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Interaction of cyclodextrins (cyclomalto-oligosaccharides) with glycolipids: n.m.r. studies of aqueous systems of cyclomaltohexaose and alkyl glycosides

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ABSTRACT

The interaction of cyclomaltohexaose (α-cyclodextrin, αCD) with alkyl glycosides (AG) in solution in D_2O was investigated by n.m.r. spectroscopy. αCD induced significant shifts of ^1H and ^{13}C signals (especially for the alkyl chain and the anomeric center) of octyl and dodecyl α- and β-D-glucopyranoside, dodecyl β-maltoside, and octadecyl β-D-glucopyranoside. The ^1H signals of the C_{12} and C_{18} AG, which were tightly bound in the original micelles, also sharpened dramatically. The CD-induced ^{13}C shifts and changes in spin–lattice relaxation times for the octyl β-glucoside were largest for C-4/C-7 of the alkyl chain as expected for inclusion of the chain in the CD cavity. The CD disrupted the AG aggregates, with inclusion of the hydrophobic chains in their macrocycles, suggesting their use for controlling the solution properties of alkyl glycosides and glycolipids.

INTRODUCTION

Alkyl glycosides (AG) are non-ionic "biological detergents" that are used widely for the solubilization of proteins and membrane components[1]. However, residual detergent may affect the physicochemical and functional properties of proteins, and their removal is usually difficult. When the alkyl glycosides are stripped from the protein, they form vesicles or micelles that are difficult to separate from the macromolecular species. Because of their ability to include long hydrophobic chains[2], cyclodextrins (CDs) should be able to disrupt these aggregates, and they have been shown to lower their surface activity and shift towards higher values the critical micellar concentration (c.m.c.) of anionic[3], cationic[3,4], and neutral detergents[5]. AGs are also useful models for studying the properties of biologically active glycolipids in solution. The possibility of controlling these properties by the addition of CDs may have practical applications.

The interaction of some AGs, in solution in D_2O at concentrations near or above the c.m.c., and cyclomaltohexaose (α-cyclodextrin, αCD) has been studied by ^1H- and ^{13}C-n.m.r. spectroscopy. The AGs studied were the most common biological detergents

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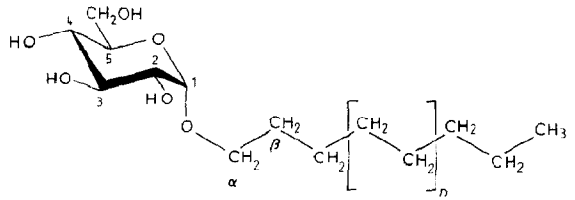
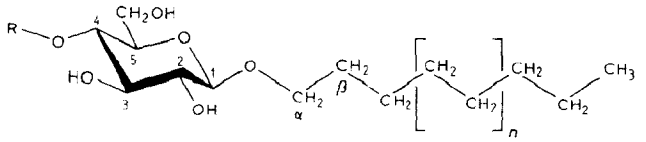


αGC_8 ($n = 1$) c.m.c. 6.3 mM[6]

αGC_{12} ($n = 3$) c.m.c. n.d.



βGC_8 ($n = 1, R = H$) c.m.c. 19 mM[6]

βGC_{12} ($n = 3, R = H$) c.m.c. 0.19[7]

βGC_{18} ($n = 6, R = H$) c.m.c. n.d.

βMC_{12} ($n = 3, R = \alpha$-D-Glc) c.m.c. 0.24[6]

Fig. 1. Alkyl glycosides and their c.m.c. values. The planar zigzag conformation of the alkyl chains is assumed only for simplicity of representation.

octyl (GC_8) and dodecyl (GC_{12}) α- and β-D-glucopyranoside, octadecyl β-D-glucopyranoside (βGC_{18}), and dodecyl β-maltoside (βMC_{12}). The structure and c.m.c. values of these compounds are given in Fig. 1.

