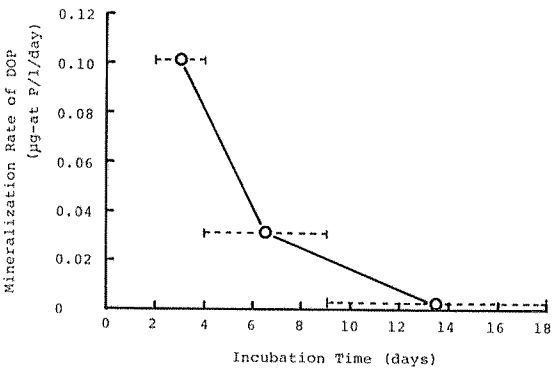

Fig. 2. The change in mean mineralization rate of DOP during the storage at 20°C in dark.

As to the change of individual fraction, decrease of Fr.1 with time stood for the lowering of the molecular size in this fraction. The lowest molecular size fraction Fr.4 also decreased with time which showed that decomposition rate of this fraction was higher than the producing rate of this fraction by the decomposition of larger molecules. Hence as a result, the amount of the fraction is considered to decreased with time.

Although the variation of medium sized fraction Fr.2 and Fr.3 is not so simple to interprete, changes until 9th day seemed to represent the lowering of the molecular

size of Fr.1 and Fr.2 and simultaneous production of Fr.2 and Fr.3. Remarkable increase of Fr.2 during the latter half of the experiment may denote not only the production of Fr.2 by the decomposition of Fr.1 but also some synthetic process of DOP molecules by use of Fr.3 and/or Fr.4. But the detailed process of synthesis could not be directly identified in this study.

These results showed that the DOP investigated was composed not only of refractory fractions but of easily decomposable fraction. This kind of labile DOP must be more important in phosphorus cycling because that may be active in phosphorus transformation through microbiological activities and liable to be used as phytoplankton nutrient.

DOP availability test

Growth of *Gymnodinium* F-4 in ASP_7M base media was compared between different phosphorus sources, which were the individual molecular fraction of DOP and Na_2-glycerophosphate (Fig. 3). The results showed that in the standard series of glycerophosphate, the growth increased with phosphate concentration until about 5 mg P/l. In this concentration range, phosphate was found to be limiting among many nutrients in this culture system.

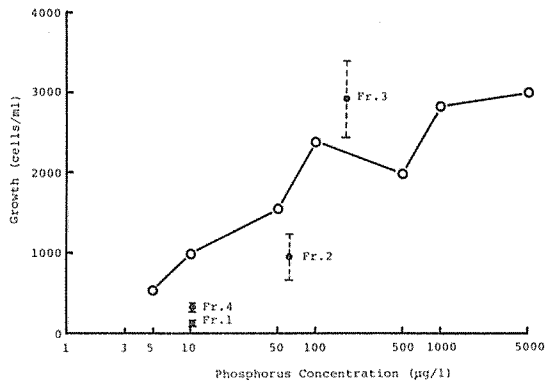

Fig. 3 The effect of individual DOP fraciton (Fr.1–Fr.4) as a sole source of phosphorus on the growth of *Gymnodinium* sp. (solid circle). The result of controlled experiment (open circle) shows the growth by Na_2-glycerophosphate at various concentrations. Both experiments were conducted using the ASP_7 M base media.

Accordingly, the DOP availability to the organism can be estimated by the relation between phosphorus concentration and growth. From this point of view, the growth by DOP Fr.1, 2, 4 except Fr.3 was generally found lower than the growth by glycerophosphate. This means that availability of these DOP fractions were low or growth inhibiting substance was contained at the same time in those fractions.

On the other hand, by DOP Fr.3 (MW; 500–10000) *Gymnodinium* showed higher growth than by glycerophosphate, which meant sufficient availability of the DOP fraction. While growth stimulating substance contained in the molecular size fraction might be also suggested because Fr.3 contained not only DOP but also whatever of the same molecular size in the sea water tested.

