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# AGGREGATIONAL STUDIES OF DIPEPTIDE FROM *CLOSTRIDIUM* STRAIN MP FLAVODOXIN

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**ABSTRACT**

With recent innovations in the field of nanosciences, research has shown tremendous advances in the arena of drug carriers. In this aspect, our work comes out with a novel dipeptide Boc-Val-Val-NHMe which has been synthesized, characterized and its aggregational behavior studied in non aqueous medium. The peptides forms inverted micelles at different temperatures ranging from 297 to 390 Kelvin. Inverted micelles are discrete particles with the hydrophilic ends clustered together leaving the hydrophobic moieties to interact with the solvent (hydrophobic) medium. These micelles find diverse applications.

**Keywords:** Micelles, critical micelle concentration, dipeptide, aggregation, aggregation number.

## INTRODUCTION

Proteins play many roles as membrane-bound receptors in mediating their functions. The aggregation of membrane active peptides in apolar media is valuable in the modeling of some interactions[1]. 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[2]. 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. The core shell architecture of polymeric micelles provides a hydrophobic core which serves as a natural carrier[3, 4]. Drug delivery systems using nanotechnology is on the raise in recent years[5]. Protein nanoparticles can be exploited to create different interactions and and subsequently can form three-dimensional networks offering a variety of possibilities for the reversible binding of active molecules, protecting them in a matrix as well as specific targeting to the site of action[6].

Self assembly of peptides resembles the modular assembly of proteins with respect to compactness, concentration of the nonpolar alkyl side chains and internal architechture. The widespread involvement of peptides in self assembling systems is due to the strong and directional nature of hydrogen bonds between –NH-CO- groups[7]. The interactions between the self assembling peptides in polar and apolar medium still need exploration. The solvophobic property of the CO-NH groups is the driving force for the aggregation in apolar medium[8]. Again, it is noteworthy that the nature, conformation and function of more complicated proteins could be understood by studying those of small peptides. Val-Val- is found in the amino acid sequence of *Clostridium* strain MP flavodoxin[9]. The aggregate formation of several peptides have been studied under various condition and for various purposes[10-14]. Interaction between the connective tissue protein collagen and cyclodextrin has been analysed[15].

In this present work, the dipeptide, Boc-Val-Val-NHMe has been synthesized, characterized and its aggregation behavior has also been analysed. It is found that this dipeptide forms micelles in a non aqueous medium, chloroform.

## MATERIALS AND METHODS

All peptides were synthesized by solution phase procedures using dicyclohexylcarbodiimide (DCC) method and the homogeneity was checked by TLC on silica gel. The structure of the synthesised peptide was confirmed by proton nmr spectra using Bruker, MSL300 P(300MHz) spectrometer.

### Synthesis and Purification of Peptides

***Boc-Val-Val-OMe*, 1a**. Boc-Val (2.1 g, 10mmol) was dissolved in 20ml of chloroform and cooled to -5ºC. Val-OMe.HCl (1.676g, 10mmol) was added followed by TEA (1.4ml, 10mmol) and N,N'-dicyclohexyl carbodiimide (DCC) (2.25g, 11mmol) and the reaction mixture was stirred at 0ºC for 3 h. After further stirring overnight at room temperature, the N,N'-dicyclohexylurea (DCU) formed was filtered and the filterate was washed with 1N HCl (3 x 20ml), 1N $Na_2CO_3$ (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, which yielded a solid mass, homogeneous on TLC (yield: 2.46g, 75%).

***Boc-Val-Val-NHMe*, 1**. 1g of **1a** (5mmol) 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, homogeneous on TLC. (Yield: 75%).

### 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. The absorbance and fluorescence intensity of a series of solutions 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. Details of the determination of the cmc using various techniques, can be to previous works[16, 17]. For the determination of the aggregation number[18] 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 [19, 20]:

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

Where $I_o$ and I are the emitted light intensities with concentrations of zero and [Q], respectively; N is the mean peptide aggregation number and $C_s$ is the tital 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 systems. The utility of ANS as a

probe and the validity of equation (1) have been already discussed [10, 11, 16, and 17].

## RESULTS AND DISCUSSION

Tables 1 show the value of the cmc and some thermodynamic parameters of the peptide derivative. Figure 1show the plot of UV absorbance against the peptide concentration at various temperatures of the peptide derivative. Cmc of the peptide increases with increase in temperature in the observed range. When compared with that of our previous peptide[13] it can be seen that the cmc of this peptide is greater than the peptide Boc-Ile-Ile-NHMe. From which it can be inferred that the solvophobicity of this peptide is comparatively lesser. Neglecting activity effects and using a biphasic micellar model[21, 22] the standard Gibbs energy change for micelle formation $Gm^{\circ}$, of peptides has been calculated from the following equation:

$$\Delta G_{m^{\circ}} = RT \ln cmc = \Delta H_{m^{\circ}} - T\Delta S_{m^{\circ}} \quad \text{----------(2)}$$

The standard enthalpy change for micelle formation, $\Delta Hm^{\circ}$ was estimated from the slope of the plot of In cmc *vs* T.

$$\Delta H_{m^{\circ}} = - RT^2 (d\ln cmc / dT) \quad \text{---------- (3)}$$

To calculate all the thermodynamic parameters, the standard states were chosen as the hypothetical states of the solutions at unit concentration.

