REVERSE MICELLE FORMATION OF THE DIPEPTIDE "Boc- Ile-Ile-NHMe"

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Abstract

The dipeptide derivative Boc-Ile-Ile-NHMe synthesized in our laboratory is found to form reverse micelles in the presence of water in chloroform solvent. Reverse micelles finds it's importance in the field of drug delivery. The critical micellar concentration (cmc) has been determined by varying the temperature for each R value, where R is the ratio of water to peptide concentration. The R value has been changed from a minimum value of 0.52 to a maximum value of 10. The aggregation number has also been determined for the micelles formed for different R values at room temperature. The change in the micropolarity of the micellar environment has also been monitored using pyrene. The thermodynamic parameters ΔG°_{m}, ΔH°_{m} and ΔS°_{m} has also been determined for the system.

Key words: Reverse Micelles, Dipeptide, Micropolarity.

I. INTRODUCTION

Amphiphilic molecules consist of a hydrophobic head (either polar or charged) and a hydrophobic tail (usually a hydrocarbon chain). They have long been of interest to biophysicists because of their ability to dissolve hydrophobic molecules in aqueous environments and to self assemble into a variety of structures such as bilayers, vesicles and micelles. Micelles have long been understood to result from hydrophobic aggregation of nonpolar tails and energetically favorable hydration of polar heads (1).

Molecular dynamic (MD) simulations have also been used to explore micelle properties. MD simulations of micelles by other groups include simple chainlike model systems (2-4) and atomic level models of real surfactants such as SDS (5), n-decyltrimethylammoniumchloride(6). The formation of micelle like aggregates in nonaqueous solvents has received less attention than the related phenomenon in water. Here, the orientation of the surfactant relative to the bulk solvent will be the opposite of that in water. Hence the term "reverse micelles". Reverse micellar systems are formed by nanometer sized droplets dispersed in organic media by the action of surfactant. Molecular systems were traditionally considered as models to study the action of enzymes at / or near biological membranes. Electrostatic interactions play an important role in the aggregation process but in the opposite sense to that in aqueous solution. A significant energetic consequence of nonaqueous micelle formation is the reduction of unfavorable interactions between the ionic head group of the surfactant and the nonpolar surfactant molecules. Such an effect might be called a "hydrophilic effect".

Unlike situations for aqueous micelles in which interactions between hydrophobic tails contribute little to the overall free energy of micelle formation, ionic, dipolar or hydrogen bonding interactions between head groups in reversed micelles are the primary driving forces favoring aggregation. Unambiguous experimental data are much less available on micelle formation in nonaqueous solvents than for aqueous systems. It is therefore far more difficult to identify trends and draw conclusions concerning the relationships between the chemical structures and critical micelle concentration and aggregation numbers in reverse micelle formation.

Reverse micelle is one of the many models thought to have properties more nearly resembling the biological cellular environment, than does the traditional dilute solution biochemical reaction systems(7). Several antibiotics such as erythromycin, oxytetracycline, benzylpenicillin and acitidione were extracted from aqueous buffers into reverse micellar solution of bis (ethylhexyl)sulfosuccinate sodium salt (AOT) in isooctane and recovered with high efficiency under mild conditions. Preliminary experiments with oxytetracycline dissolved in a fermentation broth indicate that the antibiotic can be selectively extracted from the broth and recovered efficiently without serious loss of

potency. A new method for performing the homogeneous fluoroimmunoassay (FIA) in apolar organic media has also been presented (8). This method is based on the utilization of the reverse micellar system of aerosol OT (AOT) in octane as a medium for the analysis of compounds with low water solubility. It is shown using the system for determination of a hydrophobic pesticide atrazine as an example.

The presence of small amount of water in a sea of apolar solvent environment of the surfactant can have a significant effect on some systems (9). The concept of reverse micelle has been used to synthesize nanoparticles (10-13). researchers have explored details of reverse micelles, which present isolated droplets of polar solvent sequestered from a continuous nonpolar phase by a surfactant layer(14). The solvation dynamics of silver nanoparticles have been studied using reverse micelles(15). Although water is fundamental to the stability and function of proteins (16, 17), its influence on protein folding is not well understood. Reverse micelles offer the possibility of controlling the amount of water available to proteins found in their interior(18).

The micellar inner cavity size is widely varied (from 4 to 120 or more Å) by changing the surfactant hydration degree W_o, the molar ratio of water to surfactant (mole / mole) in the system. This ratio determines most of the structural and physical properties of reverse micelle (19-21). Reverse micelles exhibit relatively ordered structures, characterized by a definite (average) radius, molecular weight and packing density. In general, micelles are an ideal substrate for the investigation of spontaneous formation of ordered structures (22). The presence of trace amount of water in in reverse micelles is of considerable importance as it changes the structural orientation upon interaction with solute and reactant molecules.