Another DOP availability test using ASP_2NTA as base media showed that the growth given by any of DOP fractions (Fr.1–Fr.4) exceeded that of controlled experiment given by K_2HPO_4 (Fig. 4). This result means firstly that all of DOP fraction were efficiently utilized as phosphorus sources. And secondly out of these four fractions, Fr.3 showed the most remarkable effect on the growth also in this experiment.

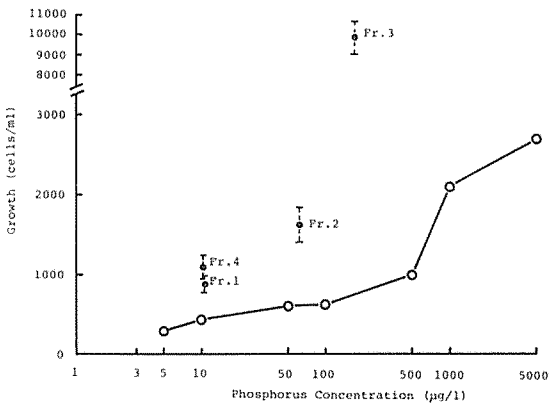

Fig. 4. The effect of individual DOP fraction (Fr.1–Fr.4) as a sole source of phosphorus on the growth of *Gymnodinium* sp. (solid circle). The result of controlled experiment (open circle) shows the growth by K_2HPO_4 at various concentrations. Both experiments were conducted using the ASP_2NTA base media.

From these results of DOP availability test, it is concluded that any of four molecular size fractions of DOP in the red tide sea water could be available to the *Gymnodinium* strain as phosphorus sources, among which DOP Fr.3 (MW; 500–10000) was especially effective on the algal growth. Almost the same kind of result was led also by DOIG et al.[16] as to the effects of soil extracts on the Florida red tide organism *Gymnodinium breve*.

DISCUSSION

The result that organic fraction occupied high percentage in total dissolved phosphorus in the sample sea water and that the molecular size distribution of DOP changed in the relatively short period may support that DOP analyzed in the present study was fresh metabolite or exudate of the red tide organism.

The changes in molecular size distribution of DOP was compared with those of DOC studied by OGURA[17), 18)] where sea water was obtained at Tokyo Bay and incubated in the same condition as the present experiment (20°C in dark) during 41 days. Types of ultrafilters employed were also the same. In the case of DOC, higher molecular fraction was dominated in the first stage in contrast to the result of DOP fractionation. The DOC fraction, molecular weight of which was higher than 10000 stood for more than 65% at the initial condition of the experiment. The highest molecular weight fraction of DOC ($MW > 100000$) did not show any remarkable decrease unlikely to the case of DOP. The amount of the lowest molecular weight fraction of DOC ($MW < 500$) remained still pretty high at even final stage of decomposition.

These comparison leads that DOP is more liable to be decomposed than DOC from

the view point of molecular size changes, although sample examined was not exactly the same. And it is also suggested that molecular weight of phosphorus containing organic matter was smaller than that of non-phosphorus containing organic matter.

In the DOP availability test conducted, it is inevitable that each molecular size of DOP fraction contains a small amount of reactive phosphate (DIP) as impurities which may derived from decomposition of DOP or desorption from ultrafilters through experimental processes. Accordingly, the effect of concomitant DIP must be properly estimated to evaluate the effect of DOP fractions. As the effect of DIP on the growth is the same as that of K_2HPO_4, corrected effect of DOP itself could be estimated.

As a result, the effect of concomitant DIP on the growth was unattainable to change the conclusion that DOP was utilized as phosphorus sources because the effect of DOP especially of Fr.3 was so obvious. For example, in case of Fig.4 the highest growth of about 10000 cells/ml was attained in the lower concomitant DIP concentration of 36.7 μg P/l. In relation to the effect of the concomitant organic matter with DOP fractions, PRAKASH and RASHID[19] also made it clear that the low molecular weight fraction of humic acid produced the greatest growth response in unialgal cultures of marine dinoflagellates.