The ΔGmº, ΔHmº and ΔSmº values of standard heat capacity change for the micellization, ΔCpº, obtained from 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, This interpretation is relevant to the formamide systems, in which some structuring by dissolved hydrocarbon also occurs. According to Evans et al., the above interpretation is erroneous or, at least misleading because at high temperatures water loses most of its structural properties and the formation of structured water in the walls of the hydrocarbon cavity is no longer possible[23]. According to Evans et al., it is sensible to attribute the micellization of the peptides in chloroform to a negative entropy change which is due to a transfer of the chloroform solvent into the peptide micelles[23]. The aggregation numbers was found to be 27 for the peptide derivative in chloroform.

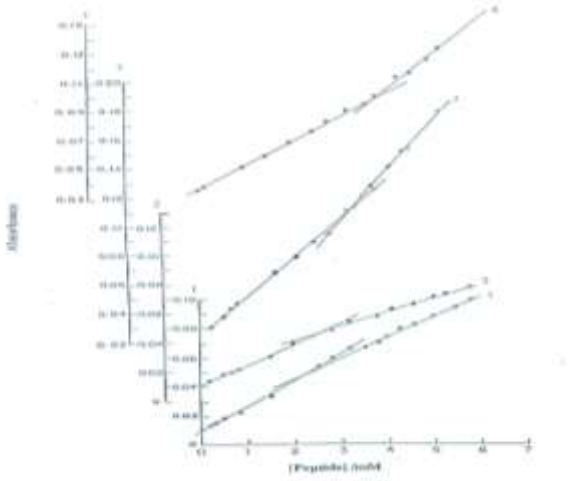

**Fig 1**: **Plot of absorbance Vs peptide concentration at different temperature for peptide 1; $\lambda_{max}$ = 262nm. Curve Nos. 1-4: 297,305,310 and 320°K. Curve numbers correspond to ordinate scale numbers.**

If we consider the structural aspects of the peptides, it can be easily seen that for the peptide dipeptide, the critical micelle concentration increases with increase in temperature (Table 1). Conventionally, solvophobic interactions provide a driving force for micellization, with steric repulsions providing an opposing force[25, 26]. In an apolar medium like chloroform, the main interaction in the peptide aggregation is believed to be intermolecular hydrogen-bond formation[27]. However, a plot of a large number of literature values for ΔGmº determined in chloroform *vs* n (where n is –NH-Co-groups) lead to a straight line with a slope of -5± 1kj mol-1 due to the stabilization of amide groups of the peptide on interaction with chloroform molecules. In the present case, the three amide groups interacting with chloroform should result in a minimum of -15± 3 kj mol-1 of energy of stabilization to the aggregate [28, 29]. the ΔGmº values obtained in the above range (**Table 1**) are in good agreement with this interpretation.

**Table 1**: **Critical Micelle Concentration and some thermodynamic parameters for The peptide 1 aggregates in chloroform solution at various temperatures.**

| Temperatur (k) | Cmc (Mm) | ΔGm º (kjmol-1) | Δ Hmº (kjmol-1) | ΔSmº (JK-1 mol-1) | ΔCp (JK-1 mol-1) |
|---|---|---|---|---|---|
| 297 | 2.25 | -15.5 | -16.8 | -6.1 | |
| 305 | 2.26 | -15.0 | -17.8 | -8.9 | -117 |
| 310 | 3.35 | -14.7 | -18.3 | -11.9 | |
| 320 | 3.75 | -14.8 | -19.5 | -17.7 | |

The polarity of the interior of the aggregate of the derivative was studied using flurescence emission of pyrene-1-buric acid (PBA). For in chloroform emission intensities of the ratio of the first to the third peak i.e.,$I_1/I_3$ is 2.19. On the addition of the dipeptide derivative the $I_1/I_3$ value of PBA varies. With the addition of peptide, the above $I_1/I_3$ ratio reduces to 2.16 where the environment of pyrene is similar to that of chloroform, since € has a value of 4.8. The ratio is shown in Table 2.

**Table: 2 $I_1/I_3$ values of pyrene in the absence and presence of the three peptides 1, 2 and 3**

| PBA in $CHCl_3$ | | Fluorescence Intensity | $I_1/I_3$ | Environment |
|---|---|---|---|---|
| Peak | λ | | | |
| 1 | 377 | 12.2 | 2.19 | --- |
| 3 | 389 | 5.56 | | |
| Boc-Val-Val-NHMe | | | | |
| 1 | 377 | 24.6 | 2.16 | 4.8 |
| 3 | 389 | 11.4 | | |

## CONCLUSION

This analysis shows the peptide, forms micelles in nonaqueous micelles. The critical micelle concentration of the micelle at various temperatures has been studied. It has been found that the solvophobicity of the peptide is lesser.

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