PHASE TRANSITIONS IN NON-IONIC DETERGENT MICELLES

E. Grell[1]*, E. Lewitzki*[1]*, R. Schneider*[2]*, G. Ilgenfritz*[2]*, I. Grillo*[3]
and M. von Raumer[4]

[1]Institute of Biophysics, Kennedyallee 70, D-60596 Frankfurt, Germany
[2]Institute of Physical Chemistry, University of Cologne, D-50939 Cologne, Germany
[3]Institute Laue-Langevin, F-38042 Grenoble, France
[4]Solvias AG, CH-4002 Basel, Switzerland

Abstract

Differential scanning calorimetry (DSC) studies of micellar, 60 mM solutions of the octaethyleneglycol alkylethers $C_{14}E_8$ and $C_{16}E_8$ provide evidence for a narrow endothermic transition at 41 and 32°C, respectively, characterized by an enthalpy change of 2 kJ mol^{-1} for both detergents. The observed thermal transition is indicative of a concerted transition of the surfactant molecules, as illustrated on the basis of a simple molecular model. The effect of co-solvents such as different alcohols on the thermal transition is investigated. Glycerol markedly lowers the transition temperature whereas the transition is absent in the presence of at least 10% ethanol. The calorimetric transition correlates with the temperature dependent increase of viscosity and static light scattering as well as with changes observed by small-angle neutron scattering (SANS). The SANS results provide clear evidence for a distinct structural change occurring at the transition temperature, which is interpreted as a sphere-to-rod transition of the detergent micelles. Moreover, the rod length increases with increasing temperature. We suggest that the process causing the thermal transition acts as the prerequisite of the growth process.

Keywords: CMC, DSC, detergent, growth, light scattering, neutron scattering, non-ionic micelle, poly(ethyleneglycol) alkylether, thermal transition, viscosity

Introduction

Detergents are widely used for the biochemical investigation of water-insoluble membrane proteins. Especially the non-ionic and comparatively mild oligo(oxyethylene) *n*-monoalkylethers (H{CH$_2$}$_x$–O–{CH$_2$CH$_2$O}$_y$H; often denoted poly(ethyleneglycol) monoalkylethers and abbreviated as C_xE_y) have been successfully applied to solubilize integral membrane proteins by forming mixed micelles with retention of enzymatic activity [1]. In order to increase protein stability or for the purpose of membrane protein crystallization cosolvents such as glycerol or heptanetriol are

---

* Author for correspondence: E-mail: ernst.grell@mpibp-frankfurt.mpg.de; Phone: +4969 6303290, Fax: +4969 6303346

sometimes added [1, 2]. Up to now, the selection of a particular detergent as well as of a suitable medium for the purpose of membrane protein solubilization is often achieved on a trial and error basis. It is therefore of interest to investigate the detailed molecular properties of this class of detergent molecules and it is hoped that the results will contribute to the understanding of the mechanism of membrane protein solubilization.

Above the critical micelle concentration (CMC), monomer detergent molecules of the type mentioned above tend to aggregate and form nanoparticulate micelles (L_1 phase [3]) of globular structure. According to the amphiphilic nature of detergent molecules, the non-polar structure segments are preferentially located in the non-polar interior and the polar head groups form an outer shell which is in contact with bulk water. The shape, size and degree of polydispersity of the micelle can depend on detergent structure, concentration, temperature and solution composition. Upon increasing temperature markedly, micellar C_xE_y solutions exhibit a phase separation at a critical temperature, T_c (cloud point temperature), where a phase with high and low detergent concentration is formed (cf. phase diagrams in [3]). At concentrations above about 20% but below T_c, many members of this class of detergents can also adopt mesophases [3]. At lower C_xE_y detergent concentrations, but still above the CMC, marked changes in basic properties such as viscosity, light and neutron scattering have been reported for some members of this class of compounds, which are indicative of a change in micellar size and/or shape [4–6]. In the lower concentration range, a new thermal transition has been found recently for $C_{14}E_8$ micelles by employing differential scanning calorimetry (DSC) [7]. This endothermic transition, characterized by a well defined transition temperature and a ΔH value around 2 kJ mol^{-1}, has been attributed to a conformational rearrangement of the assembled detergent molecules, involving particularly the polar head group and its interaction with water molecules [7]. Here, we report the results of investigations concerning changes in physical and structural properties of C_xE_y micelles in aqueous solution, related to this thermal transition. In addition, the effect of co-solvents has been studied.