N.m.r. spectra can provide information on the aggregation[8] and the inclusion state[2] of hydrophobic or amphiphilic molecules. In preliminary surface tension[5] and ^{1}H-n.m.r. studies[9], αCD was shown to interact with *n*-alkyl β-D-glycosides to a greater extent than cyclomaltoheptaose (βCD) and cyclomalto-octaose (γCD), probably because of better fit of the *n*-alkyl chains in the cavity of αCD. Therefore, the present study was focused on aqueous systems of AG and αCD.

EXPERIMENTAL

Materials. — αGC_8 and αGC_{12} were synthesized essentially according to the imidate method[10], βGC_8, βGC_{12}, and βMC_{12} were obtained from Sigma and recrystallized from ethyl acetate, and βGC_{18} was prepared by the Koenigs–Knorr procedure, in the presence of Ag_2O as catalyst[10]. The purity of the products was determined by elemental analysis, melting points, optical rotation, and n.m.r. spectroscopy[10]. αCD and γCD (Chinoin, Budapest) were used without further purification, taking into account their water content (determined by drying at 105° for 12 h).

Methods. — Solutions of the AG and the CD were prepared starting from solutions of the AG in D_2O (99.7%) near the known (or expected) c.m.c. values (αGC_8,

5mM; αGC_{12}, 5.7mM; βGC_8, 37.5mM; βGC_{12}, 0.59mM; βGC_{18}, 0.19mM; βMC_{12}, 0.59mM), and increasing amounts of the CD were added in order to obtain CD/AG ratios (R) in the range 0–20. N.m.r. spectra were obtained at 23° with a Bruker CXP-300 instrument (300 MHz for ^1H, and 75 MHz for ^{13}C). The chemical shifts for the ^1H signals were referenced to the signal of residual HOD in the solvent, and the ^{13}C signals to external sodium 2,2,3,3-tetradeuterio-3-trimethylsilylpropionate. The apparent dissociation constants of "complexes" were calculated from values of CD-induced ^1H shifts[11]. The ^{13}C spin–lattice relaxation times (T_1) were calculated by fitting the experimental values obtained by the inversion recovery method, using the calculation routines of the Bruker DISCXP Program, version 86011.2 for the Aspect 2000 computer (standard deviations, 0.03–0.09).

RESULTS AND DISCUSSION

Spectra of the hydrocarbon moieties. — As illustrated in Fig. 2 for βGC_8 (*a*) and βGC_{12} (*b*), protons H-2 (βCH) to H-*n* of the hydrocarbon moiety of the AG resonated in the region 1.0 –1.9 p.p.m.; H-1 (αCH) resonated in the region 3.6–4.0 p.p.m.[12]. In the absence of αCD, only signals of the glycosides with the shortest hydrocarbon chain, *i.e.*, βGC_8 (Fig. 2*a*, R = 0) and αGC_8 (spectrum not shown), are sufficiently sharp to show multiplicity due to coupling of vicinal protons. As the chain length increased, this multiplicity was lost because of signal broadening, associated with strongly reduced mobility of the original AG aggregates. This effect is illustrated in Fig. 2*b* for βGC_{12} (R = 0). Similarly, the ^1H signals of αGC_{12}, βGC_{18}, and βMC_{12} (spectra not shown) were broad. The βCH_2 signal of βGC_{18} was practically absent from the spectrum.

