Properties of alkyl β-D-glucoside and alkyl β-D-maltoside micelles

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Characterization of alkyl β-D-glucoside (C_nG) and alkyl β-D-maltoside (C_nM) micelles has been studied. A (−) to (+) bisignate CD of (4*Z*,15*Z*)-bilirubin-IXα (BR) is induced by C_nG or C_nM micelles, indicating that BR bound to the glycolipid micelles preferentially takes an (*S*)-helix configuration. The intensity of the CD induced by the C_nM micelle is stronger than that induced by the C_nG micelle. In order to understand such a phenomenon, the characterization of the C_nG and C_nM micelles has been investigated. The fluorescent probe method using 1,3-bis(1-pyrenyl)propane (P3P), pyrene and 1-pyrenecarbaldehyde suggests that C_nM provides less lipophilic and less fluid micelles compared with C_nG. Such a difference in micelle properties is reflected in the solubilization phenomena of C_nG and C_nM micelles. The C_nG micelles solubilize well the lipophilic solutes such as P3P and 5,10,15,20-tetraphenylporhyrin. The less fluid microenvironment and higher microscopic concentration of the glucopyranose unit of the C_nM micelle seems to be preferable for forming an optically active BR–glycolipid complex.

Non-ionic amphiphiles having sugar head groups, glycolipids, are very important as surfactants whose micelles extract membrane proteins without denaturation.[1] Glycolipids are also important as biodegradable detergents.[2] Few studies on the characterization of the glycolipid micelles, however, have been reported. Shinoda *et al.*[3] have determined the critical micelle concentrations (cmc) of 1-octyl (C_8G), 1-decyl ($C_{10}G$) and 1-dodecyl β-D-glucosides ($C_{12}G$) from the measurements of surface tensions to be 2.5×10^{-2}, 2.2×10^{-3} and 1.9×10^{-4} mol dm^{-3}, respectively. Light scattering measurements provide a similar cmc value of C_8G and an average aggregation number of 87, which is larger than those of other micelles formed by surfactants having an octyl group.[4] The shape of the C_8G micelle has been assumed to be ellipsoidal.[4] The cmc and the aggregation number of C_8G obtained from fluorescence methods[5] are in good agreement with those reported previously. The cmc value of 1-nonyl β-D-glucoside (C_9G) has also been determined to be 6.5×10^{-3} mol dm^{-3}.[6] Although the reason for the sudden decrease in solubilities of $C_{10}G$ and $C_{12}G$ in water has not been clarified yet, formation of a bilayer structure has been speculated in the $C_{10}G$ and $C_{12}G$ systems.[6] Lipids having a maltopyranose residue show cmc values similar to those of the corresponding glycolipids. For example, the cmc values of 1-octyl (C_8M), 1-decyl ($C_{10}M$) and 1-dodecyl β-D-maltosides ($C_{12}M$) are 2.65×10^{-2}, 2.20×10^{-3} and 1.6×10^{-4} mol dm^{-3}, respectively.[7] The aggregation number of the $C_{12}M$ micelle has been estimated to be 110 from fluorescence quenching[8] and 5×10^4 Da, which corresponds to an aggregation number of 98, from gel filtration.[1] The measurements of surface pressures of $C_{12}M$–dodecyl octaoxyethylene glycol monoether and of ΔpK_a values of 4-heptadecyl-7-hydroxycoumarin and 4-(octadecyl)-1-naphthoic acid in the aqueous $C_{12}M$ solutions suggest that the $C_{12}M$ micelle possesses an 'aqueous-like' microenvironment.[9] The difference in nature between the C_nG and C_nM micelles has been found to be the dependence of the lipid concentration on the micelle sizes.[10,11] At concentrations above cmc, $C_{12}M$ provides micelles having a constant size while the C_8G micelles gradually enlarge with increasing C_8G concentration. A large difference has also been observed in the induced circular dichroism (ICD) of cresol red.[10] The signal intensities of ICD in the $C_{12}M$ micellar system are much larger than those in the C_8G system.

In this study, we carried out the characterization of the glycolipid micelles formed by C_nG and C_nM. The marked differences between these two types of micelles were observed in the ICD of (4*Z*,15*Z*)-bilirubin-IXα (BR) and fluidities, polarities and solubilization rates of lipophilic solutes.

