Micelle Formation of Boc-Val-Val-Ile-OMe Tripeptide in Chloroform and its Conformational Analysis

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The tripeptide derivative **1** (Boc-Val-Val-Ile-OMe) forms micelles in chloroform as confirmed by UV–VIS, fluorescence and NMR spectroscopic techniques; the conformational analysis of the tripeptide **1** was determined by ^{1}H NMR spectroscopy.

The ordered aggregates of peptide derivatives in an apolar medium like cyclohexane and benzene have recently been reported by Ihara *et al.*[1] The driving force for micelle formation in an apolar medium is attributable to the highly solvophobic property of NHC(=O)– groups which provide low-solubility parameters.[2] We have already reported[3] the micelle formation of tetrapeptides in dilute aqueous solution which are stabilised by intermolecular hydrogen bonds. To the best of our knowledge, peptides do not form micelles. Moreover, the micelle formation of peptides in chloroform has rarely been reported in the literature. However, in this communication, we report that the tripeptide Boc-Val-Val-Ile-OMe, **1**, with three amide groups in chloroform can be self-assembled with defined conformational preference in the micellar state. The self-assembling peptide in an apolar medium will provide a good model system to study β-structure nucleation of proteins, where the β-structure forms in the highly hydrophobic environment.[4–6] The sequence of peptide **1** has several noteworthy characteristics. It was designed to be soluble and to adopt preferentially a β-sheet conformation in organic media.[5] In addition, the constituent amino acids, both Val and Ile, have β-branching which will have a preferred conformation that 'cup' against each other back-to-front with favourable packing.[6] This sequence is found in the β-sheets region of protein triosephosphate isomerase.[6]

Peptide **1** was prepared by the solution-phase method using the dicyclohexylcarbodiimide (DCC)–hydroxybenzotriazole (HOBT) strategy.[7] The aggregation studies were carried out using *tert*-butylphenol as an external probe. The concentration of the probe was kept sufficiently low (2×10^{-5} mol dm^{-3}) during UV–VIS and fluorescence spectroscopic measurements so that the ratio between the probe and peptide micellar concentrations is $\ll 1$, and therefore, the question of perturbation of peptide micellar structure by the probe will not arise. Among two major peaks of the probe (243 and 276 nm), the high-energy peak, *i.e.* 243 nm (λ_{max}) was chosen for the aggregation studies. Fig. 1 shows the absorbance *vs.* peptide concentration in chloroform solution at 25 °C. In Fig. 1, there is a clear break at 2.5 mmol dm^{-3}, the critical micelle concentration (CMC) of the peptide. The CMCs of the peptide obtained at 5 and 25 °C using fluorescence techniques (see Fig. 2) are 1 and 2 mmol dm^{-3}, respectively. Amide ^{1}H NMR chemical shifts have been increased at concentrations above the CMC of the peptide (Fig. 3). Therefore, the CMC of the peptide also has been determined from the plot of amide proton chemical shift *vs.* peptide concentration [Fig. 3(*b*)]. The CMC values obtained by these three methods are in good agreement with each other. Regarding details for the CMC determination, we refer to our earlier works.[8–10]

Temperature dependence of the three amide protons chemical shift has been studied at 10, 15, 20, 25 and 40 °C. In all the cases, linear plots with negative coefficients, *i.e.* dδ/dT of the order of $\geq 2 \times 10^{-3}$ ppm °C^{-1} are obtained and are given in Table 1. In $CDCl_3$ the values ≤ 0.002 ppm °C^{-1} may be associated with the solvent exposed or strongly intramolecularly hydrogen bonded NH's while higher dδ/dT are assigned to intermolecularly hydrogen bonded NH.[11] The high dδ/dT values for the amide protons indicate that these protons are involved in intermolecular hydrogen bonding. The C$^\alpha$H and NH regions of peptide **1** have been assigned, and part of it has been in Fig. 4(*a*). The ^{1}H NMR assignments were determined by using the 2D COSY technique and the NMR parameters are listed in Table 1. The broad nature of the NMR signals of this peptide is due to aggregation. However,