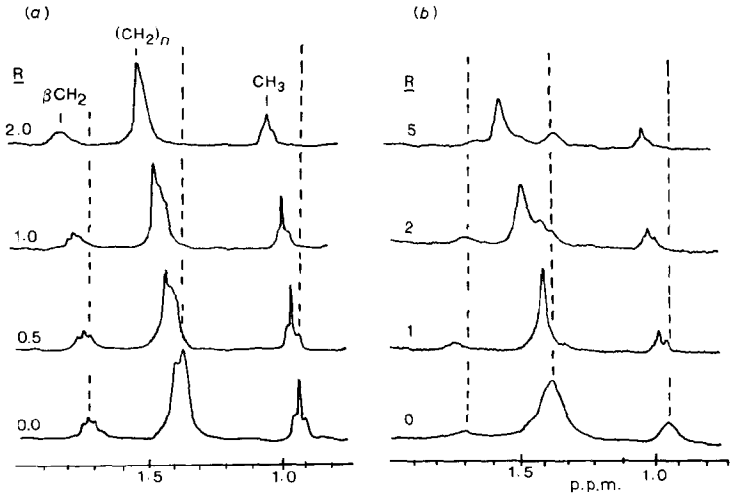


Fig. 2. ^1H-N.m.r. spectra (300 MHz, D_2O) of the alkyl moieties of βGC_8 (*a*) and βGC_{12} (*b*), alone and in the presence of αCD at different CD/AG molar ratios (R). The αCH_2 signal of the alkyl chain is in the 3.6–4.0 p.p.m. region (see Fig. 5).

TABLE I

αCD-induced chemical shifts ($\Delta\delta$, p.p.m.) of the ^{1}H resonances of the hydrocarbon moieties of alkyl glycosides[a]

R	CH_3 αGC_8	$(CH_2)_n$	βCH_2	CH_3 βGC_8	$(CH_2)_n$	βCH_2	CH_3 βGC_{12}	$(CH_2)_n$	βCH_2
0	0	0	0	0	0	0	0	0	0
0.5	−11.2	−23.5	−10.0	nd	nd	nd	−4.1	−5.8	−6
0.7	nd	nd	nd	nd	nd	nd	−8.3	−6.5	−12
1.0	−11.7	−23.5	−14.7	−5.1	−6.9	−3.1	−9.4	−7.7	−9
2	−15.8	−27.0	−18.2	−9.7	−12.5	−7.0	−20.0	−32.5	0
3	−21.7	−32.4	−22.3	−14.2	−19.2	−10.5	nd	nd	nd
4	nd	nd	nd	−16.9	−21.4	−12.2	nd	nd	nd
5	−21.7	−29.9	−19.9	−23.8	−26.2	−17.8	−24.5	−55.4	+18
10	−29.3	−33.4	−20.5	nd	nd	nd	−26.0	−61.6	+21
20	−39.9	−39.3	−26.4	nd	nd	nd	−26.1	−63.5	+24
	αGC_{12}[b]			βGC_{18}			βMC_{12}		
0	0	0	0	0	0	0	0	0	0
1	nm	nm	nm	±2	−1.6	nm	−6.4	−8.8	0
2	−38.0	−65.1	nm	±2	−2.3	nm	−7.3	−10.5	+3
3	−40.4	−69.9	nm	±2	−2.3	nm	−9.3	−15.5	+9
4	nd	nd	nd	±2	−2.0	nm	−10.4	−26.5	+12
5	−42.2	−78.1	nm	±2	−2.0	nm	−10.4	−30.9	+18
10	−41.6	−78.5	nm	±2	−2.0	nm	−11.4	−49.0	+18
20	nd	nd	nd	±2	−2.0	nm	−10.3	−55.2	+21

[a]With reference to the solvent (HOD) signal: +, upfield; −, downfield. R = CD/AG molar ratios. For $(CH_2)_n$, the shifts are for the major peak of a group of unresolved or partially resolved signals. When more than one set of signals were present, only the sets of major signals were considered. nd = not determined; nm = not measurable. [b]Shifts relative to βGC_{12}, R = 0.

On the addition of αCD, both the chemical shift and the shape of the ^{1}H signals of the AG were affected. The CD-induced shifts, reported in Table I and shown in Fig. 2 for βGC_8 and βGC_{12}, revealed a general downfield displacement of the signals associated with the CH_3, $-(CH_2)_n-$, and βCH_2 groups of the AG. For low CD/AG ratios (up to R = 1), the $-(CH_2)_n-$ signal of βGC_8 (as well as that of αGC_8; spectrum not shown) was resolved into more than two components. The βCH_2 signal of βGC_{12} behaved atypically, *i.e.*, it moved downfield up to R = 1, then upfield for $R > 1$. For βMC_{12}, this signal was practically invariant up to R = 1, then moved upfield. For αGC_{12} and βGC_{18}, the βCH_2 signal was not observed. On the other hand, the chemical shifts of the other signals of βGC_{18} were not affected significantly by the CD.