Results and discussion

CD spectra of BR

BR is a tetrapyrrole bile pigment produced by metabolism of heme.[12] Although BR does not have a chiral centre, the intramolecular hydrogen bonds cause the formation of conformational enantiomers (Fig. 1). In solution, a rapid interconversion between (*S*)- and (*R*)-helix BR occurs. Although BR is not optically active in solution, BR becomes optically active when the interconversion is restricted by complexation with chiral hosts.[13] Lightner *et al.*[14] have found that the (−) to (+) bisignate CD signals of BR appear in water when BR complexes with α-, β- or γ-cyclodextrin. The CD spectroscopic data reveal that BR bound to cyclodextrin essentially takes the (*S*)-helix configuration [(*M*)-helicity]. Cyclodextrin-induced CD is observed only when the carboxyl groups of BR are dissociated. We concluded that this conformational enantiomerism is promoted by a hydrogen-bonding interaction between the CO_2^- groups of BR and the secondary OH groups of cyclodextrin and that the cyclic cavity of cyclodextrin does not play an essential role for the enantiomerism of BR.[15] We also found that the bisignate CD Cotton effect is observed when non-cyclic oligosaccharides such as maltose, maltotriose and maltoheptaose are used in place of cyclodextrins.[16] No effect was measured in the case of the monosaccharide, glucose. In this study, we examined the micellar effects on the conformational enantiomerism of BR using mono- and di-saccharides with hydrophobic alkyl chains as chiral surfactants.

The ICD spectra of BR in the C_8G and C_8M micellar solutions at pH 11 are shown in Fig. 2. In both cases, the (−) to (+) bisignate Cotton effect was observed in the CD spectra of BR. According to the exciton-coupling theory applied to bisignate CD Cotton effects,[17] it can be concluded that BR bound to glycolipid micelles selectively has an (*M*)-helicity. The intensity of ICD obtained in the C_8M micellar system is stronger than that in the C_8G system. In the case of glucose itself, no ICD of BR is observed even at very high concentrations of the sugar. At concentrations below cmc, neither C_8G nor C_8M induce the bisignate CD signals.