Experimental

Materials and methods

Chemicals

Octaethyleneglycol tetradecyl- and hexadecylether ($C_{14}E_8$ and $C_{16}E_8$) of quality grade BioChemika, histidine, EDTA, ethyleneglycol of grade MicroSelect, glycerol of grade puriss. p.a., ethanol abs. of grade Spektranal and D_2O 99.9% have been purchased from Fluka (Taufkirchen).

Calorimetry

Isothermal titrations have been performed at 25°C by employing the MCS ITC and DSC measurements with the MCS DSC instrument (MicroCal, Northampton). All

solutions have been carefully degassed under reduced pressure. For the DSC scans between 10 and 100°C a temperature gradient of 30°C per h has been chosen.

Viscosimetry

Viscosities at different temperatures have been determined by employing a falling ball viscosimeter (Haake, Karlsruhe), equipped with four different balls for determinations in the range between 0.3 and 200 mPas. Viscosimetry of $C_{14}E_8$ has been performed with a single and of $C_{16}E_8$ with two different balls (cf. different symbols for data in Fig. 1). Setting of the temperature program and the time measurement is controlled with a computer. Water and viscosity standard oils from Physikal. Techn. Bundesanstalt (Braunschweig) have been used for the purpose of calibration. Relative viscosities have been calculated as η/η_0, where η_0 is the viscosity of the solvent.
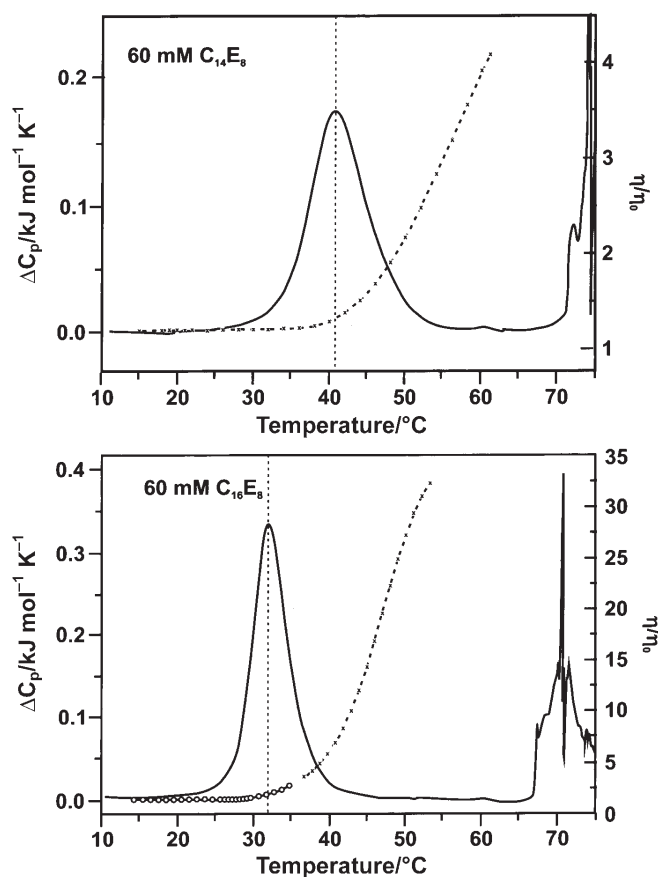

Fig. 1 DSC curves of 60 mM $C_{14}E_8$ and $C_{16}E_8$ between 10 and 70°C (solid line) as well as the temperature dependence of the relative viscosity of the micellar system (bold broken line). The thin broken line characterizes the transition temperature T_t

Static light scattering

Measurements have been performed with a Spex 212 fluorimeter (both monochromators set at 3000 Å) from Instruments S.A. (Grasbrunn), equipped with a thermostated cuvette holder and a setup for magnetic stirring within the semi-micro 1 mL quartz cuvette (Hellma, Müllheim). The temperature gradient (30°C h^{-1}) has been achieved with a programmable RE 306 ecoline thermostate from MWG Lauda (Lauda). Temperature has been measured under stirring inside the cuvette with a Pt 100 and a 2180A RTD digital thermometer from Fluke (Ismaning).

