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The Aggregation and Thermodynamic Studies on the Peptide Micelle Boc-Val-Ile-NHMe in Non-aqueous Medium.

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ABSTRACT

A novel dipeptide *viz* Boc-Val-Ile-NHMe was synthesized in our laboratory and characterized. The aggregational properties of this peptide have been examined in nonaqueous media at different temperatures using various spectroscopic techniques. The critical micelle concentration (cmc) of these peptides has been determined in nonaqueous media at different temperatures using UV-VIS and fluorescence spectroscopic techniques. The cmc's obtained by these methods are in good agreement with each other. The thermodynamic parameters ΔGm^{o}, $\Delta H_m{}^{o}$, $\Delta S_m{}^{o}$, ΔC_p for the peptide has also been estimated. The aggregation number of this peptide has been determined by fluorescence techniques using the Mg salt of 8-anilino-1-naphthalenesulphonic acid (ANS) and N-Cetylpyridinium chloride (CPC) as probe and quencher, respectively.

Keywords: Peptides and protein; Cmc; Aggregation number, enthalpy, entropy, free energy.

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INTRODUCTION

Micelles as drug carriers are advantageous when compared with other altrernatives as soluble polymers and liposomes. Micellar systems increase the bioavailability of drugs [1, 2]. It is well known in the case of protein folding that all the information required to define a tertiary fold is encoded in the aminoacid sequence [3]. As membrane-bound receptors, proteins play several important roles in mediating their functions; the aggregation of membrane active peptides in apolar media is valuable in the modeling of some interactions [4]. Also all proteins comprises of both polar and nonpolar regions, each of which has a specific role to play in the self assembling process of the protein. Bioactive peptides serve to maintain and regulate life activities. They are widely used as drugs, healthcare products and food additives.

There is considerable interest in understanding protein aggregation both in vivo and in vitro [5]. Self aggregation of biomolecules is the process by which specific components of the concerned molecules assemble into well defined aggregates. Peptide self assembly is very similar to the modular assembly involved in protein folding in terms of compactness, the concentration of the nonpolar alkyl side chains and internal architecture. The strong and directional nature of hydrogen bonds between –NH-CO- groups in the peptides contributes to their widespread involvement in self assembling systems [6]**.** Especially in apolar medium, the solvophobic nature of amide groups is an advantage because of their low solubility in apolar liquids [7, 8]. The molecular interactions between the self assembling peptides in both polar and apolar media are yet to be understood in detail. The forces and changes involved in surfactant aggregation in nonpolar non aqueous solvents differ considerably from those for water based systems. The orientation of surfactant relative to the bulk solvent will be the opposite to water [9]. In apolar medium the driving force for aggregate formation is attributable to the high solvophobic property of –NH-CO- groups [10]. Also simple peptide aggregates could provide a framework for understanding the nature, conformation and function of more complicated proteins. Peptide micelles are also predicted to be potential drug carriers [11, 12]. One of the peptide synthesized was found to form reverse micelles [13].

The micelle formation of amphipathic molecules in apolar solvents has been debated in the past because the plot of optical properties vs amphipathic concentration is not sharp enough to get the clear break point, and as a result this gives variable cmc values. In this paper, the peptide derivative Boc-val-Ile NHMe has been synthesized and characterized. Also, the analysis of its aggregation and micellization behavior in nonpolar solvent has also been done.

MATERIALS AND METHODS

The peptide was synthesized by solution phase procedure using dicyclohexylcarbodiimide (DCC) method and the homogeneity was checked by TLC on silica gel and NMR.

Synthesis and Purification of Peptide

Boc-Val-Ile-Ome

4.34g (20 mmol) of Boc-Val was coupled with 20mmol of (1.83g) of Ile-OMe.HCl, 20mmol of triethylamine (TEA) (2.8ml) and 22mmol of DCC (4.33g) and the reaction mixture was stirred at 0ºC for 3 hrs. After further stirring overnight at room temperature, the N, N'-dicyclohexylurea (DCU) formed was filtered and the filterate was washed with 1N H_2SO_4 (3 x 20ml), 1N Na2CO3 (3 x 20ml) and water (2 x 10ml). 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. A solid mass homogeneous on TLC was obtained and was characterized by 90 MHz NMR spectrometer. (Yield: 5g, 72%).

Boc-Val-Ile-NHMe

1.73g of peptide (5mmol) was Boc-Val-Ile-NHMe 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 24h and washed with ether to obtain a white solid. The final yield was homogeneous on TLC and was characterized by NMR spectrophotometer. (Yield: 1.3g, 75%).