Due to the polar core, reverse micelles can solubilise water in apolar solvents. This solubilised water is referred to as water pool(23). The water in the water pool is partly “bound” to the polar wall, and is relatively “free” only above a certain critical concentration into which as the water content increases, the reverse micelles swell in size (14, 15) termed as “Swollen Micelles”(16).

It is well known that the reverse micellar system reliably resembles the microenvironment that enzymes find in the cell. Reverse micelle consists of micropools of water lined by a monolayer of an amphiphile all dispersed in an apolar solvent (24). For a hydrophilic enzyme found in the cytosol or the matrix of an organelle, reverse micelle provide the water pools as a mimetic environment, where the property of the water resembles that of the water closely associated with the cell. The reverse micelles can host all kinds of substrate molecules whether hydrophilic, hydrophobic or amphiphilic and this is one of the important advantages over an aqueous medium.

Reverse micelles possess macroscopic properties that make them an ideal system for enzymological studies. A reverse micellsr solution is thermodynamically stable and optically transparent and large amounts of host molecules can be accommodated without disturbing the macroscopic properties. It is widely accepted that reverse micelles consist of spherical nanometer sized water droplets coated with a close packed surface monolayer of surfactant molecules, oriented in such a way that the surfactant head groups are hydrated at the surface of the water droplet, with the apolar tails protruding out into and solvated by the organic solvent.

Biomolecular shape and therefore function is emergent from a dynamic interplay with water. However, dynamic interactions involving water are extremely complicated and difficult to imagine (25). Hence the relationship between the microscopic interactions of water with biomolecules and macroscopic behaviour is unclear. Mixing of water and headgroups decreases as the size of the micelle increases.

Error Estimation

Estimates listed for error of measured properties are the standard errors of the mean. The standard error is equal to the standard deviation divided by the square root of the number of the independent samples.

II. EXPERIMENTAL

A. Materials and Methods

The peptide was synthesized by solution phase procedure using DCC and the homogeneity was checked by TLC on silica gel.

B. Synthesis and purification of the peptide

Boc – Ile – Ile – Ome: 1a. Boc-Ile (4.62g, 20mmol) was dissolved in 20ml of chloroform and cooled to $-5°C$. Ile-Ome.HCl (1.83g, 20mmol) was added followed by (20 mmol, 3.63g) of DCC and 20 mmol of TEA (2.8ml) and the mixture was stirred at 0°C for 3h. After further stirring overnight at room temperature, the N, N′-dicyclohexylurea (DCU) formed was filtered and the filterate was washed with 1N HCl (3×20 ml), Na_2CO_3 (3×20 ml) and water (2×20 ml). Evaporation of chloroform under vacuum yielded a solid mass, which was dissolved in acetonitrile. The undissolved DCU present in the solution was filtered. The filterate was evaporated in vacuum, which yielded a solid mass, homogeneous on TLC (yield: 5.03g, 70%).

Boc – Ile – Ile –NHMe.1. 1.8g of peptide 1a was dissolved in 1ml of absolute methanol and saturated with methylamine gas. Methylamine was generated by dropping saturated solution of methylamine hydrochloride over NaOH. Methanol was evaporated after 24 h and washed with ether to obtain a solid homogeneous on TLC. This was characterized by 90 MHz NMR (Yield: 1.5g, 83%).

C. Sample Preparation

The injection method has been followed to prepare the reverse micellar solution. Calculated amount of water was added into the stock solution of the peptide in chloroform. Water added to the chloroform solution was not solubilised spontaneously by the micelles. Mechanical disruption of the water drops by shaking the mixture was necessary to obtain a transparent solution.

D. Determination of the Critical Micelle Concentration and the aggregation Number

In the determination of the critical micelle concentration (cmc) of the peptides by UV – VIS and fluorescence spectroscopic techniques, a series of peptide solutions with varying peptide concentrations were prepared. The absorbance (at (λ_{max}) and fluorescence intensity were plotted as a function of peptide concentration. The abrupt changes in the value of the initial slopes at a particular concentration were considered as the cmc of the peptide. For details of determination of the cmc using various techniques, we refer to our previous works (26, 27). For the determination of the aggregation number (28) of the peptide, a semi magnesium salt of 8 - anilino-1 -naphthalene sulphonic acid (ANS) and N- Cetyl pyridinium chloride (CPC) were used as the external probe and quencher, respectively. The technique assumes that the numbers of both probe and quencher molecules per micelle have Poisson's Distributions which leads to the following expression (28, 29):

$$\ln (I_0/I) = N [Q]/(C_s - cmc) \quad \ldots (1)$$

where I_0 and I are the emitted light intensities with quencher concentrations of zero and [Q] respectively, N is the mean aggregation number and C_s is the total concentration of the peptide. N is calculated from the slope of the plot of *ln* (I_0/I) against [Q] for fixed C_s. The probe ANS was used at a concentration small enough to prevent eximer (exciplex) formation. All the experiments were performed in the presence of HPLC grade solvents. The utility of ANS as a probe and the validity of the equation (1) have already been discussed (30, 31).