The half-widths of the signals of the C_8 glycosides were largely unaffected by the CD; the slight broadening observed for $R > 1$ (as in Fig. 2*a*, for R = 2) was associated with incipient precipitation. In contrast, the original broad signals of βGC_{12} (Fig. 2*b*) sharpened dramatically on the addition of αCD. The major CH_3 and $-(CH_2)_n-$ signals of this glycoside were shifted downfield as for βGC_8. However, a signal of medium

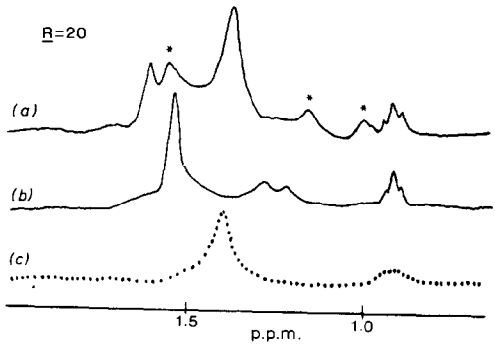


Fig. 3. ^{1}H-N.m.r. spectra of AG in the presence of a large excess of CD ($R = 20$): (*a*) βGC_{18} + αCD; (*b*) βMC_{12} + αCD; (*c*) βMC_{12} + γCD. Signals attributed to an AG species in slow exchange with the major one are labelled with asterisks.

intensity remained (or showed up) essentially in the same region as in the absence of CD.

As illustrated in Fig. 3 for βGC_{18} (*a*) and βGC_{12} (*b*), "new" signals (labelled with an asterisk) were evident in the presence of a large excess of αCD ($R = 20$). Since the triplet-shaped signal at $\sim$1 p.p.m., slightly upfield of the major CH_3 signal, was attributed to another methyl group, the new signals appeared to have been due to species that exchanged slowly with the major signal on the n.m.r. time-scale. Excess of γCD did not cause any significant shift or sharpening of the signal for βMC_{12} (Fig. 3*c*) and the other alkyl glycosides (spectra not shown).

Where the induced shifts could be measured over a sufficiently wide range of R values (as for the C_{12} glycoside), the corresponding plots (Fig. 4) were typical titration curves, with inflexions corresponding to the apparent stoichiometry of the complexed species. The apparent dissociation constants of these "complexes", calculated from the CD-induced shifts[11], were of the order of 10^{-5}.

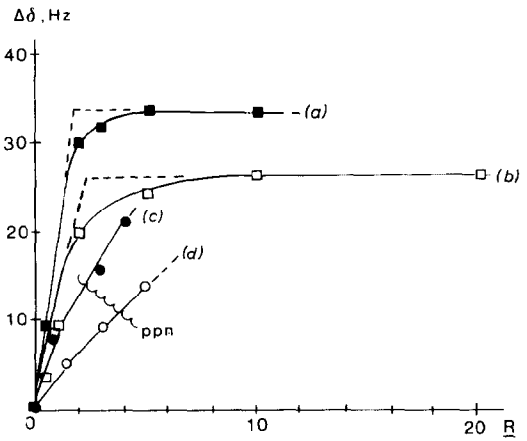


Fig. 4. ^{1}H-N.m.r. titration curves with αCD of αGC_{12} (*a*), βGC_{12} (*b*), αGC_8 (*c*), and βGC_8 (*d*). For the C_8 glucosides, some precipitation occurred at $R > 3$.