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Determination of the Critical Micelle Concentration and the Aggregation Number

A series of peptide solutions with varying peptide concentrations were prepared to determine the critical micelle concentration (cmc) of the peptides by UV-VIS and fluorescence spectroscopic techniques. The absorbance 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 the determination of the cmc using various techniques, we refer to previous works and also those of Mandal *et al* ET AL [14-16].

For the determination of the aggregation number of the peptides, a semi magnesium salt of 8-anilino-1-napthalenesulfonic acid (ANS) and N-Cetyl pyridinium chloride (CPC) were used as the external fluorescent probe and quencher, respectively. The technique assumes that the numbers of both probe and quencher molecules per micelle have poisson distributions which leads to the following expression [17, 18].

$$In\ (Io/I) = N\,[Q] / (C_s - cmc) \text{ ---------- (1)}$$

Where Io and I are the emitted light intensities with quencher concentrations of zero and [Q], respectively; N is the mean peptide aggregation number and Cs is the total concentration of the peptide. N is calculated from the slope of the plot of *In* (I_o/I) against [Q] for fixed C_s. The probe ANS was used at a concentration small enough to prevent excimer (exciplex) formation. All the experiments were performed in the presence of HPLC grade solvents and there were no trace amounts of water in the system. The utility of ANS as a probe and the validity of equation (1) have been recently discussed [10, 19].

RESULTS AND DISCUSSION

Table 1 shows the value of the cmc and some thermodynamic parameters of the peptide derivative. Figure 1 shows the plot of UV absorbance against the peptide concentration at various temperatures of the peptide derivative. Figure 2 shows the nmr spectra of the peptide derivative. The cmc value of the dipeptide has been found to be 1.4 mM at 22^{o}C. The cmc of the peptide increases with increase in temperature indicating that the micelle formation is being hindered by the increase in temperature. The aggregation number is found to be 69 for the peptide derivative in chloroform. The cmc value of the present peptide derivative is lower than that of our previous peptide Boc-Val-Val-NHMe [15]. The standard Gibbs energy change for micelle formation using biphasic micellar model and neglecting activity effects is given by:

$$\Delta G_m^{\circ} = RT\ In\ cmc = \Delta H_m^{\circ} - T\Delta S_m^{\circ} \text{ ---------- (1)}$$

The standard enthalpy change for micelle formation, ΔH_m° was estimated from the slope of the plot of ln cmc *vs.* T.

To calculate all the thermodynamic parameters, the standard states were chosen as the hypothetical states of the solutions at unit molar concentration. In our present work, the aggregation number has not been taken into consideration as a thermodynamic variable in since the peptide derivative, in apolar solvent possess quite high aggregation numbers which are almost independent of temperature. ΔCp°, the standard heat capacity change for micellisation obtained from the slope ΔH_m° *vs* T is also given in the Table.

It is assumed that a major factor driving the surfactant molecules into aggregation in water is a positive enthalpy change, presumably associated with the breakdown of the structured water which surrounds the hydrocarbon chain in the unassociated species. The micellisation of peptides in chloroform is attributed to a negative entropy change where there is a transfer of a chloroform molecule into the peptide micelle, as per Evans *et al*. Table also shows that ΔH_m° and ΔS_m° values for the micelles of the peptide in chloroform are always negative in the temperature range of the investigation. The negative value of ΔS_m° may arise from the values of ΔH_m° owing, to some extent, to the reestablishment of the hydrogen bonds in the solvent. Therefore the results indicate that the driving force for micellization for the peptide in chloroform is enthalpic nature. This analysis has also been applied by Evans and Ninhan [20] to changes in protein conformation and to other biochemically, important self assembling processes. Comparing the results of thermodynamic experiments on model organic compounds, it is apparent that the heat capacity changes play a crucial role in characterizing hydrophobic interactions [21, 22].

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Table 1: Critical micelle concentration and some thermodynamic parameters for the peptide aggregates in chloroform at various temperatures.