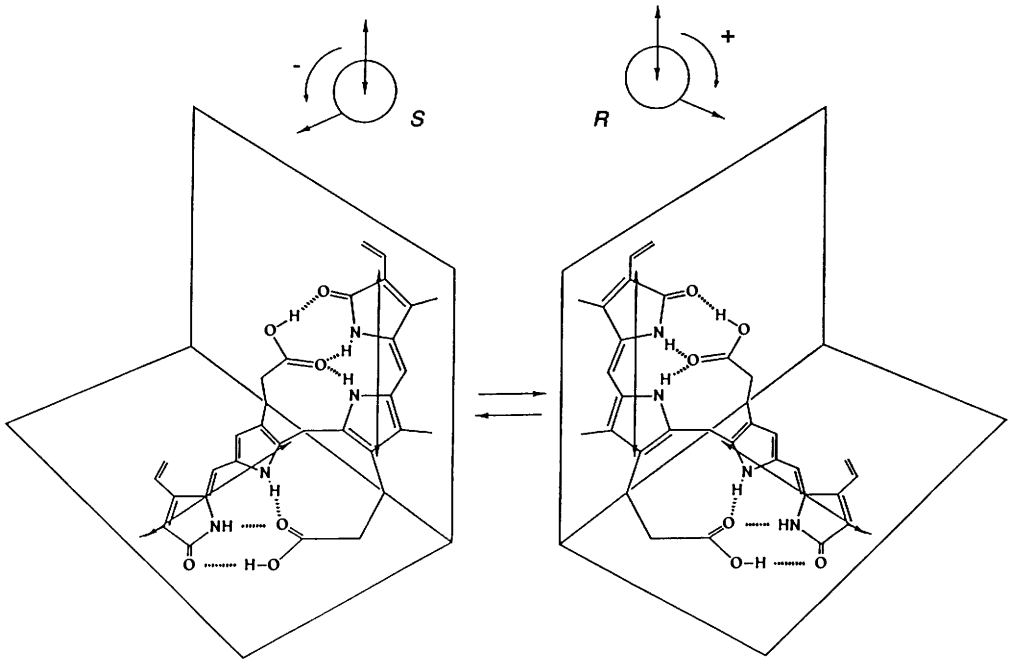


Fig. 1 Interconversion between conformational enantiomers of BR

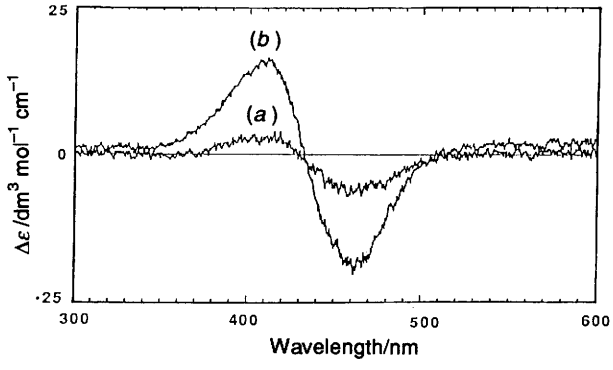


Fig. 2 CD spectra of BR (2×10^{-5} mol dm^{-3}) in the (*a*) C_8G and (*b*) C_8M micellar solutions at pH 11. The concentrations of C_8G and C_8M were 3.5×10^{-2} and 4.0×10^{-2} mol dm^{-3}, respectively.

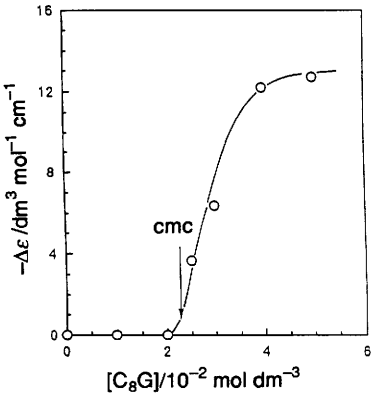


Fig. 3 CD intensity of BR (2×10^{-5} mol dm^{-3}) as a function of concentration of C_8G. The $\Delta\varepsilon$ values ($\Delta\varepsilon = [\theta]/3300$ dm^3 mol^{-1} cm^{-1}) were measured at 470 nm.

The C_8G concentration effect on the CD intensity is shown in Fig. 3. The micelle formation is essential to induce the optical activity of BR. The interactions between BR and bile salts have extensively been studied.[18] It has been reported that BR is bound to bile salts at the concentrations below cmcs of the salts

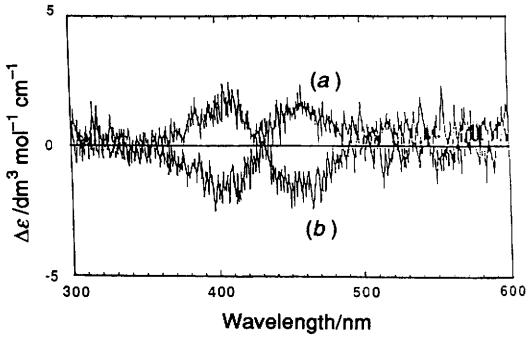
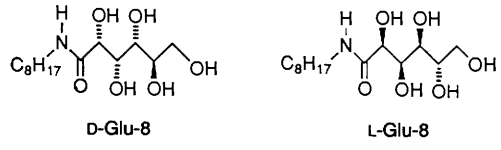


Fig. 4 CD spectra of BR (2×10^{-5} mol dm^{-3}) in (*a*) D-Glu-8 (2×10^{-2} mol dm^{-3})–SDS (2×10^{-2} mol dm^{-3}) and (*b*) L-Glu-8 (2×10^{-2} mol dm^{-3})–SDS (2×10^{-2} mol dm^{-3}) co-micellar solutions at pH 11 and 25 °C



through hydrophobic[19] and/or hydrogen-bonding interactions.[13] In both cases of C_8G and C_8M, no evidence for binding at concentrations below their cmcs was obtained from the CD measurements. This may be ascribed to the weaker interaction between glycolipid in the monomer and/or pre-micelle forms and BR compared with the BR–bile salt systems. The cyclic structures such as glucopyranose and maltopyranose residues are not essential to induce CD of BR. The mirror-image CD spectra of BR were recorded when co-micelles of D-Glu-8 and sodium dodecyl sulfate (SDS) and of L-Glu-8 and SDS were used (Fig. 4). Peracetylated D-Glu-8 and L-Glu-8 did not induce CD of BR, indicating that the secondary OH groups of the sugar attached to the chiral centres are important to cause the conformational enantiomerism of BR. The pK_a values of BR have been estimated to be 8.12 ± 0.23 and 8.44 ± 0.33 using a rapid partition method,[20] where the effects of the intermolecular hydrogen bonds of BR on the pK_a values can be neglected.

