CONFORMATIONAL FLEXIBILITY IN ENKEPHALINS: SOLVENT DEPENDENT TRANSITIONS IN PEPTIDES WITH GLY–GLY SEGMENTS DETECTED BY CIRCULAR DICHROISM

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1. Introduction

The widespread interest in developing structure–activity correlations for opioid peptides [1–3] has led to a large number of investigations on the preferred solution conformations of enkephalins [4–11]. The results of these studies have led to proposals ranging from β-turn conformations. With Gly^2–Gly^3 [14] or Gly^3–Phe^4 [4–10] as the corner residues. Evidence for the lack of preferred conformations in solutions, resulting from dynamic averaging between an ensemble of structures, has also been presented [11]. While a consensus has yet to be reached on the solution conformations of enkephalins, there is general agreement that receptor interactions presumably involve a folded, defined conformation of the pentapeptide [12–14]. The recognition of multiple receptor sites for opioid ligands [15–17], has provided a further impetus for studies of conformational flexibility in enkephalins, with the possibility that interaction at μ and δ sites may be mediated through different conformations of the peptides [18]. Here, we describe an unusual solvent-dependent conformational transition, in the protected fragment Boc-Gly–Gly–Phe–Met-NH_2 and Met^5-enkephalinamide which is abolished on restricting conformational freedom by substitution of Gly by α-aminoisobutyryl (Aib) residues [19,20].

Abbreviations: Aib, α-aminoisobutyryl; DCC, *N,N'*-dicyclohexylcarbodiimide; TLC, thin-layer chromatography; Boc, t-butyloxycarbonyl; TFE, trifluoroethanol

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2. Experimental

All peptides were synthesized by solution phase procedures using DCC or DCC-1-hydroxybenzotriazole mediated couplings. Peptides were characterized by 270 MHz ^1H NMR and checked for homogeneity by TLC on silica gel. CD spectra were recorded on a JASCO J-20 spectropolarimeter using cells of 1 mm pathlength. Molar ellipticities $[\theta]_M$ were calculated using the formula:

$$[\theta]_M = (\theta_{obs} \times M_r)/10\, C\, .\, l \text{ deg} \,.\, \text{cm}^2 \,.\, \text{dmol}^{-1}$$

where

θ_{obs} = observed reading in degrees;
C = concentration in g/ml;
l = pathlength in cm.

^1H NMR studies were carried out on a Bruker WH-270 FT-NMR spectrometer at the Bangalore NMR Facility. Amide NH resonances of Phe and Met were unambiguously identified by spin decoupling experiments, which established the connectivities between $C^{\beta}H_2$, $C^{\alpha}H$ and NH protons. Variable temperature measurements were carried out in $(CD_3)_2SO$ over 20–80°C, at 10 mg peptide/ml.

3. Results and discussion

Fig.1 shows the CD spectrum of Boc-Gly–Gly–Phe–Met-NH_2 (1) in methanol, trifluoroethanol (TFE) and a 1:1 (v/v) mixture of these solvents. In MeOH, a weak negative band at 235 nm and a strong

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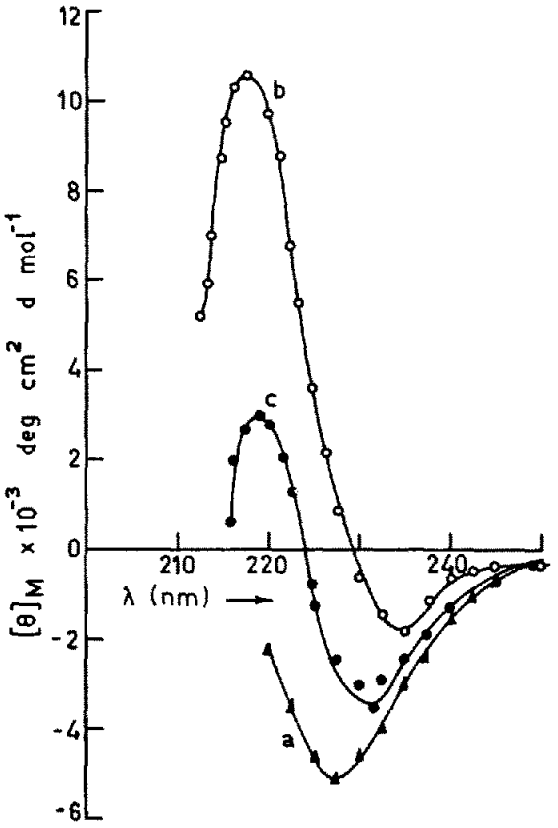

Fig.1. CD spectra of Boc-Gly–Gly–Phe–Met-NH_2 1 (1.96 mM) in: (a) TFE; (b) MeOH; (c) TFE–MeOH, 1:1 (v/v).