III. RESULTS AND DISCUSSION

Conventionally, solvophobic interactions provide a driving force for micellization with steric repulsion providing an opposing force (32 – 35). Peptide 1 was found to form reverse micelles with the addition of water in chloroform. The ratio of concentration of water to the concentration of the peptide is defined as the hydration degree R. Neglecting activity effects and using a biphasic micellar model (21), the standard Gibb's free energy change for micellar formation $\Delta G°_m$ of the peptides has been calculated from the following equation:

$$\Delta G°_m = RT \ln cmc = \Delta H°_m - \Delta S°_m \quad \ldots (2)$$

The standard enthalpy change for micelle formation, $\Delta H°_m$ was estimated from the slope of the plot of *ln cmc vs* T using the following equation

$$\Delta H°_m = RT^2 (d \ln cmc/dT) \quad \ldots (3)$$

To calculate all the thermodynamic parameters, the standard states were chosen as the hypothetical states of the solution at unit molar concentration. In recent publications we have considered aggregation number as one of the thermodynamic variables in the

calculation of the above thermodynamic parameters because the aggregation numbers of such peptides is sufficiently low. However, the aggregation number has not been taken into consideration as a thermodynamic variable in the present work because this peptide possesses quite high aggregation number.

A. Critical Micellar Concentration

From table 4 it is seen that the room temperature cmc value increases as the R factor is increased, till R = 2.22. Beyond this, the room temperature cmc value decreases till R = 10. Also the same trend is followed for the aggregation number as well. It is already

Table 1. Thermodynamic parameters of the reverse micelles of peptide 1

R value W_c/P_c	Temperature (°C)	$\Delta G°_m$ (kJ mol^{-1})	$\Delta H°_m$ (kJ mol^{-1})	$\Delta S°_m \times 10^{-3}$ (kJ mol^{-1})
0.52	25	– 16.25	18.42	116.36
	30	– 16.04	19.05	115.83
	35	– 17.40	19.68	120.43
	40	– 18.08	20.32	122.71
1.11	25	– 13.65	23.58	124.96
	30	– 15.51	24.38	131.68
	35	– 16.31	25.19	134.76
	40	– 16.88	26.02	137.08
1.48	25	– 13.30	68.54	274.68
	30	– 15.16	70.86	283.93
	35	– 16.15	73.22	290.20
	40	– 13.61	75.62	285.10
2.22	25	– 13.17	77.17	302.17
	30	– 15.11	79.25	311.44
	35	– 15.59	81.88	316.50
	40	– 15.17	84.56	318.65
4.44	26	– 14.09	20.03	114.15
	30	– 15.76	20.57	119.94
	35	– 16.46	21.25	122.47
	40	– 17.06	21.25	124.67
6.67	26	– 14.59	20.03	114.15
	30	– 15.76	20.57	119.94
	35	– 16.46	21.25	122.47
	40	– 17.06	21.65	124.67
10	26	– 15.08	23.74	130.21
	30	– 13.43	24.38	124.80
	35	– 14.23	25.19	130.15
	40	– 14.75	26.02	130.27

suggested that a higher water to surfactant ratio results in larger and more spherical aggregates (36). This is not followed further when the R factor is increased. This may be due to the fact that the structuring of water molecules is stabilized and hence the constant value of $\Delta H°_m$ and $\Delta S°_m$ for R = 4.44 and R = 6.67. Again for R = 10, it is seen that both $\Delta H°_m$ and $\Delta S°_m$ increases which implies that the stability of the system has come down, which reflects the unstable interaction between the water molecules and the charged groups of the peptides.

Analyzing each R value, it is seen that as the temperature is increased, the cmc value decreases when compared with that at room temperature. This indicates that micellization is favored with increase in temperature. But for R = 10, it is seen that the cmc increases when temperature is increased to 30°C, beyond which the cmc falls, the reasons which have been discussed already.