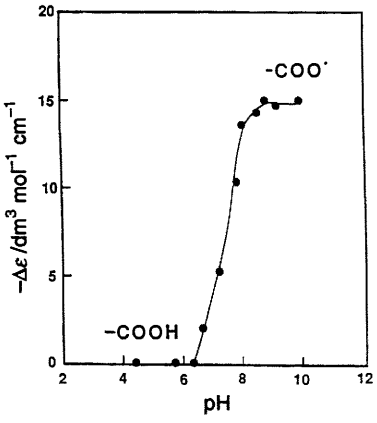


Fig. 5 CD intensity of BR (2×10^{-5} mol dm^{-3}) as a function of pH of the aqueous C_8M (10^{-3} mol dm^{-3}) solution. The CD intensities were measured at 470 nm.

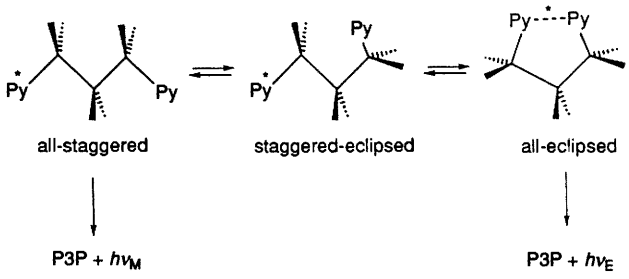
Therefore, it is quite reasonable to conclude that BR is in a dianion form in water at pH values above 9. Deprotonation of the pyrrinone or pyrrole residue occurs at pH > 12,[21] the trianion of BR does not participate in the present systems. A 'lock and key' mechanism cannot be applied to the conformational enantiomerism of BR induced by the glycolipid micelles because of the fluctuating nature of the micelle. It is reasonable to assume, therefore, that the hydrogen-bonding interaction between the CO_2^- groups of BR and the secondary OH groups of the sugar head groups is essential to induce the optical activity of BR, though the main binding factor for forming the BR–micelle complex seems to be hydrophobic and/or van der Waals interactions. The pH titration curve on the CD intensity of BR in the $C_{12}M$ micellar system is shown in Fig. 5, which indicates that a neutral form of BR provides only a racemic BR–micelle complex. This result also supports hydrogen bonding between the CO_2^- groups of BR and the OH groups of the glycolipid as the force required to induce the helicity of BR. We have demonstrated that hydrogen bonding between the CO_2^- groups of BR and the secondary OH groups of β-cyclodextrin is a driving force for conformational enantiomerism of BR induced by β-cyclodextrin.[15,16] A recent study shows that micelles provide an environment which is preferable for forming hydrogen bonds in water.[22] Since glucose itself does not induce CD of BR, the effect of micellization on ICD of BR is extremely remarkable in the case of C_nG. The enhanced binding of the BR dianion with the sugar molecule, the dehydration from the sugar head groups and the restricted motion of the sugar molecule, which occur upon micellization, may promote the formation of the intermolecular hydrogen bonds to fix partially the conformation of BR.

In order to improve our understanding of these effects, we tried to characterize these micelles.

Microscopic fluidities of C_nG and C_nM micelles

Since the formation of the pyrene (Py) excimer proceeds at a diffusion-controlled rate, this photochemical process can be used to estimate microscopic fluidities of micellar interiors.[23] However, a statistical distribution of the probe molecules among micelles complicates the treatment of the results.[24] We used 1,3-bis(1-pyrenyl)propane (P3P), which forms an intramolecular excimer *via* a process exhibited in Scheme 1, as a convenient fluorescent probe for evaluating relative microscopic fluidities of micelles.[25] The time-dependent fluorescence intensity change of the P3P excimer is expressed by eqn. (1)