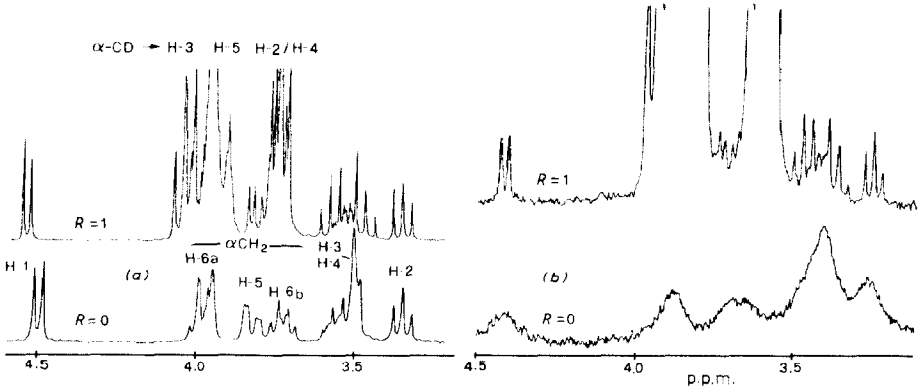


Fig. 5. ^{1}H-N-m.r. spectra of the carbohydrate moieties (lower spectra), and of the carbohydrate moieties + αCD (upper spectra) for βGC_8 (*a*) and βGC_{12} (*b*). Assignments indicated for βGC_8 are for the glucose moiety on the spectrum for $R = 0$, and for αCD on the spectra for $R = 1$.

^{1}H-N.m.r. spectra of the carbohydrate moieties and of the CD. — As illustrated in Fig. 5 for βGC_8 (*a*) and βGC_{12} (*b*), the spectral behaviour of the carbohydrate moieties of the AG qualitatively paralleled that of the corresponding alkyl moieties. Thus, in the absence of CD, βGC_8 (Fig. 5*a*) showed well-resolved signals, whereas the signals of βGC_{12} (Fig. 5*b*) were broad in the absence, and sharp in the presence, of αCD. However, the CD caused some sharpening of the carbohydrate signals of βGC_8 (Fig. 5*a*), in contrast to the slight broadening observed for the corresponding alkyl moieties (Fig. 2*b*).

The CD signals (except those of H-1 and H-2) severely overlapped those of the carbohydrate moiety of the AG. Since the CD spectrum changed in a complex way in the presence of the AG, no attempts were made to obtain difference spectra. The only significant shift clearly observed was for the signal of H-1 of βGC_8 (-0.04 p.p.m. for $R = 1$; Fig. 5*a*); the corresponding signal of αGC_8 could not be observed because of severe intrinsic broadening, which persisted up to $R = 3$, after which some precipitation occurred. Also, the spectrum of αCD was clearly affected by the AG. Overlap with signals of the carbohydrate moiety of the AG prevented accurate measurement of the induced shifts, which were small for the highest R values. However, where these shifts could be evaluated, it was apparent that interaction with AG affected not only the signals of H-3 and H-5 (as typical on formation of inclusion complexes)[13], but also that of H-1, and, perhaps, those of H-2 and H-4.

^{13}C-N.m.r. spectra. — Because of sensitivity limitations, ^{13}C spectra could be obtained only for solutions of βGC_8 (Fig. 6*a* and *b*) and βGC_{12} (insert *c* in Fig. 6). Assignment of signals was made by comparison with those[12] of αGC_8. Measured T_1 values are shown in the spectra for each carbon in the absence (*a*) and in the presence (*b*) of CD ($R = 2$). Addition of the CD to GC_8 shifted essentially only the signals of the alkyl moiety, and spread the resonances for C2/C-8 over a wider frequency range, with partial resolution of the C-2, C-4, and C-5 signals (since C-1 is barely affected by the CD, it was assumed that the unaffected signal in the 27 p.p.m. region was that of C-2). Signal