Small angle neutron scattering (SANS)

Experiments have been carried out at the Institute Laue-Langevin in Grenoble (ILL, France) on the D22 spectrometer. The wavelength has been set at 8 Å; two sample-to-detector distances of 2 and 10 m with collimation of 11.2 and 17.6 m, respectively, have been used to cover a large q-range from 0.004 to 0.3 Å^{-1}, with q, the wave vector $q=(4\pi/\lambda)\sin(\theta/2)$ Å^{-1} and θ the scattering angle. Samples prepared in D$_2$O have been filled in 1 mm thick quartz cells (Hellma, (Müllheim) and mounted to a programmable and thermostated cell holder. Different temperatures from 15 to 60°C and an acquisition time of 15 min per sample at every temperature have been chosen.

The raw data have been radially averaged. The absolute scaling has been obtained after subtraction of the empty cell contribution, electronic background and normalisation with water using standard ILL procedures [8].

For colloidal suspensions with spherical geometry, the absolute intensity $I(q)$ is equal to the product of the particle form factor $P(q)$ and the structure factor $S(q)$. In dilute solutions with no interaction between particles $S(q)$ is assumed to be identical to one. Under such conditions, the scattering intensity is given by the form factor.

Results

CMC and demicellization enthalpy determination

In order to characterize micellar solutions under defined conditions its is important to know the CMC in the chosen medium as well as whether the structure of the formed micelles sensitively depends on detergent concentration. This is done by carrying out isothermal microcalorimetric titrations in aqueous solution with and without of 25% glycerol (mass/mass) at 25°C. The detergent C$_{14}$E$_8$ is added from the titration syringe in portions of 5 µL of a 0.5 mM solution to the calorimetric cell, containing the identical buffer solution. This solution predominantly consists of micelles. Upon the first additions, a heat signal characteristic of an exothermic process is observed and is attributed to the demicellization process. Upon further additions, this signal decreases gradually until it reaches a constant value representing the heat of dilution of the C$_{14}$E$_8$ micelles. The point of inflection of the dependence of the measured heat change on the detergent concentration is found at a C$_{14}$E$_8$ concentration of 9 µM, which corresponds to the CMC. The enthalpy of demicellization is –11.5 kJ mol^{-1}. In the presence of 25% glycerol (mass/mass) the CMC is similar; only the demicellization

enthalpy is lower. Similar calorimetric titrations performed with $C_{16}E_8$ indicate that its CMC is even lower than that of $C_{14}E_8$. A quantitative determination, however, was no more possible by applying the same technique. These results clearly show that solutions with millimolar concentrations of these detergents are far above their CMCs, implying therefore, that nearly all $C_{14}E_8$ and $C_{16}E_8$ molecules adopt an aggregation state in form of a micelle. Up to concentrations of about 10 mM, the molar heat of dilutions are almost constant at 25°C. Thus there is no evidence for a concentration dependent change of the structure of the micelles or of their degree of aggregation.

Thermochemical characterization by DSC and correlation with viscosity and light scattering changes

In 60 mM solutions as well as at lower concentrations in 30 mM histidine/HCl, 0.1 mM EDTA pH 6.8 and in a temperature range below T_c, the micellar phases of $C_{14}E_8$ and $C_{16}E_8$ show a typical thermal transition in the DSC experiment (Fig. 1). This transition, characteristic of an endothermic process, is similar to the phase transitions reported for phospholipid samples [9]. The transition is markedly broader for $C_{14}E_8$ (half line width $\Delta T_{1/2} \cong 9$°C) than for the micelles of $C_{16}E_8$ micelles ($\Delta T_{1/2} \cong 5.5$°C). The transition temperature T_t for $C_{16}E_8$ ($T_t=32$°C) is lower than that for $C_{14}E_8$ ($T_t=41$°C) and, evidently, appears to depend on the length of the hydrophobic alkyl residue of the detergent molecule. The enthalpy changes of both transitions are similar, namely 2.1 ± 0.2 kJ mol^{-1}. Both transitions are reversible as long as the DSC scans are kept below T_c. Although the phase separation at T_c is reversible, too, its re-equilibration is very slow under conditions where no stirring is possible, as in the DSC cell. If the DSC run is performed up to about 100°C, an additional thermal signal, likely to correspond to the cloud point, is detected around 71 for $C_{16}E_8$ and 74°C for $C_{14}E_8$ (Fig. 1).