$$I(t) = A \exp(-k_1 t) + B \exp(-k_2 t) \qquad (1)$$



Scheme 1

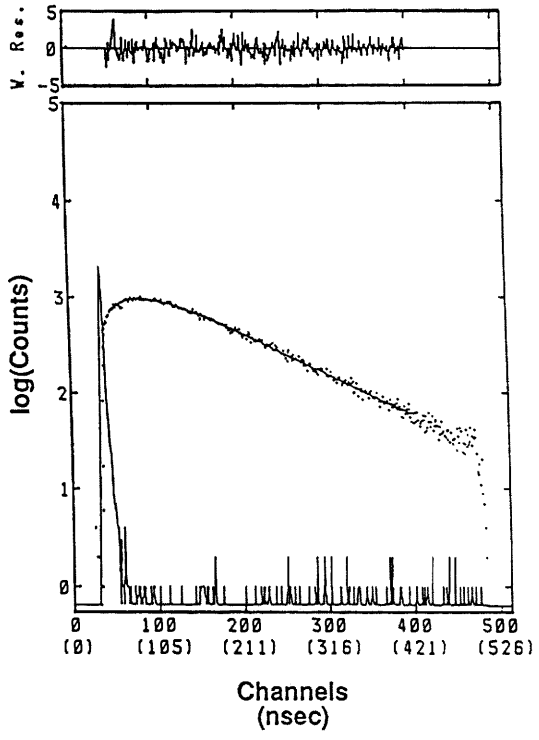


Fig. 6 Rise and decay curve of the intramolecular excimer fluorescence of P3P (5×10^{-6} mol dm^{-3}) in aqueous C_9G (10^{-2} mol dm^{-3}) solution. P3P was excited at 333 nm (the light pulse is shown as a peaked curve by a solid line) and the excimer fluorescence was extracted by passing the fluorescence through a Toshiba VY-47 filter (> 470 nm, 50% transmittance at 470 nm).

and the rise time of the P3P intramolecular excimer is defined by eqn. (2) where k_1 and k_2 are the rate constants for

$$\tau_+ = 1/k_1 \qquad (2)$$

formation and decay of the intramolecular excimer of P3P, respectively. A rise time (τ_+) of the intramolecular excimer fluorescence of P3P depends on the viscosity of the medium.[25b] Therefore, τ_+ can be used as a measure for estimating relative microscopic fluidities of micelles. For the use of P3P as the fluorescent probe, it is necessary for the probe molecules to be bound to micelles and that no microcrystals of the probe exist in the system. Then we applied the freeze–thaw effect[25b,26] to solubilize P3P in the glycolipid micelles and the resulting micellar solutions were passed through the filters to remove remaining microcrystals of P3P. The P3P molecule is so lipophilic that this probe molecule should be bound to the hydrophobic part of the micelle.

The rise and decay curve of the fluorescence of the P3P intramolecular excimer in the C_9G micellar solution is shown in Fig. 6. The simplex treatment[27] of the time-dependent

Table 1 Rise times (τ_+) of P3P intramolecular excimer fluorescence in C_nG and C_nM micellar solutions at 25 °C

Lipid	Lipid concentration/ 10^{-2} mol dm^{-3}	τ_+/ns
C_8G	4	36
C_9G	1	37
$C_{10}G$	0.35	34
C_8M	4	80
$C_{10}M$	1	82
$C_{12}M$	1	83

fluorescence intensity curve provides τ_+ of 37 ns. The τ_+ values obtained for various micellar solutions are summarized in Table 1. The τ_+ values obtained for the C_nG are 34–37 ns while those for the C_nM are 80–83 ns. Evidently the results indicate that the micellar interior of the C_nM micelle, where the P3P molecule is located, is more rigid than that of the C_nG micelle.

The ^{1}H NMR spectra of C_8G and C_8M (4×10^{-2} mol dm^{-3}) in D_2O were measured to obtain information about the fluidities of these micelles. However, no marked difference in linewidths of the signals were observed between C_8G and C_8M.

Microscopic polarities of C_nG and C_nM micelles

The Ham effect of Py monomer fluorescence has widely been used for estimating microscopic polarities of micelles.[28] The ratio of the intensity of the first vibronic fluorescence band (I_1) to that of the third one (I_3) of the Py monomer decreases with decreasing polarity of the medium. The I_1/I_3 values in various solvents are listed in Table 2; these values depend on spectrofluorometer used. Table 2 also exhibits the I_1/I_3 values obtained for the micellar systems. The Py molecule is so lipophilic that this probe is expected to give information about the polarity of the micellar interior. The I_1/I_3 values in the C_nG micelles are *ca.* 1.0 while those in the C_nM micelles are 1.09–1.16, indicating that the polarity of the interior of the C_nM micelle is slightly higher than that of the C_nG micelle.