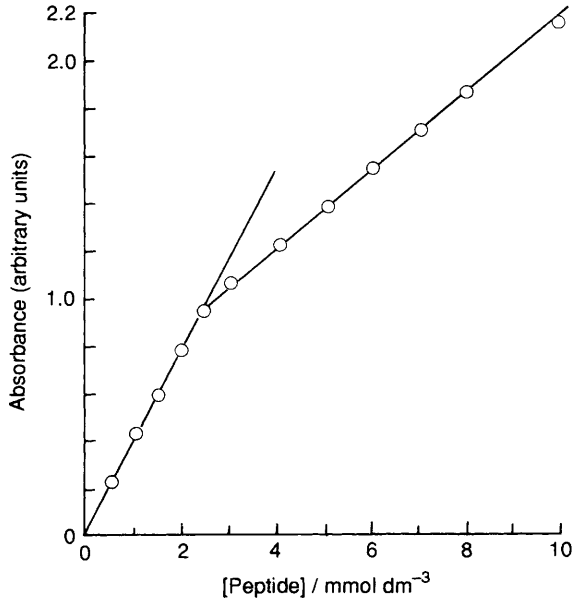


Fig. 1 Plot of absorbance for *tert*-butylphenol at 243 nm *vs.* peptide concentration at 25 °C in chloroform solution; [*tert*-butylphenol] = 2×10^{-5} mol dm^{-3} (fixed)

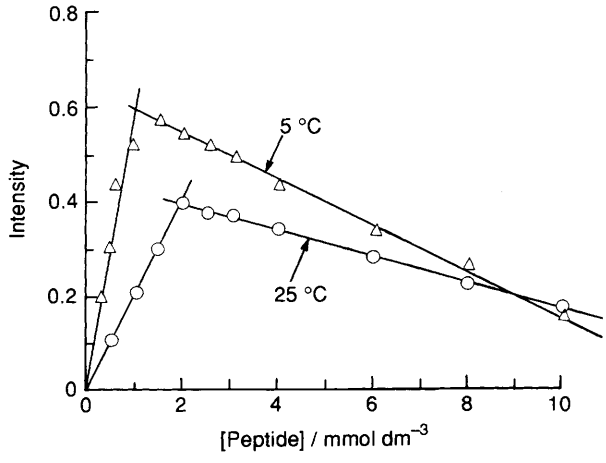
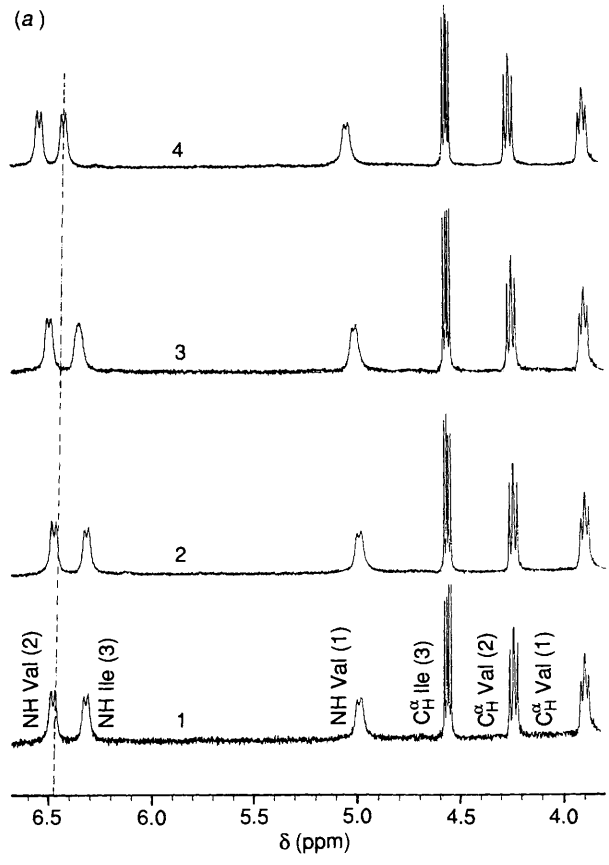