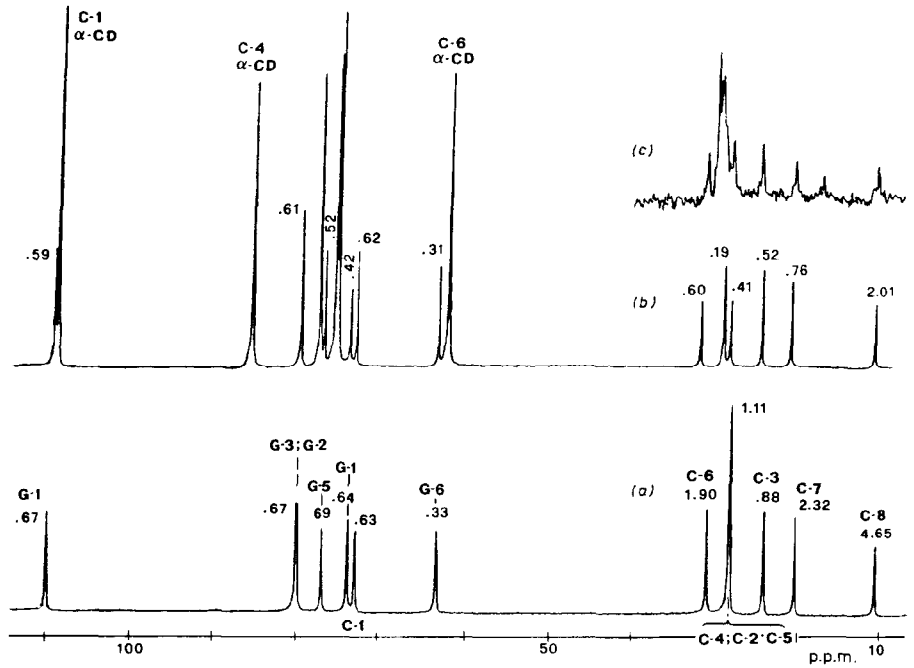


Fig. 6. ^{13}C-N.m.r. spectra (75 MHz, D_2O) of βGC_8, alone (*a*) and in the presence of αCD, $R = 1$ (*b*). Signals G and C are for the glycose and alkyl moieties, respectively. Values of spin–lattice relaxation (T_1) are given for signals of the AG. The partial spectrum (*c*) is for βGC_{12}.

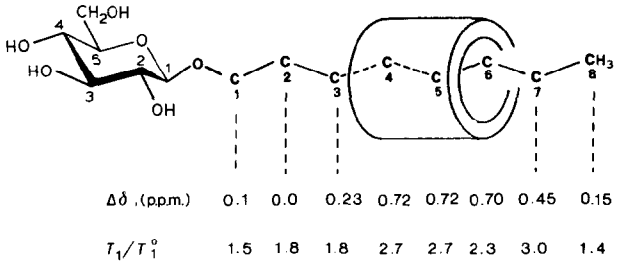


Fig. 7. α-CD-induced ^{13}C-n.m.r. shifts [$\Delta\delta$ (p.p.m.) for $R = 1$], and changes in spin–lattice relaxation times (T_1/T_1^0). $\Delta\delta$ values are with reference to signal G-4 of the glucose moiety at $R = 0$; T_1 is for $R = 1$, and T_1^0 for $R = 0$.

spreading also occurred in the spectrum of βGC_{12} (Fig. 6*c*), where a set of weaker signals also showed up above the noise level. The CD-induced shifts [$\Delta\delta$ (p.p.m.) referred to $G_4 = 0$], and changes in T_1 values (expressed as T_1/T_1° ratios, where T_1 is the spin–lattice relaxation time at $R = 1$, and T_1° is the corresponding value for $R = 0$) for βGC_8 are reported in Fig. 7, which also shows that the largest effects may correspond to a statistical environment of the C-4/C-7 segment of the alkyl chain of the AG such as expected for its inclusion in the CD.

DISCUSSION

At AG concentrations near or above the c.m.c. values and in the absence of CD, a significant proportion of the AG molecules are more or less tightly aggregated in micelles. The more loosely associated molecules are those of the AG with the highest c.m.c., *i.e.*, those with the shortest alkyl chain (C_8), especially if β-linked[6]. Such a loose association is reflected by the relative sharpness of the corresponding n.m.r. signals.

Addition of αCD affected only the chemical shift of the hydrogens of the alkyl moieties (and at least of H-1 of the carbohydrate moieties) of the C_8 and C_{12} glycosides, but, for the C_{12} and C_{18} glycosides, it also caused a significant sharpening of all the AG signals. The fact that γCD (the macrocycle of which is too large for a tight inclusion of *n*-alkyl chains)[2] does not affect the AG spectra strongly suggests that the αCD-induced spectral changes are triggered by the formation of inclusion complexes with the hydrophobic chains. Perhaps surprisingly, for βGC_{18} the signal sharpening was comparatively less evident, and was not paralleled by significant CD-induced shifts. However, the micelles of this long-chain AG are expected to be more tightly associated than those of its C_{12} analogue, and the present concentration of βGC_{18} used could be so much above the (presently unknown) c.m.c. value as to be practically insensitive to the CD.