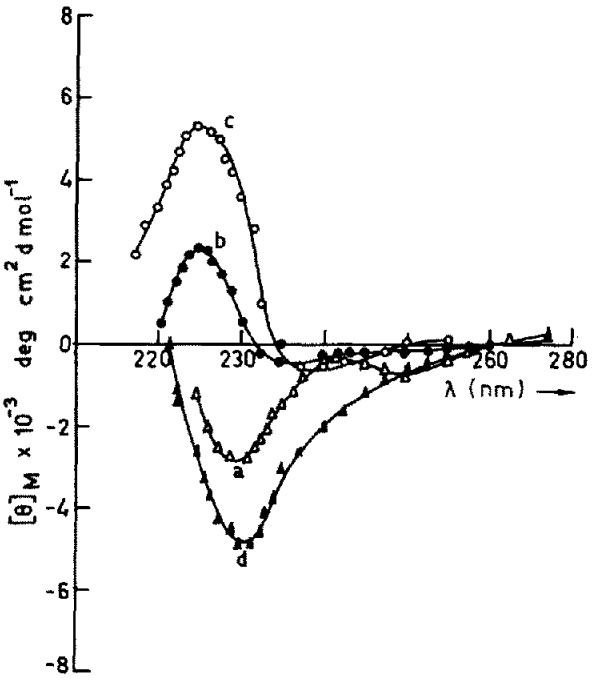

Fig.2. CD spectra of Tyr–Gly–Gly–Phe–Met-NH_2 (1.74 mM) in: (a) MeOH and (b) TFE; Boc-Tyr–Gly–Gly–Phe–Leu-NH_2 (1.53 mM) in (c) MeOH and (d) TFE.

positive band at 218 nm is observed, whereas in TFE there is a dramatic change, with only a negative band at 228 nm. This suggests that the peptide undergoes a large conformational change on going from MeOH to TFE. Interestingly, the spectrum in a 1:1 solvent mixture (fig.1c), exhibits CD bands characteristic of both solvents, suggesting that different conformational states may be populated. A similar solvent dependent reversal of the signs of the CD bands is also observed in Met^5-enkephalinamide (Tyr–Gly–Gly–Phe–Met-NH_2) and Boc-Tyr–Gly–Gly–Phe–Leu-NH_2 (fig.2). Aib residues, in which both C^α hydrogens of Gly are replaced by methyl groups, can be used to restrict the conformational flexibility of small acyclic peptides [21]. Fig.3 shows the CD spectra of 3 tetrapeptides, in which the Gly residues are separately and simultaneously replaced by Aib residues. Both Boc-Aib–Gly–Phe–Met-NH_2 (2) and Boc-Aib–Aib–Phe–Met-NH_2 (3) show positive CD bands at 218 nm in MeOH as observed for **1**. However, in the case of Boc-Gly–Aib–Phe–Met-NH_2 (**4**) a negative band at 216 nm is observed. In all the Aib-substituted peptides there is no change in the sign of the CD band on going from MeOH to TFE. Small shifts in band position and changes in intensity are observed. The relevant CD parameters for these peptides are summarized in table 1. The CD spectra were concentration-dependent over 1–2 mM in TFE.

The above results establish that Boc-Gly–Gly–Phe–Met-NH_2 (**1**), Met^5-enkephalinamide and Boc-Tyr–Gly–Gly–Phe–Leu-NH_2 show pronounced solvent-dependent conformational equilibria. Substitution of Gly by Aib abolishes this degree of flexibility. Earlier studies of Aib peptides, have clearly established the propensity of Aib–X and X–Aib sequences to adopt β-turn conformations, stabilized by an intramolecular 4 → 1 hydrogen bond [21–25]. ^{1}H NMR studies of the tetrapeptides have been carried out to delineate the intramolecularly hydrogen-bonded NH groups. Temperature coefficients ($d\delta/dT$) of the Phe and Met NH-groups, determined in $(CD_3)_2SO$, are summarized in table 1. In 2 the Phe