B. Thermodynamic parameters

Table 1 shows that $\Delta H°_m$ and $\Delta S°_m$ values for the reversed micelles of the peptide in chloroform are always positive in the temperature range of investigation. A negative enthalpy change is attributed to the disordering of the solvent molecules around the exposed solvophilic groups (21). Since $\Delta H°_m$ is positive throughout it indicates the ordering of solvent molecules around the solvophilic groups which means structuring of water molecules which are in contact with the polar groups of the peptide.

C. Aggregation Number

The aggregation numbers observed for the present system are very large. It is seen from table 4 that the aggregation number increases with increase in water content till R = 2.22 (along with the increase in cmc). When the water content is further increased, the aggregation number decreases (along with a decrease in cmc values). Generally the aggregation number increases with increase in cmc values. However, when the water content is more, the above trend is not followed.

With pyrene, the polarity increases in water content, which is reflected by the I_1 / I_3 values given in table 3. When the micelle formation at temperature 25°C is correlated for different R values, it is seen from table 2, that micelle formation is hindered with increase in water as cmc is increasing till R = 2.22. Beyond this, the cmc value of the peptide increases with further addition of water. This indicates that the micelle formation of the peptide is hindered. This might be due to the " swollen micelle" formation (35).

IV. SUMMARY AND CONCLUSION

The dipeptide system synthesized in our laboratory has been found to form reverse micelles. The thermodynamic parameters of the system has been calculated and presented. The thermodynamic parameters for seven different R factor has been calculated. It is seen that a higher water to surfactant ratio results in larger and more spherical aggregates is being confirmed till a particular R value only and beyond that, the trend is not followed, the reasons of which are discussed. Further work is yet to be continued.

Table 2. Cmc values of the reverse micelles of peptide 1 at different temperatures for different R values

R value W_c/P_c	Temperature (°C)	Cmc (mM)	$\Delta G°_m$ (kJ mol^{-1})
0.52	25		1.4
	30	1.7	– 16.04
	35	1.1	– 17.40
	40	0.95	– 18.08
1.11	25	4.2	– 13.65
	30	2.1	– 15.51
	35	1.7	– 16.31
	40	1.5	– 16.88
1.48	25	4.6	– 13.30
	30	2.4	– 15.16
	35	1.8	– 16.15
	40	5.3	– 13.61
2.22	25	4.9	– 13.17
	30	2.4	– 15.11
	35	2.2	– 15.59
	40	2.9	– 15.17

R value W_c/P_c	Temperature (°C)	Cmc (mM)	$\Delta G°_m$ (kJ mol^{-1})
4.44	26	3.4	– 14.09
	30	1.9	– 15.76
	35	1.6	– 16.46
	40	1.4	– 17.06
6.67	26	2.8	– 14.59
	30	2.6	– 15.76
	35	2.4	– 16.46
	40	2.3	– 17.06
10	26	2.2	– 15.08
	30	4.8	– 13.43
	35	3.8	– 14.23
	40	3.4	– 14.75

Table 3. I_1/I_3 values for different R values of dipeptide 1

R value	I_1/I_3	Environment
0.5	2.25	6.15
1.0	2.2	6.15
1.5	2.21	6.15
2.22	2.23	4.8
4.44	2.22	6.15
6.67	2.25	6.15
10.0	2.24	6.15

Table 4. Aggregation numbers for different R values at room temperature (25°C)

R value	Room Temerature cmc	Aggregation number
0.5	1.4	19.6
1.0	4.0	43.0
1.5	4.6	56.6
2.22	4.9	61.0
4.44	3.4	57.6
6.67	2.8	49.7
10.0	2.2	40.2

Table 5. Comparison of the thermodynamic parameters with temperature and R value

R value	Temperature							
	25°C		30°C		35°C		40°C	
	$\Delta H°_m$ kJ mol^{-1}	$\Delta S°_m$ kJ mol^{-1}	$\Delta H°_m$ kJ mol^{-1}	$\Delta S°_m$ kJ mol^{-1}	$\Delta H°_m$ kJ mol^{-1}	$\Delta S°_m$ kJ mol^{-1}	$\Delta H°_m$ kJ mol^{-1}	$\Delta H°_m$ kJ mol^{-1}
0.52	18.42	116.36	19.05	115.83	19.68	120.43	20.32	122.71
1.11	23.58	124.96	24.38	131.68	25.19	134.76	26.02	137.08
1.48	68.54	274.68	70.86	283.93	73.22	290.20	75.62	285.10
2.22	77.17	302.17	79.25	311.44	81.88	316.50	84.56	318.65
4.44	20.03	114.15	20.57	119.94	21.25	122.47	21.65	124.67
6.67	20.03	114.15	20.57	119.94	21.25	122.47	21.65	124.67
10.0	23.74	130.21	24.38	124.80	25.19	130.15	26.02	130.27

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