It is known that the fluorescence intensity of 1-pyrenecarbaldehyde (PyCHO) decreases with decreasing polarity of the medium.[29] Table 3 shows the relative fluorescence intensities of PyCHO in H_2O–methanol where the fluorescence intensity in 100% methanol is used as a standard. The relative fluorescence intensities obtained for the glycolipid micelles are also summarized in Table 3. The I/I_{MeOH} value tends to decrease with increasing alkyl chain length of C_nG or C_nM. Therefore, the PyCHO molecule may be located at the site near the micellar surface. The results shown in Table 3 show that the micellar surface of the C_nM micelle is more polar than that of the C_nG micelle. The fluorescences from Py and PyCHO provide an image that a micelle/water interface of the C_nM micelle is an aqueous-like environment because of well extended hydration to the large maltopyranose head groups while the micellar core of the C_nM micelle shows almost the same polarity as that of the corresponding C_nG micelle. Drummond *et al.*[9] also concluded from the measurements of the surface pressure that the interface of the $C_{12}M$ micelle has a microenvironment which is considerably aqueous-like in nature. On the other hand, the micelle/water interface of the C_nG micelle seems to be more hydrophobic because of the less extended hydration to the smaller glucopyranose residues.

Solubilizing ability of glycolipid micelles

The fluidities and the polarities reveal that the C_nG micelles can provide a more lipophilic and fluid microenvironment in water compared with the C_nM micelles. If it is true, the differences between the C_nG and C_nM micelles should be reflected in the solubilization of lipophilic solute in these micelles.[25a]

Table 2 I_1/I_3 values of pyrene (10^{-6} mol dm^{-3}) monomer fluorescence in organic solvents and C_nG and C_nM micelles at 25 °C[a]

Medium	Relative permittivity	I_1/I_3
Hexane	1.89	0.55
Diethyl ether	4.20	0.98
Butan-1-ol	4.39	0.97
Chloroform	4.90	1.23
THF	7.58	1.23
3-Methylbutan-1-ol	14.7	0.94
2-Methoxyethan-1-ol	16.9	1.44
Propan-2-ol	18.3	1.01
Butan-2-one	18.5	1.30
Propan-1-ol	22.2	0.99
Ethanol	23.8	1.12
Methanol	33.1	1.32
Acetonitrile	37.5	1.64
DMSO	48.9	1.86
C_8G		1.01
C_9G		1.00
$C_{10}G$		1.00
C_8M		1.16
$C_{10}M$		1.13
$C_{12}M$		1.09

[a] The concentrations of the lipids were 4×10^{-2} mol dm^{-3} for C_8G and C_8M, 10^{-2} mol dm^{-3} for C_9G, $C_{10}M$ and $C_{12}G$ and 3.5×10^{-3} mol dm^{-3} for $C_{10}G$.

Table 3 Relative fluorescence intensities of PyCHO (10^{-5} mol dm^{-3}) in aqueous methanol and glycolipid micelles at 25 °C[a]

Medium	Relative permittivity	I/I_{MeOH}
50% (v/v) Methanol	56.6	4.19
60% (v/v) Methanol	51.9	3.57
70% (v/v) Methanol	47.2	2.95
80% (v/v) Methanol	42.5	2.54
90% (v/v) Methanol	37.8	1.71
100% (v/v) Methanol	33.1	1.00
C_8G		1.32
C_9G		0.98
$C_{10}G$		0.62
C_8M		1.42
$C_{10}M$		1.23
$C_{12}M$		1.00

[a] The concentrations of the lipids were 4×10^{-2} mol dm^{-3} for C_8G and C_8M, 10^{-2} mol dm^{-3} for C_9G, $C_{10}M$ and $C_{12}G$ and 3.5×10^{-3} mol dm^{-3} for $C_{10}G$.

P3P forms microcrystals when small amounts of P3P in acetone are injected into water. The P3P microcrystals show broad absorption bands at 352.5 and 335 nm. When the C_8M micelles exist in the system, the P3P molecules are gradually solubilized in water to exhibit the absorption maxima at 346 and 330 nm due to solubilized P3P as shown in Fig. 7. The time courses of the optical densities at 346 nm after injection of P3P in acetone into the micellar solutions are shown in Fig. 8 to compare the rate of solubilization of the C_nG micelles with that of the C_nM micelles. In the case of the C_8G micellar system, considerably rapid solubilization occurs and the optical density at 346 nm slightly decreases after the solubilization is completed. The rapid solubilization also occurs in the C_9G micellar system. In this case, however, the marked disappearance of absorption bands due to solubilized P3P occurs after the solubilization process. Precipitation of the P3P-bearing C_9G micelles proceeds in this system. In contrast to these phenomena in the C_nG micelles, very slow solubilization occurs in both C_8M and $C_{12}M$ micellar systems and no destruction of the C_nM