No significant excess heat signal can be detected unambiguously in the DSC curve between the phase transition and T_c under the sensitivity conditions of the applied thermoanalytical equipment. In contrast to this observation, the temperature dependence of the relative viscosity (η/η_0) of the aqueous micellar system increases with increasing temperature for $C_{14}E_8$ as well as for $C_{16}E_8$ micelles (Fig. 1). No marked viscosity change, however; has been observed below a characteristic temperature (about 30 for $C_{16}E_8$ and 38°C for $C_{14}E_8$). This characteristic temperature clearly correlates with T_t of the thermal transitions indicated by the DSC results of both detergents (Fig. 1). The molecular reason for the viscosity increase observed here could be due to a change in the structure or shape of the micellar state, induced by the thermal transition. This structure change can be due to particle aggregation or size increase (growth) of the micelles, as suggested earlier [4–6]. A noteworthy difference between both detergents above T_t is that the viscosity contribution of the more hydrophobic $C_{16}E_8$ is higher than that of $C_{14}E_8$. The particles formed by $C_{16}E_8$ thus may be larger or longer or more interconnected. The experimentally observed temperature dependent increase of the relative viscosity from a certain onset temperature on is very similar to the increase in static light scattering, detected in the same temperature range (Fig. 2). Although employing this additional method, the thermal transition is not directly observed. But also here, the micelles are likely to exhibit a change in size
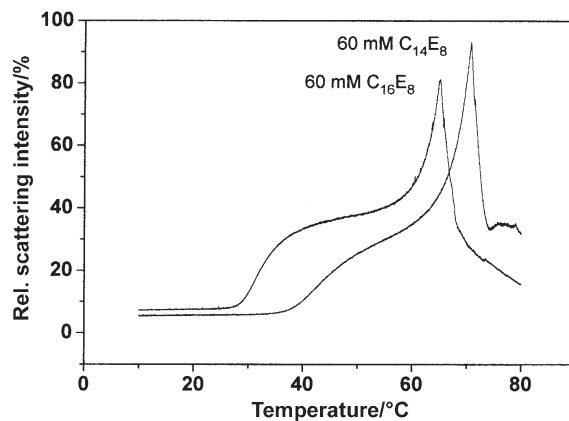
Fig. 2 Temperature dependence of static light scattering of 60 mM $C_{14}E_8$ and $C_{16}E_8$, shown as as relative scattering intensity *vs.* temperature

and/or shape above the thermal transition temperature. The onset temperatures of the scattering increase for $C_{14}E_8$ and $C_{16}E_8$ are similar to those found in the viscosity studies. Above T_t, $C_{16}E_8$ exhibits a higher scattering intensity than $C_{14}E_8$, which, in agreement with the corresponding viscosity levels, is indicative of the existence of larger or more interconnected high temperature structures for the more hydrophobic detergent. In addition, it is important to note that the high temperature scattering maximum shown in Fig. 2 (for $C_{16}E_8$ and $C_{14}E_8$ around 65 and 72°C, respectively) is an apparent one and does not correspond to T_c, although it nearly reaches its value. It is related to the increasing high turbidity due to intense particle scattering of the concentrated detergent solutions, already strongly detectable below T_c. This intense particle scattering is accompanied by a considerable loss of stray light, responsible for the marked intensity decrease above 65 and 70°C, respectively.