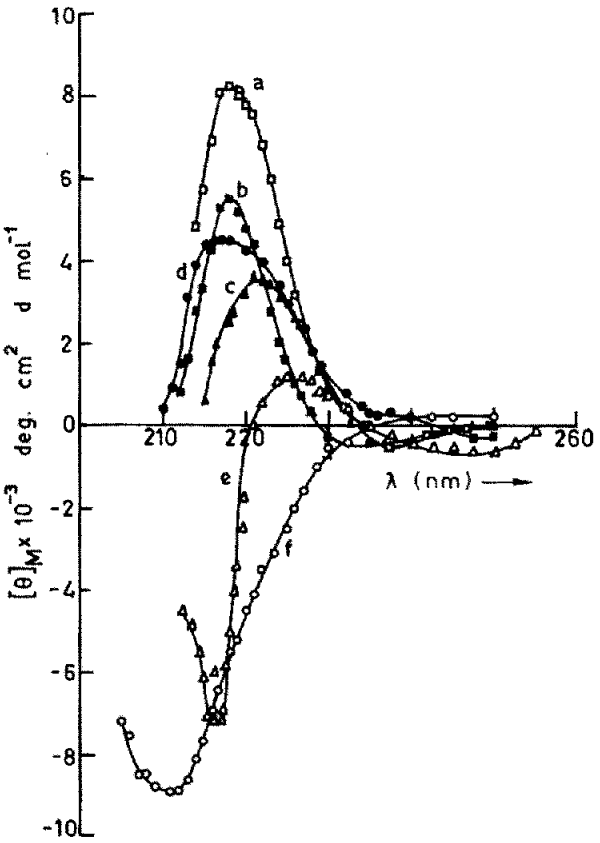

Fig.3. CD spectra of protected tetrapeptide amides. Boc-Aib–Gly–Phe–Met-NH_2 **2** in (a) MeOH and (b) TFE; Boc-Aib–Aib–Phe–Met-NH_2 **3** in (c) MeOH and (d) TFE; Boc-Gly–Aib–Phe–Met-NH_2 **4** in (e) MeOH and (f) TFE.

NH has a low $d\delta/dT$ value (2.77×10^{-3} ppm/°C) characteristic of a solvent shielded or intramolecularly hydrogen-bonded NH group, whereas the $d\delta/dT$ value for the Met NH is indicative of a solvent-exposed proton [26]. The $d\delta/dT$ values in table 1 also suggest that in **3** and **4** both Phe and Met NH groups are intramolecularly hydrogen bonded. These results, together with the known stereochemical preferences of Aib residues [21–25] lead us to conclude that in **2**, β-turn structures having Aib–Gly as the corner residues are significantly populated. This conformation is stabilized by a 4 → 1 hydrogen bond between the Boc CO- and Phe NH-groups (fig.4a). In peptides **3** and **4** the NMR data favours incipient 3_{10} helical structures formed by consecutive type III β-turns having X–Aib and Aib–Phe as the corner residues (X = Aib in **3** and Gly in **4**). These conformations require that both Phe and Met NH groups are intramolecularly hydrogen-bonded (fig.4b). Such structures have been unambig-

Table 1
CD parameters and NMR temperature coefficients (NH protons) in enkephalin fragments

Peptide	Solvent (mM)	λ_{max} (nm)	$[\theta]_M \times 10^{-3}$ (deg . cm^2 . dmol^{-1})	$d\delta/dT \times 10^3$ (ppm/°C)
Boc-Gly–Gly–Phe–	MeOH (1.96)	218	+10.6	
Met-NH_2 **1**	TFE (1.96)	227.5	−5.2	Phe 5.55[a]
	MeOH + (1.96)	218	+2.9	Met 5.55
	TFE	231	−3.5	
Boc-Aib–Gly–Phe–	MeOH (1.86)	218	+7.84	Phe 2.77
Met-NH_2 **2**	TFE (1.86)	218	+5.42	Met 3.70
Boc-Aib–Aib–Phe–	MeOH (1.77)	221	+3.5	Phe 2.31
Met-NH_2 **3**	TFE (1.77)	218	+4.52	Met 1.85
Boc-Gly–Aib–Phe–	MeOH (1.86)	216	−7.25	Phe 1.85
Met-NH_2 **4**	TFE (1.86)	212	−8.96	Met 2.32
Tyr-Gly–Gly–Phe–	MeOH (1.74)	225	−2.9	Phe 3.70
Met-NH_2	TFE (1.74)	230	+2.4	Met 4.63
Boc-Tyr–Gly–Gly–	MeOH (1.53)	225	+5.3	–
Phe–Leu-NH_2	TFE (1.53)	232	−4.8	