Fig. 2 Plot of fluorescence intensity for *tert*-butylphenol *vs.* peptide concentration in chloroform solutions at 5 (△) and 25 °C (○); [*tert*-butylphenol] = 2×10^{-5} mol dm^{-3} (fixed); λ_{ex} = 262 nm and λ_{em} = 408 nm

the NH and C$^{\alpha}$H resonances of individual residues are well separated indicating one predominant conformation. The difference nuclear Overhauser effect (NOE) spectra are shown in Fig. 4(*b*)–(*g*). The observed positive NOEs for peptide **1** in $CDCl_3$ suggest that rotational correlation times are short enough to be in the region $\omega\tau_c \ll 1$ at 270 MHz.[12] The NOE data of the peptide is given in Table 2. Two interresidue NOEs ($C_i^{\alpha}H \longleftrightarrow N_{i+1}H$) are clearly observed. The observed NOEs in $CDCl_3$ are fully consistent with the β-structure conformation because in such a conformation, the



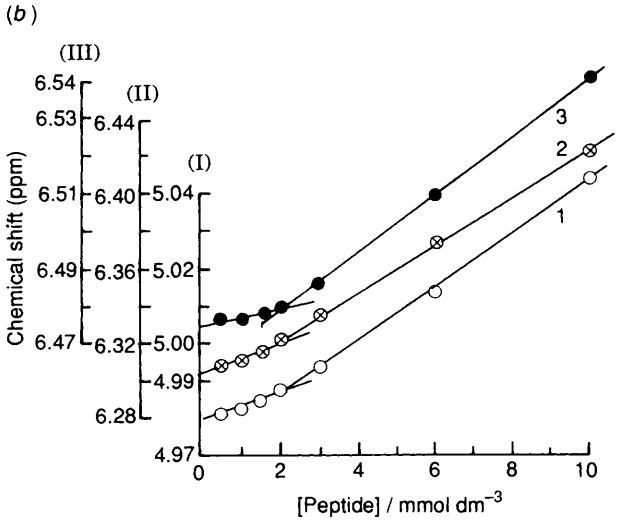


Fig. 3 (*a*) 400 MHz ^{1}H NMR spectra of different concentrations of peptide **1** in $CDCl_3$ at 25 °C; 1, 0.5; 2, 2; 3, 6 and 4, 10 mmol dm^{-3} peptide. (*b*) Plot of three amide protons chemical shift *vs.* peptide concentration at 400 MHz and at 25 °C. Curve numbers correspond to ordinate scale numbers; ○, NH Val (1); ⊗, NH Ile (3); ●, NH Val (2)

Table 1 ^{1}H NMR parameters and calculated φ values from $J_{NH\text{-}H\alpha}$/Hz for peptide **1**

Residue:	Val (1)	Val (2)	Ile (3)
Parameters			
δNH^a	5.00	6.50	6.38
$\delta H\alpha^a$	3.91	4.25	4.57
$d\delta/dT^b$	−0.002	−0.003	−0.004
$^3J_{NH\text{-}H\alpha}$/Hz^c	7.9	8.6	8.1
ϕ^d	−119.9	−126.9	−121.9
	−89.9	−96.9	−91.8

[a] δ Values are expressed as ppm downfield from internal $SiMe_4$ and reported for a peptide concentration of *ca.* 10 mmol dm^{-3} in $CDCl_3$. [b] $d\delta/dT$ Values are expressed as ppm K^{-1} measured at the concentration of 10 mmol dm^{-3}. [c] Errors in *J* values are ±0.4 Hz. [d] Possible φs were obtained from Karplus equation with sets of constants used by Pardi *et al.* The selected values of φs are underlined.

Table 2 Difference[a] NOE data for peptide **1**

Resonance irradiated	NOE observed	% Enhancement
Val (2) NH	Val (1) C$^{\alpha}$H	10.9
Ile (3) NH	Val (2) C$^{\alpha}$H	12.5
Val (1) NH	—	—
Ile (3) C$^{\alpha}$H	—	—
Val (2) C$^{\alpha}$H	Ile (3) NH	8.3
Val (1) C$^{\alpha}$H	Val (2) NH	7.3

[a] NOE experiments were carried out at a probe temperature of 293 K and reported peptide concentration of *ca.* 20 mmol dm^{-3}.