Effect of co-solvents

Organic solvents can interact differently with the hydrophilic (head) and hydrophobic (tail) part of detergent molecules and are therefore likely, depending on their mode of interaction, to modify properties of detergent micelles. Therefore, DSC, viscosity and static light scattering studies have been carried out in the presence of different alcohols such as glycerol, ethylene glycol and ethanol. If 25% glycerol (mass/mass) is added to the media mentioned above, the thermal transition of the $C_{14}E_8$ micelles is still observable in the DSC curve but the transition temperature is significantly lowered by about 10°C. The ΔH value, however, remains unchanged although the transition is now somewhat broader. Corresponding results are obtained for $C_{16}E_8$ where T_t is now at 23°C for a 60 mM solution. In the presence of 25% ethylene glycol (mass/mass), the decrease of the transition temperature of micellar $C_{14}E_8$ is comparatively small and the transition is only slightly broader than in its absence. In contrast to these DSC observations, no thermal transition is detectable for $C_{14}E_8$ in the presence of ethanol, provided the ethanol content is larger than about 10% (mass/mass).

Again, these results correlate very well with the temperature dependent viscosity and static light scattering changes. For 60 mM $C_{14}E_8$ in the presence of 25% glycerol (mass/mass), the onset temperature of the viscosity and light scattering increase is found around 28°C, again 10°C lower than in the absence of co-solvents. Accordingly, 25% ethylene glycol (mass/mass) does not markedly affect the viscosity of 60 mM $C_{14}E_8$ above the thermal transition, compared to its absence. Again in accordance with the DSC experiment, the typical viscosity increase of the aqueous $C_{14}E_8$ solution is absent upon the addition of 25% ethanol (mass/mass).

Structural features

Because an increase of viscosity and static light scattering has been found above the thermal transition temperature of $C_{16}E_8$ and $C_{14}E_8$, neutron scattering studies have been carried out at different temperatures between 15 and 60°C with $C_{14}E_8$ in order to find out whether a change in micellar size and/or shape is observable above T_t. For contrast reasons, the samples have been prepared in D_2O, which does not alter markedly T_t according to a DSC control experiment. Typical normalized scattering curves are shown in Fig. 3 for 60 mM $C_{14}E_8$ at 15 and 60°C, above and below the DSC transition. The behaviour at
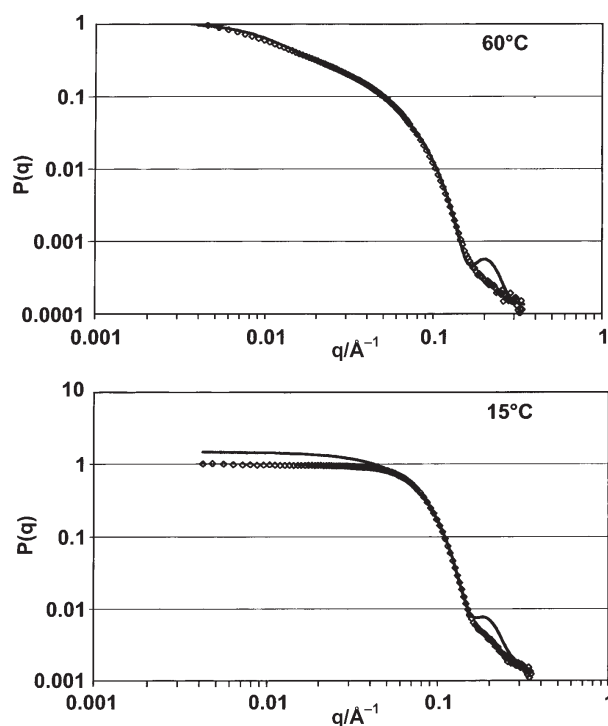

Fig. 3 Experimental SANS curves of 60 mM $C_{14}E_8$ in D_2O at 15 and 60°C. Double logarithmic plot of the intensity, given by the structure factor $P(q)$ *vs.* the wave vector q. The solid lines correspond to a fit for a mondisperse sphere at 15°C and cylinder at 60°C

low q values depends strongly on temperature. These differences clearly indicate that a change in structure occurs. Indeed, at 15°C, the scattering curve reaches a plateau and the typical spectrum of spherical particles is found. At 60°C, above T_t, the continuous increase of the intensity at low q values and a q^{-1} slope in the log-log representation is observed, which is characteristic of elongated particles.

