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Conformational characteristics of receptor-selective opioid peptides

^{1}H n.m.r. and c.d. spectroscopic studies of δ-kephalin and [Val^4]morphiceptin

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An investigation on the conformations of highly receptor-selective opioid peptides was carried out to gain further understanding of the structure–activity relationship of endogenous enkephalins. The preferred conformations of a highly μ-selective [Val^4]morphiceptin and a highly δ-selective δ-kephalin have been probed by ^{1}H n.m.r. solvent-, concentration- and temperature-dependences of amide protons to take the folded conformations stabilized by an intramolecular hydrogen bond and the anti-parallely extended dimeric structures respectively. Their possible stereo-conformations were proposed, based on the analyses of the vicinal coupling constants ($J_{HNC_\alpha H}$). The conformational difference between the μ- and δ-selective opioid peptides was further ascertained by the c.d. measurements. The c.d. spectra of the μ-selective peptides show negative bands in the range of 210–230 nm, while those of the δ-selective ones show the opposite positive bands. A correlation between c.d. spectra and receptor-selectivity was possible.

INTRODUCTION

Since the discovery of two naturally occurring pentapeptides, [Leu^5]- and [Met^5]enkephalins (Hughes *et al.*, 1975), numerous synthetic, pharmacological and physicochemical studies have focused on opioid peptides which are of interest as possible substitutes for alkaloid opiate drugs and for their biological importance as natural analgesics (Morley, 1980; Clement-Jones & Besser, 1984; Hansen & Morgan, 1984; Schiller, 1984; Rapaka, 1986). For understanding the physiological response triggered by the binding of these molecules, information about the three-dimensional structures of the receptor and the opioid ligand is needed. In order to gain the stereostructural information on opioid peptides, many investigations have been undertaken using various techniques such as n.m.r., c.d., and X-ray crystallography (for review see Schiller, 1984; Rapaka, 1986). Despite numerous studies, which have often resulted in conflicting conclusions, the conformation of opioid peptides remains an intriguing subject. For example, two kinds of conformations have been proposed as the stable enkephalin form; a β-turn folded form and a dimeric anti-parallel β-sheet structure (Higashijima *et al.*, 1979; Ishida *et al.*, 1984; Renugopalakrishnan *et al.*, 1985; Doi *et al.*, 1987). This conformational variation of these linear opioid peptides may in part reflect the multiplicity of receptors, such as μ-, δ- and κ-receptors (Paterson *et al.*, 1984).

Recently, peptides which exhibit high selectivity for each opioid receptor have been reported (Kosterlitz *et al.*, 1980; Chang *et al.*, 1981; Fournie-Zaluski *et al.*, 1981; Gacel *et al.*, 1981; Handa *et al.*, 1981; Mosberg *et al.*, 1983; Zajac *et al.*, 1983; Sakaguchi *et al.*, 1986). The elucidation of the conformational characteristics of these peptides, therefore, appears to be very important for understanding the substrate-specificity of each opioid receptor, and for the establishment of guidelines for the development of potent and selective analgesics.

Bearing this in mind, the following receptor-selective opioid peptides were chemically synthesized: Tyr-D-Ala-Gly-Phe-D-Leu (DADLE) and Tyr-D-Thr-Gly-Phe-Leu-Thr (δ-kephalin) as δ-selective peptides; Tyr-D-Ala-Gly-(N-Me)Phe-Gly-OH (DAGO), Tyr-Pro-Phe-Pro-NH_2 (MC, morphiceptin) and Tyr-Pro-Phe-Val-NH_2 (VMC, [Val^4]morphiceptin) as μ-selective ones.

This paper deals with the conformational characteristics of the highly μ-selective VMC and the δ-selective δ-kephalin in solution, based on the ^{1}H n.m.r. analyses, and with the c.d. spectra representing the characteristics of μ- and δ-selective peptide conformations.

MATERIALS AND METHODS

Materials

DADLE, δ-kephalin, DAGO, MC and VMC were chemically synthesized from respective amino acid components by normal liquid-phase methods. All deprotected peptides were purified by gel filtration on a Sephadex LH-20 column in 100% CH_3OH, followed by reverse-phase chromatography on an ODS column (YMC-Pack S-343), using 10 mM-$CH_3CO_2NH_4$ (pH 4.2)/CH_3CN (3:2, v/v) solvents. If necessary, further purification was performed by reverse-phase h.p.l.c. (10–40% CH_3CN, linear gradient) in 10 mM-$CH_3CO_2NH_4$ buffer. The final products were obtained

Abbreviations used: VMC, [Val^4]morphiceptin (Tyr-Pro-Phe-Val-NH_2); δ-kephalin, Tyr-D-Thr-Gly-Phe-Leu-Thr; [Leu^5]enkephalin, Tyr-Gly-Gly-Phe-Leu; [Met^5]enkephalin, Tyr-Gly-Gly-Phe-Met; DADLE, Tyr-D-Ala-Gly-Phe-D-Leu; DAGO, Tyr-D-Ala-Gly-(N-Me)Phe-Gly-OH; DPDPE, Tyr-D-Pen-Gly-Phe-D-Pen.

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as lyophilisates. The homogeneity of the peptides was established by t.l.c., h.p.l.c. and amino acid analyses (Hitachi L8500 amino acid analyser). All peptides synthesized were more than 95% pure, as judged from the h.p.l.c. elution profiles.

Tyr-D-Pen-Gly-Phe-D-Pen (DPDPE), a highly δ-selective peptide (Mosberg *et al.*, 1983; Corbett *et al.*, 1984), was purchased from Bachem AG (Bubendorf, Switzerland). The solvents for ^1H n.m.r. measurements $(C^2H_3)_2SO$ (≥ 99.8% pure) and ^2H_2O (≥ 99.75% pure) were purchased from Merck (Darmstadt, Federal Republic of Germany). $(C^2H_3)_2SO$ was stored with synthetic zeolite to remove water contamination. All other materials used for this work were commercial preparations (reagent grade) and were used without further purification.