One proof of the formation of true inclusion complexes is the selective shift of the resonances of H-3 and H-5 of the CD, *i.e.*, those inside the cavity[12]. The signal of H-3 (as well as, to a minor extent, that of H-5) of αCD shifted in the presence of the alkyl glycosides. However, the signal of H-1 (and perhaps also those of H-2 and H-4) also shifted, suggesting that both the cavity and the external part of the CD are perturbed in the interaction with AG and the solvent. Also, the observed CD-induced ^{1}H-shifts relative to the HOD signal undoubtedly reflect not only changes in the microenvironment of the AG molecules (from the self-associated to the inclusion state), but also (or prevalently) a change in the "structure" of the D_2O molecules.

At least for βGC_8, the CD-induced ^{13}C shifts and changes in T_1 values (Figs. 6 and 7), which are largest for the resonances of C-4/C-7 of the alkyl chain, suggest that these carbon atoms have penetrated into the cavity of the CD. This microenvironment for the CH_2-4/7 could also explain the "disproportionation" of the $-(CH_2)_n-$ ^{1}H signals (Fig. 2*a*) in terms of a shift-reagent effect of the CD[13] on those CH_2 groups that spend most time in the cavity of the CD, rather than the co-existence of at least one other AG species in slow exchange with the major species. On the other hand, signals attributable to new species (probably AG–CD 1:2 complexes) showed up clearly in the spectra of the C_{12} and C_{18} glycosides.

The present study strongly supports the concept that αCD can disrupt the microaggregates of alkyl glycosides (and conceivably also those of more complex glycolipids) in aqueous systems. As depicted in Fig. 8, the CD can control the aggregation and surfactant properties of these amphiphilic substances by stripping monomeric molecules from the aggregates and/or from the water surface. These complex systems are being studied further.

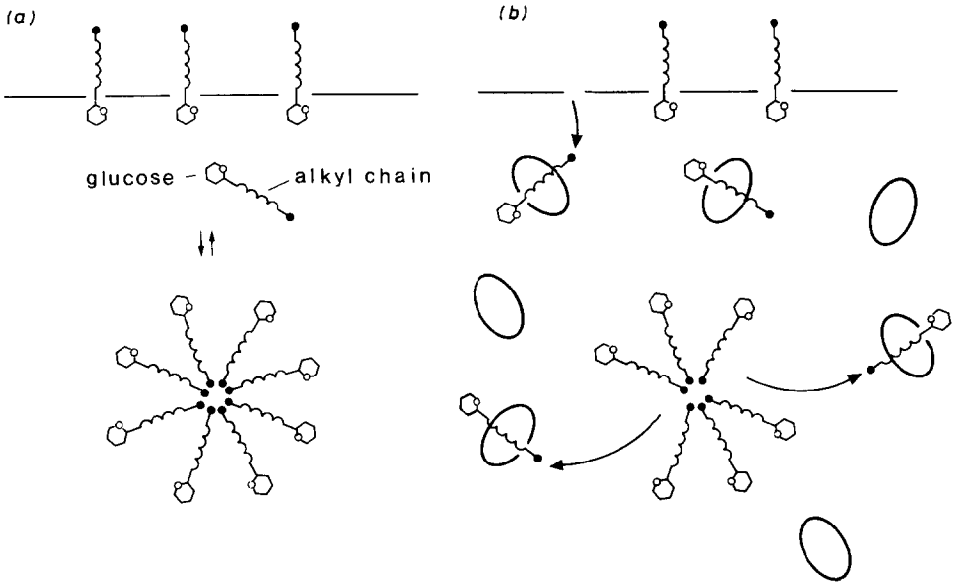


Fig. 8. Idealized picture of an aqueous system of alkyl glycosides, in the absence (*a*) and in the presence (*b*) of αCD.

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