For the evaluation we assume that there is no interaction between the micelles so that we can focus on the form factor. Thus, the contribution from interacting particles, as treated for example by Glatter *et al.* [6], is therefore neglected. We have applied a form factor for spherical geometry [10] to treat the data below T_t and one for cylindrical shape [10] above T_t. The ideal form factors have been smeared by a Log-Normal distribution to describe a radius polydispersity. The obtained theoretical curves are also shown in Fig. 3, where a good agreement is achieved. Nevertheless, it is important to note that the particles, in particular rods, are in reality of polydisperse nature and that the shape of globular micelles such as that of $C_{12}E_8$ is rather oblate than spherical [11]. The side maxima, shown by the theoretical curve in Fig. 3 at q values around 0.2 Å^{-1}, are smeared out in reality according to the reasons given before. Based on this simple analysis, we obtain for the sphere model at 15°C a radius of about 30 Å and for the cylinder model at 60°C a cylinder radius of about 25 and a length around 450 Å. A more detailed analysis will be given elsewhere [12]. These SANS results clearly support the hypothesis of a structural change in form of a sphere-to-rod transition. It is interesting to note that the radius of the cylindric micelle is smaller than that of the sphere which can be understood as a consequence of denser packing in the cylinder.

Discussion

According to our studies, only DSC allows to detect the narrow endothermic phase transition of the C_xE_y micelles. The subsequent, temperature dependent processes such as observed by viscosity, static light and neutron scattering, attributed to micellar growth, do not lead to a narrow thermal response resolvable by the applied DSC instrumentation. Accordingly the enthalpy change of the micellar growth appears to be comparatively small. It is important to note here, that the resolved DSC peak is comparatively sharp although the measured enthalpy change is very small, which can only be understood on the basis of a cooperative transition. Above T_t, the neutron scattering results are indicative of the formation of cylinder-shaped rods. Because the approximate onset temperatures of the growth processes, detectable by viscosity, static light and neutron scattering, clearly correlate with the onset of the endothermic transition in the DSC curves of the investigated micellar C_xE_y solutions, we conclude that the latter transition is a pre-requisite for the growth process, leading to rod formation. The overall process therefore can be interpreted as a sphere-to-rod transition, suggested also for smaller C_xE_y detergents [6]. This interpretation is consistent with recent studies by dynamic light scattering [13], where the particle size distribution has been determined above and below T_t for 30 mM solutions. Below T_t, the apparent hydrodynamic radius for $C_{14}E_8$ micelles is 35.5 Å, above T_t a component

with a much larger value around 110 Å could be resolved [13]. These results agree with the particle dimensions obtained here from the neutron scattering data of $C_{14}E_8$.

With regard to a molecular interpretation of the micellar transition, a dominating change in the properties of the head group has been suggested for $C_{14}E_8$ [7]. The results given here clearly indicate that an elongation of the non-polar tail also markedly affects the transition temperature. Increasing the temperature in the transition range could lead to a removal of water molecules, associated with the polar head groups at the interface. Formally, this corresponds to the breaking of intermolecular hydrogen bonds. This loss of solvate molecules is likely to induce a conformational change of the octa-oxyethylene group, accompanied by a corresponding volume decrease, as schematically shown in Fig. 4a. Following the concept of Israelachvili [14], a volume decrease of the polar part of the detergent molecule will lead to a decreased stability of a spherical (A in Fig. 4a), but to a stabilization of a planar arrangement (B in Fig. 4a) of the detergent molecule. A reduction of curvature, i.e. a tendency towards a more planar arrangement, will of course favour association and the subsequent growth of micelles. This leads to the formation of long cylindrical micelles (rods), as shown schematically in Fig. 4b. In such a non-spherical arrangement, the packing of the detergent molecules can be denser as a consequence of the smaller head group volume. It is thus not surprising that the rod, respectively cylinder radius of the elongated micelle is smaller than that of the globular micelle, predominating below the transition temperature.