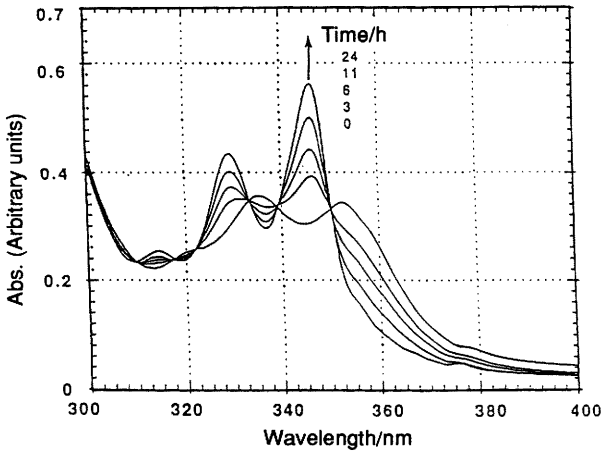


Fig. 7 Progressive absorption spectral changes of P3P (5×10^{-6} mol dm^{-3}) after P3P in acetone was injected into the aqueous C_8M (4×10^{-2} mol dm^{-3}) solution at 25 °C

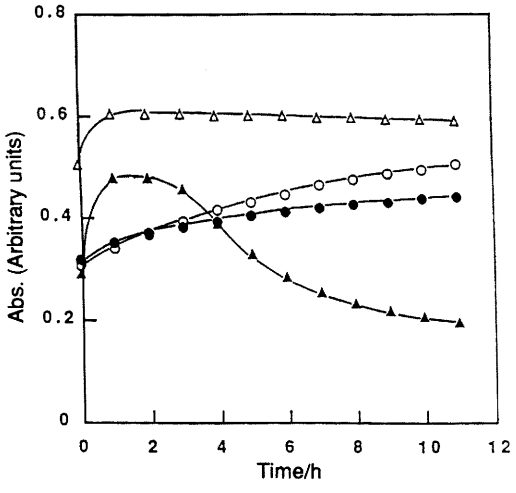


Fig. 8 Progressive changes in the optical densities at 346 nm due to P3P (5×10^{-6} mol dm^{-3}) after P3P in acetone was injected into the C_8G (△), C_9G (▲), C_8M (○) and $C_{12}M$ (●) micellar solutions. The concentrations of the lipds were 4×10^{-2} mol dm^{-3} for C_8G and C_8M and 10^{-2} mol dm^{-3} for C_9G and $C_{12}M$.

micelles occurs. The P3P-bearing C_nM micelles are more stable than the P3P-bearing C_nG micelles.

Essentially the same results were obtained in solubilization of 5,10,15,20-tetraphenylporphyrin (TPP). TPP is not soluble in water and TPP dispersed in water shows very weak and broad absorption bands. Upon solubilization in micelles, the characteristic absorption bands of TPP appear. The solubilization rate can be followed by monitoring the optical density of the Soret band of TPP at 417 nm. The results are shown in Fig. 9. In both C_8G and C_9G micellar systems, the relatively rapid solubilization of TPP is followed by precipitation of the TPP-bearing C_nG micelles. Meanwhile TPP is solubilized very slowly in both C_8M and $C_{12}M$ micelles. The problem is the reason(s) for destruction of the C_nG micelles after solubilization of the lipophilic solute. The solute-bearing C_nG micelles formed by injection of this solute in organic solvent seem to be in a thermodynamically non-equilibrium state. Probably the lipophilic solute molecule at the water/micelle interface gradually penetrates into the lipophilic micellar interior. Since the solubility of C_nG in water is considerably poor, the lipophilic solute-bearing C_nG micelles may gradually precipitate. However, it is true that the solubilization ability of the C_nG micelles is much higher than that of the C_nM micelles.

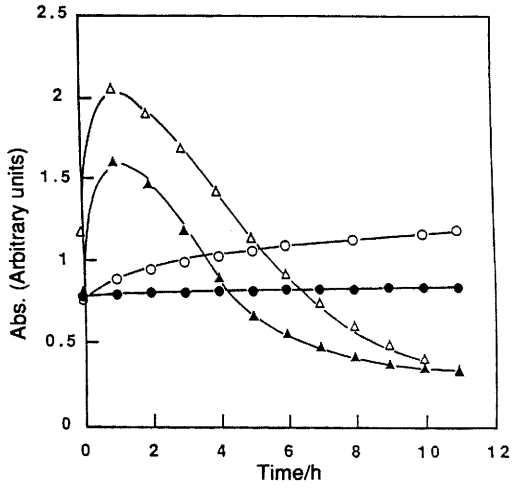


Fig. 9 Progressive changes in the optical densities at 417 nm due to TPP (5×10^{-6} mol dm^{-3}) after TPP in DMF was injected into the C_8G (△), C_9G (▲), C_8M (○) and $C_{12}M$ (●) micellar solutions. The concentrations of the lipids were 4×10^{-2} mol dm^{-3} for C_8G and C_8M and 10^{-2} mol dm^{-3} for C_9G and $C_{12}M$.