When DOP was utilized by the algae, it is not readily clear whether DOP was directly used or utilized through DIP after mineralization during the incubation time. But taking account of the another result that the same *Gymnodinium* strain can grow by adenylic acid or guanylic acid as a sole source of phosphorus in the same culture condition (Table 2), the direct utilization of DOP is also strongly suggested. If some parts of DOP were utilized indirectly, this result is interpreted ecologically as that DOP fraction was utilized after all in relatively short period of time. In the actual eutrophic condition, microbial activities, for example *in situ* phosphatase activity, are so high that the proportion of the indirect utilization of DOP might not be small.

Among dinoflagellate species which composed of red tide, some were experimentally proved to utilize not only orthophosphate but organic phosphorus compounds such as glycerophosphate and adenylic acid as phosphorus sources.[20] On the other hand, chemical species of DOP in sea water have not yet been obvious. Furthermore, *in situ* uptake of DOP by red tide organism has not almost been elucidated. Hence that the known organic phosphorus compound is utilized by culture experiment does not mean that DOP in sea water can be available *in situ* by phytoplankton.

However, the direct utilization of DOP suggested in this study seems to be very important because the process means shortcut pathway of phosphorus cycle. The pathway of phosphorus; phytoplankton → DOP → phytoplankton, may be more advantageous to phytoplankton growth compared with usual pathway via mineralization of organic phosphorus such as; phytoplankton → DOP → DIP → phytoplankton. This kind of shortcut pathway seem to play a important role especially at blooming of red tide organisms.

ACKNOWLEDGMENT

We wish to thank Professor T.ENDO of Hiroshima University for his helpful advice and for valuable discussion. Thanks are also due to Miss. M.FUJII and to Miss S. KOBATAKE of the same university for their assistance of arranging the manuscript.

SUMMARY

Dissolved organic phosphorus (DOP) is one of the major forms of phosphorus in coastal sea water. However, principal constituents, utilization and decomposition of DOP in the sea are little known. This paper dealt with molecular size fractionation of DOP in red tide sea water, changes of molecular size distribution during decomposition and availability of DOP to phytoplankton.

For molecular weight (MW) fractionation, ultrafiltration using Diaflo membrane UM-05, UM-10, MX-100 (retaining MW approx. 500, 10000, 100000 respectively) was employed and DOP was fractionated into four fractions according to MW. In the initial red tide sea water, the lowest MW fraction (MW < 500) was most predominant, followed by the highest MW fraction (MW > 100000). During the incubation, DOP as a whole gradually decomposed to DIP. The lowest MW fraction mineralized immediately and the highest MW fraction also decomposed. While the intermediate MW fraction showed a tendency to concentrate on the second highest MW fraction (10000 < MW < 100000).

The availabilty test of each molecular weight fraction of DOP to red tide organism *Gymnodinium* sp. revealed that every DOP fraction was utilized as a sole source of phosphorus, among them DOP Fr.3 (500 < MW < 10000) showed the most successful effect on the growth. These results suggested the actual cycling and important role of DOP in the eutrophic coastal region especially at blooming of phytoplankton.

REFERENCES

1) STRICKLAND, J.D.H. and AUSTIN, K.H. : *J. Fish. Res. Board Can.*, **17**, 337–345 (1960).
2) MATSUDA, O., ENDO, T. and KOYAMA, H. : *J. Fac. Fish. Anim. Husb. Hiroshima Univ.*, **14**, 217–240 (1975).
3) STUMM, W. and STUMM-ZOLLINGER, E. : in "Water Pollution Microbiology" (MITCHEL, R. ed.), pp. 11–42, Wiley-Interscience, New York (1972).
4) HOOPER, F.F. : in "Environmental Phosphorus Handbook" (GRIFFITH, E.J. et al. ed.) , pp. 179–201, John Wiley & Sons, New York (1973).
5) COSGROVE, D.J. : in "Advance in Microbial Ecology" (ALEXANDER, M. ed.) Vol.1, pp. 95–134, Plenum Press, New York (1977).
6) WATT, W.D. and HAYES, F.R. : *Limnol. Oceanogr.*, 8, 276–285 (1963).
7) LEAN, D.R.S. : *J. Fish. Res. Board Can.*, **30**, 1525-1536 (1973).
8) LEAN, D.R.S. and NALAWAJKO, C. : *J Fish. Res. Board Can.*, **33**, 1312–1323 (1976).
9) MINEAR, R.A. : *Environmental Sci. & Technol.*, **6**, 431–437 (1972).
10) PINTER, I.J. and PROVASOLI, I. : in "Symposium on Marine Microbiology" (OPPENHEIMER, C.H. ed.), pp. 114–121, Charles C. Thomas Publisher, Springfield (1963).
11) JOHANNES, R.E. : in "Advance in Microbiology of the Sea" (DROOP, M.R. and WOOD, E.J.F. ed.), Vol.1, pp. 203–213, Academic Press, London (1968).