The effect of co-solvents can be interpreted accordingly. Ethylene glycol strongly interacts neither with water nor with $C_{14}E_8$. In the presence of glycerol, a strong interaction between the trivalent alcohol and water leads to a decreased water content at the interface of the micelle. A reduction of the number of water molecules bound to the polar head groups favours the planar arrangement energetically. It is
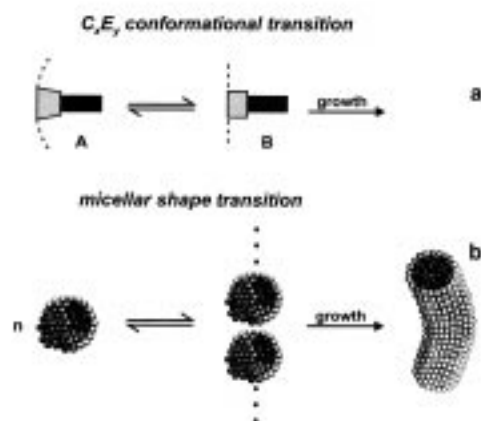

C_xE_y conformational transition

micellar shape transition

Fig. 4 Schematic illustration of a molecular interpretation of the thermal transition on the basis of a single detergent molecule as head group conformation and simultaneous volume change from state A to B, where state B favours the planar molecular arrangement (a); schematic illustration of the consequences of the thermal transition (sphere-to-rod transition) (b);

therefore not surprising that a lower thermal transition temperature is found in the presence of glycerol. On the other hand, ethanol already exhibits a short alkyl chain, although it is short. If the alkyl group interacts with the detergent molecules, it can be incorporated at the interface in an oriented manner so that the ethyl residue sticks into the non-polar alkyl chain region of the micelle. This can lead to an apparent volume increase of the head group, now stabilizing the spherical arrangement of the detergent molecules. The degree of stabilization is so high that the thermal transition is no more observable in the usual temperature range. We thus have to assume that the globular micelles remain unchanged in the presence of ethanol upon increasing temperature. This is consistent with our viscosity and static light scattering observations.

In summary, the thermal transition detected by DSC for this class of non-ionic detergents allows to characterize quantitatively structural changes in detergent micelles. Knowing now its existence, the behaviour of micelles with regard to their temperature induced growth can be explained qualitatively on the basis of a very simple model related to solvent/head group interaction at the interface.

* * *

References

1 J. C. Skou and M. Esmann, Biochim. Biophys. Acta, 567 (1979) 436.

2 H. Michel, In Crystallization of Membrane Proteins (H. Michel ed.), CRC Press, Boca Raton 1991, p. 73.

3 D. J. Mitchell, G. J. T. Tiddy, L. Waring, T. Bostock and M. P. McDonald, J. Chem. Soc., Farad. Trans. 1, 79 (1981) 975.

4 W. H. Richtering, W. Burchard, E. Jahns and H. Finkelmann, J. Phys. Chem., 92 (1988) 6032.

5 R. Strey, Ber. Bunsenges. Phys. Chem., 100 (1996) 182.

6 O. Glatter, G. Fritz, H. Lindner, J. Brunner-Popela, R. Mittelbach, R. Strey and U. Egelhaaf, Langmuir, 16 (2000) 8692.

7 E. Grell, E. Lewitzki, M. von Raumer and A. Hörmann, J. Therm. Anal. Cal., 57 (1999) 371.

8 R. E. Ghosh, S. U. Egelhaaf and A. R. Rennie, Institute Laue-Langevin report no. ILL98GH14T; A cumputing Guide for Small-Angle Scattering Experiments.

9 A. Blume, In Physical Properties of Membranes, Functional Implications (C. Hidalgo ed.), Plenum Press, New York 1988.

10 Lord Rayleigh, Proc. Roy. Soc. London, Ser. A, 84 (1911) 25; O. Glatter and O. Kratky, In Small Angle X-ray Scattering, Academic Press, London 1955.

11 C. Tanford, Y. Nozaki and M. F. Rohde, J. Phys. Chem., 81 (1977) 1555.

12 E. Grell, H. Ruff, E. Lewitzki, R. Schneider, G. Ilgenfritz, M. Kriechbaum, P. Laggner, I. Grillo, manuscript under preparation.

13 H. Ruf, Langmuir, in press.

14 J. Israelachvili, In Intermolecular and Surface Forces, 2nd ed., Academic Press, London 1991.