[a] Temperature coefficients determined for Boc-Gly–Gly–Phe–Met-OMe

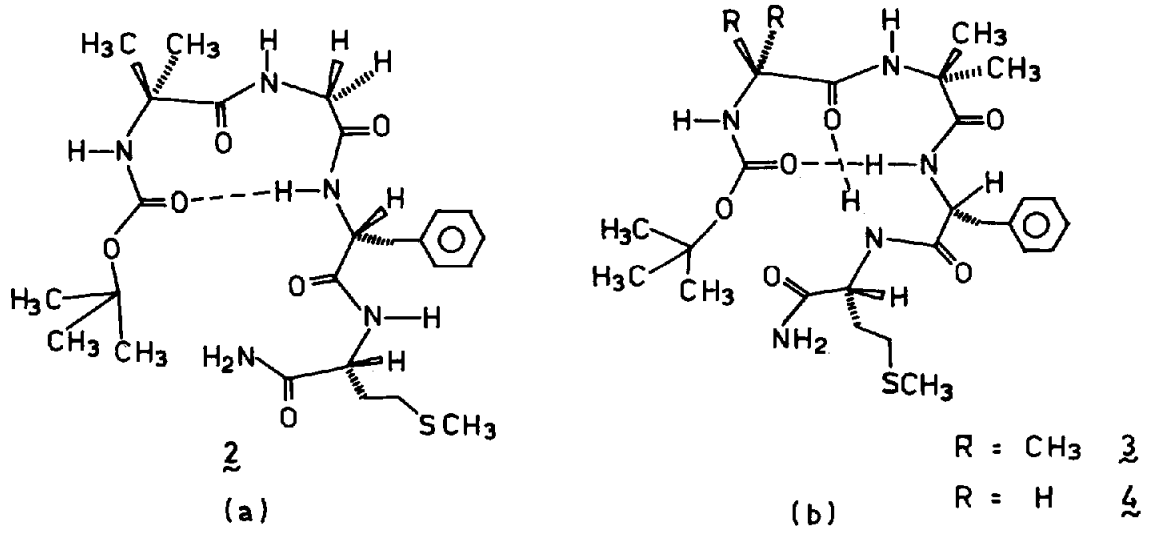

Fig.4. Proposed conformations for (a) Boc-Aib–Gly–Phe–Met-NH_2 **2** and (b) Boc-Aib–Aib–Phe–Met-NH_2 **3** or Boc–Gly–Aib–Phe–Met-NH_2 **4**.

uously established in solution and solid state for Aib containing oligopeptides [21–25].

The CD data presented above show that peptides with the Gly–Gly sequence exhibit similarities to the Aib analogs **2** or **4**, depending on the solvent conditions. It thus appears that both Gly^2–Gly^3 and Gly^3–Phe^4 β-turn conformations for enkephalins may be energetically close, leading to large solvent-dependent changes in conformer populations. This may account for the apparently conflicting proposals put forward in the literature. Theoretical studies have favoured Gly^2–Gly^3 β-turn structures [14,27] and a type I′ Gly^2–Gly^3 β-turn has been observed in the solid state [28]. Spectroscopic studies in solution have, however, been interpreted in terms of Gly^3–Phe^4 β-turns [10] or random conformations [11]. The reversal in signs of the CD bands in Tyr–Gly–Gly–Phe–Met-NH_2 as compared to **1** may reflect the influence of the Tyr residue on the energetics of the different β-turn structures. The studies on the model tetrapeptides reported here eliminate contributions due to the aromatic ring of Tyr, a feature considered in earlier studies of enkephalins [18]. Detailed interpretations of enkephalin CD spectra must also consider the changes in the signs of the bands for the various categories of β-turns, predicted from theoretical calculations [29]. The possibility that different well-defined, folded conformational states are populated in peptides with sequences related to enkephalins, is relevant in view of recent reports of multiple receptor sites for opioid ligands [15,17]. Biological responses induced by opioid peptides could, in principle, be modulated by altering populations of folded conformations.

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