Temperature (K)	cmc (mM)	ΔG_m° ($KJmol^{-1}$)	ΔH_m° ($KJmol^{-1}$)	ΔS_m° ($JK^{-1}mol^{-1}$)	ΔC_p° ($JK^{-1}mol^{-1}$)
295	1.40	-16.1	-19.5	-11.6	-131
305	1.83	-16.0	-20.8	-16.1	
310	2.1	-15.9	-21.5	-18.3	
320	3.12	-15.1	-22.2	-22.7	

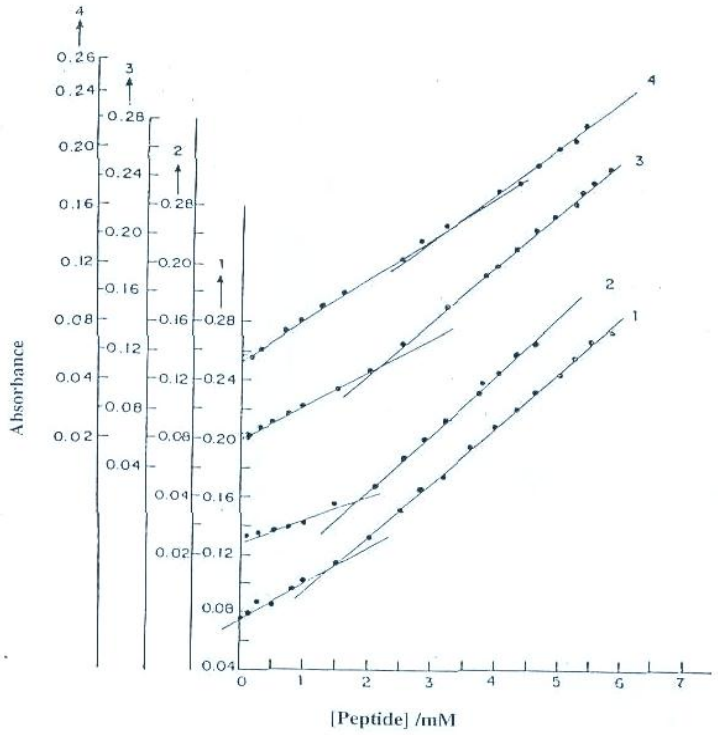

Figure1: Plot of absorbance vs peptide concentration at different temperature for peptide 2; λ_{max} = 262nm. Curve Nos. 1-4: 299,305,310 and 320° K. Curve numbers correspond to ordinate scale numbers.

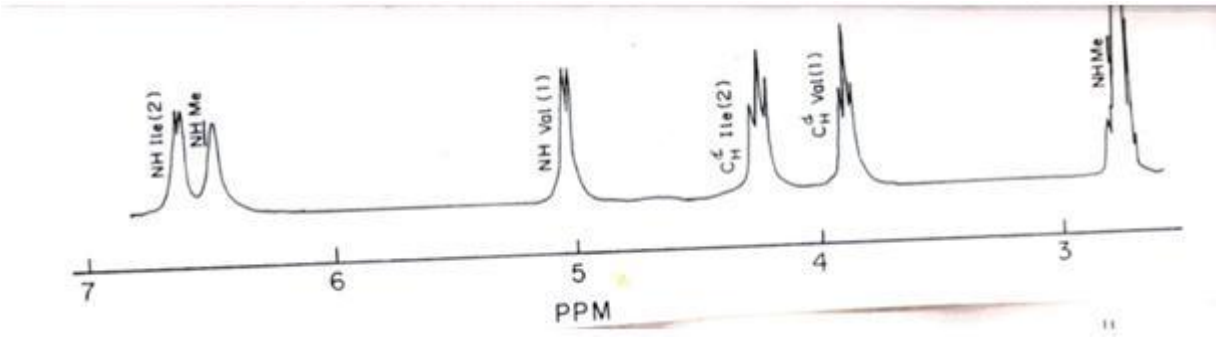

Figure 2: Proton nmr spectra of the peptide Boc-Val-Ile-NHMe

If we attribute a negative ΔG_m° to hydrogen bonding with solvent molecules, then the process does not result in more ordered molecules around the exposed groups (which can interact with the chloroform molecules). Then such a process would also decrease ΔC_p° values [23, 24] since release of chloroform solvation leads to a reduction in the number of heat absorbing bonds. In the present case, the negative value of ΔCp° implies that the chloroform is less ordered around the peptides micelles. Hence the negative ΔH_m° may be attributed to the relatively well solvated peptide molecules in the micellar state.

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CONCLUSION

The experimental observations we conclude that for the dipeptide, there is an increase in the value of critical micellar concentration with increase in temperature. This indicates that the micelle formation is being hindered by the increase in temperature. The potential of the peptide micelle to be utilized as a carrier of drug is yet to being worked upon. The possible potential of the peptide to be used as a drug carrier is to be analyzed insilico.

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