Comparison of C_nG and C_nM micelles

The fluorescent probe method clearly indicates that the C_nG micelles have more fluid interiors and less polar surfaces compared with the C_nM micelles. Namely, C_nG forms micelles having a character of an 'oil-droplet'. Meanwhile, since the maltopyranose residue of C_nM is large and extensively hydrated, many water molecules are located at the micellar surface. The water molecules are extensively bound to the maltopyranose residues through hydrogen bonding. The water molecules bound to the sugar head groups may also interact with surrounding water molecules to form hydrogen-bonding networks. We imagine that the hydrogen-bonding networks at the C_nM micellar surfaces reduce the mobility of the C_nM molecules and reduce the rates of solubilization. The less fluctuating nature of the micelle, as well as the higher local concentration of the glucopyranose unit in the C_nM micellar system, may be preferable to form the hydrogen-bonded complex of optically active BR and C_nM compared with the C_nG micellar system.

Experimental

C_8G (Nacalai), C_9G, $C_{10}G$, $C_{10}M$ (Sigma), $C_{12}M$ (Aldrich) and SDS (Nacalai, >99% purity) were purchased and used without further purification. C_8M was prepared and purified according to the procedures described in the literature.[1] Octyl-D-(D-Glu-8) and -L-gluconamides (L-Glu-8) were synthesized by the literature methods.[30] BR (Sigma) was dissolved in chloroform and washed with aqueous sodium carbonate. Chloroform was removed under reduced pressure and the residue was washed with a small amount of methanol. P3P was prepared in a previous study[25b] and its purity was checked by measuring the melting point. PyCHO[31] and TPP[32] were prepared by literature methods. Py (Nacalai) was purified by passing it through a silica gel column with cyclohexane.

Fluorescence spectra (uncorrected) were recorded on a Shimadzu RF-5000 spectrofluorometer whose cell holders were thermostatted. Fluorescence decay curves were measured using an Ortec-PRA single photon-counting apparatus and the data were analysed by a Simplex method with an NEC 9801 microcomputer. CD spectra were recorded on a JASCO J-500A spectropolarimeter with a data processor.

BR (0.1 mol dm^{-3}) in an N_2-saturated aqueous NaOH solution was injected into the N_2-saturated micellar solution.

The final concentration of BR was 2.5×10^{-5} mol dm^{-3} and the pH of the sample was 11. Since BR reacts photochemically, all vessels of the samples containing BR were covered with kitchen foil to protect them from light exposure. Since BR is easily oxidized by oxygen remaining in solution, the measurements were carried out as rapidly as possible. The sample was put in a cell holder and allowed to stand for 10 min to reach a constant temperature. Then the CD spectrum was measured within 30 min. An almost identical result was obtained when the measurement was repeated.

A certain amount of P3P in acetone was injected into the micellar solution using a microsyringe to prepare 5×10^{-6} mol dm^{-3} P3P solution and the resulting solution was bubbled with N_2 gas. The micellar solution thus prepared was rapidly frozen with liquid N_2 and then thawed. This freeze–thaw treatment was repeated three times. The freeze–thaw treatment has been found to be useful to solubilize lipophilic solutes in surfactant micelles.[25b,26] After this treatment, the solution was passed through a filter (MILLEX-SR 0.5 μm FILTER) to remove insoluble P3P. The final amount of acetone was 1% (v/v).

The micellar solutions of Py and PyCHO were prepared by the procedures similar to those for P3P. The final concentrations of Py and PyCHO were 1×10^{-6} and 1×10^{-5} mol dm^{-3}, respectively. The Py and PyCHO solutions contained 0.1% (v/v) acetone and 1% (v/v) methanol, respectively, because these solvents were used for preparing the stock solutions of these fluorescent probes.

The solubilization phenomena were monitored by measuring the absorption spectral change of P3P or TPP after the solute in organic solvent was injected into the micellar solution.

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