12) FOGG, G.E.: *Water Res.*, 7, 77–91 (1973).
13) POMEROY, L.R., MATHEWS, H.M. and MIN, H.S. : *Limnol. Oceanogr.*, 8, 50–55 (1963).
14) MENZEL, D.W. and CORWIN, N. : *Limnol. Oceanogr.*, **10**, 280–282 (1965).
15) MURPHY, J and RILEY, J.P. : *Anal. Chim. Acta.*, 27, 31–36 (1962).
16) DOIG, M.T. and MARTIN, D.F. : *Water Res.*, 8, 601–606 (1974).
17) OGURA, N. : *Marine Biology*, 24, 305–312 (1974).
18) OGURA, N. : *Marine Biology*, **31**, 101–111 (1975).
19) PRAKASHI, A. and RASHID, M.A. : *Limnol. Oceanogr.*, **13**, 598–606 (1968).
20) MAHONEY, J.B. and MACLAUGHLIN, J.J.A. : *J. exp. mar. Biol. Ecol.*, **28**, 53–65 (1977).

海水中溶存有機態リン（DOP）の分子量分画と植物プランクトンによる利用

松田　治・近江謙一・佐々田　憲

DOPは海水中リンの基本的形態の一つであり，沿岸海域のリン循環の中で重要な地位にあると考えられる。しかし，その実体と現場での生成・消費・分解機構には不明な点が多い。そこでDOPの基本的性状を限外濾過法により，特に分子量サイズの面から検討した。さらにDOP分解時の分子量変化と植物プランクトンによるDOP画分の利用に関する実験を行って，DOPの現場における役割の一端を明らかにした。

限外濾過膜として3種のDiaflo膜，即ちUM－05，UM－10，XM－100，（Amicon社；分画分子量それぞれ約500，10000，100000）を用い，限外濾過用セル（Amicon社202型）を使用してDOPを4種に分画した。試水として1975年6月の赤潮海水を用い，20℃暗所に保蔵した際のDOP分子量分布の経時変化を18日間観察した。次にDOPの分子量別各画分のみをリン源として鞭毛藻*Gymnodinium* F－4株を培養し，その増殖を比較することにより，DOPが植物プランクトンのリン源として，どの程度有効であるかを判定した。

実験初期には試水中溶存態リンの中でDOPが86％と非常に高い割合を占めていたが，分子量別では低分子DOP画分Fr.4（MW＜500）が38％と最も優勢で，これに次ぎ高分子画分Fr.1（MW＞100000）の比率が高かった。DOPは次第に分解してDIPが増加したが，分解時の変化を分子量別に見ると，大部分の高分子画分Fr.1は低分子化し，Fr.4は最も速やかに無機化することが判明した。中間的分子量をもつFr.2（MW；10000－100000），Fr.3（MW；10000－500）も9日目頃まで次第に低分子化したと考えられる。赤潮海水中DOPの分子量別画分のみをリン源とした培養実験の結果からは，4画分とも*Gymnodinium*のリン源として利用され，そのうち特にDOP Fr.3が顕著な増殖効果をもつことが明らかになった。これらは赤潮発生時などにおけるリン循環の短絡経路を示唆するものと考えられる。