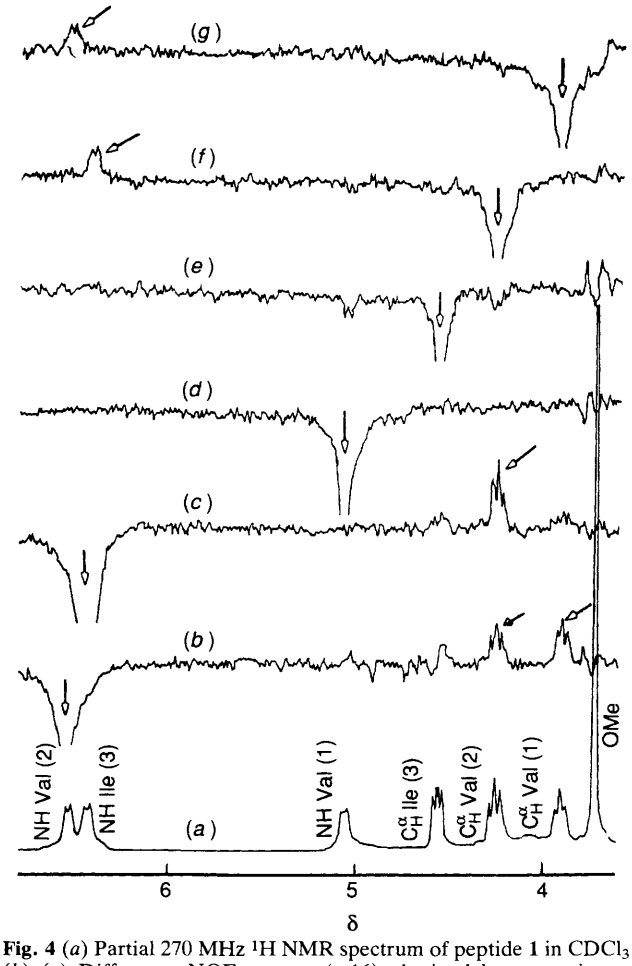


Fig. 4 (*a*) Partial 270 MHz ^{1}H NMR spectrum of peptide **1** in $CDCl_3$. (*b*)–(*g*) Difference NOE spectra (×16) obtained by saturation of specific resonances indicated by downward arrows. The enhancements are indicated by slanted arrows.

*i*th and (*i* + 1)th residues adopt structures with $\phi = -120° \pm 10°$ and $\psi = 130° \pm 10°$ resulting in a close approach (2.1–2.4 Å) of $C_i^{\alpha}H$ and N_{i+1} protons.[13] In all cases NOEs in the direction of $N_{i+1}H \rightarrow C_i^{\alpha}H$ are always greater in magnitude than those for the reverse direction. This is because of the availability of alternative relaxation pathways for the NH protons.

The vicinal coupling constant of amide protons of the peptide **1** is given in Table 1. From the ^3J(HN-αH) values, the dihedral φ for rotation about the N–C bond was obtained using Pardi's equation.[14] The selected φ values are underlined in the Table 1. These values fall in the β-sheet regions of the Ramachandran plot[15] confirming the presence of β-sheet conformation. Apart from these results the relatively low-field positions of $C^{\alpha}H$ and NH protons of the amino acid residue in $CDCl_3$ are presumably a consequence of such a conformation.[16] An ideal β-sheet structure results in a fairly close approach of the >C=O of the amino acids in peptides. In principle, this should result in electrostatic destabilisation due to unfavourable dipole–dipole interaction. Such an unfavourable interaction could be compensated by strong linear >C=O···H–N hydrogen bond formation. In a low dielectric solvent like $CDCl_3$, the formation of such intermolecular hydrogen bonds determines the conformation. This condition persists in the interior region of the proteins where the estimated dielectric constant is very low.[17] Thus, we conclude that the micellisation of this peptide in an apolar medium has a stabilising β-structure conformation.

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