^1H n.m.r. measurements

Stock solutions of VMC and δ-kephalin (30 mM) were prepared in both $H_2O/^2H_2O$ (9:1, v/v) and $(C^2H_3)_2SO$ solvents. The respective concentrations were gravimetrically determined by dry weight, and then adjusted to 30 mM solutions by diluting the solvents using a volumetrically variable pipette (Eppendorf 4710). The molar fractions of the mixed solution were determined volumetrically starting from one solvent and then adding a pre-set quantity of the other solvent solution. The pH value for the aqueous solution was directly measured in n.m.r. tubes at 25 °C with a pH meter, and was adjusted to 5.5 by adding NaO^2H or ^2HCl solution.

^1H n.m.r. spectra were recorded on a Varian XL-300 pulse FT spectrometer (300 MHz for ^1H resonances) equipped with a temperature-control accessory (accurate to within ±1 °C). For ^1H n.m.r. spectral measurements of the aqueous solution, water suppression was achieved by the symmetric 1331 pulse sequence. The internal references for chemical shifts (δ) were 2,2-dimethyl-2-silapentane 5-sulphonate for the aqueous solution, and tetramethylsilane for the $(C^2H_3)_2SO$ solution (accurate to within ±0.001 p.p.m. and ±0.3 Hz). Assignments of ^1H resonances were made with the aid of spin multiplicities, spin decoupling, and two-dimensional proton–proton correlation, and by referring to the related paper (Grathwohl & Wüthrich, 1976; Roques *et al.*, 1980; Sudha & Balaram, 1983; Casey, 1985).

C.d. spectroscopy

C.d. spectra were recorded with a Jasco J-500 spectrometer equipped with thermostatted cell assembly. A slit program was used to obtain a wavelength accuracy of > 0.5 nm. The instrumental was flushed with dry N_2 and the spectra were recorded using quartz cuvettes of 10 mm path length. All spectra were recorded at least twice, with noisy regions being run up to four times. The spectra depicted are averages over all runs. The experimental uncertainty was less than 5.5% in the entire spectral range examined. The cuvette holder was thermostatted, and all measurements (200–300 nm) were recorded at 20 °C. Molar ellipticities [θ] were calculated using the formula: $[\theta]_M = \theta_{obs.} \times M_r/10 \cdot c \cdot l$ degrees·cm²·dmol⁻¹, where $\theta_{obs.}$ = observed reading (degrees), c = concentration (g·ml⁻¹) and l = path length (cm). The sample concentrations were gravimetrically adjusted to 1.1×10^{-4} M. The following two kinds of solvents were used: 20 mM-Tris/HCl buffer (pH 7.4); 20 mM-Tris/HCl buffer + 2 mM aqueous lysophosphatidylcholine. All c.d. spectra of freshly prepared solutions were measured within 2 h.

RESULTS AND DISCUSSION

Solution conformations of VMC and δ-kephalin by ^1H n.m.r. analyses

The ^1H n.m.r. spectra of VMC and δ-kephalin in $(C^2H_3)_2SO$ solution are shown in Fig. 1 together with their peak assignments. The spectrum of VMC shows the equilibrium between *cis* and *trans* rotamers around the Tyr (C′)–Pro(N) bond. The *cis*/*trans* ratio was 4/5 in $(C^2H_3)_2SO$ and 1/2 in ^2H_2O solutions at 30 mM concentrations. Owing to the existence of the *cis*-*trans* equilibrium, several signals overlapped and the accurate determination of all parameters (chemical shifts and coupling constants) was not always possible.

Solvent and temperature dependences of NH protons

The temperature and solvent-composition dependences of amide proton chemical shifts have been used for distinguishing between 'exposed' and 'intra- or intermolecularly hydrogen-bonded' NH groups of peptides (Wüthrich, 1976). The exposed protons are more influenced than the hydrogen-bonded amide protons by any change in external environment such as temperature or solvent. The temperature dependences of the chemical shifts of amide protons in $(C^2H_3)_2SO$ and ^2H_2O solvents were measured at temperatures between 30 and 80 °C with increments of 10 °C. The temperature coefficient ($d\delta/dT$ p.p.m.·°C⁻¹) for each proton, as calculated from the least-squares line, are listed in Table 1. The NH_3^+ protons of Tyr¹ residue were not observed because of the fast exchange with solvent protons. The NH protons of

Table 1. Temperature coefficients for the amide protons of VMC and δ-kephalin in $(C^2H_3)_2SO$ and $H_2O/^2H_2O$ (9:1, v/v) solutions

The $-d\delta/dT$ values of less than approx. (3.5–4.5) × 10³ p.p.m./°C could be considered as participating in molecular hydrogen bond (Ohnishi & Urry, 1969; Urry & Long, 1976; Khaled *et al.*, 1977; Zetta & Cabassi, 1982).

Amide protons	Solvent...	$10^{-3} \times -d\delta/dT$ (p.p.m./°C)	
		$H_2O/^2H_2O$	$(C^2H_3)_2SO$
VMC			
Phe³ *cis*		8.0	4.3
trans		10.1	4.0
Val⁴ *cis*		7.3	5.4
trans		5.0	4.7
$CONH_2$ (I)		1.7	4.4
$CONH_2$ (II)		4.1	5.0
δ-Kephalin			
D-Thr²		8.2	8.1
Gly³		5.8	4.6
Phe⁴		6.4	4.9
Leu⁵		8.1	5.1
Thr⁶		3.8	0.9

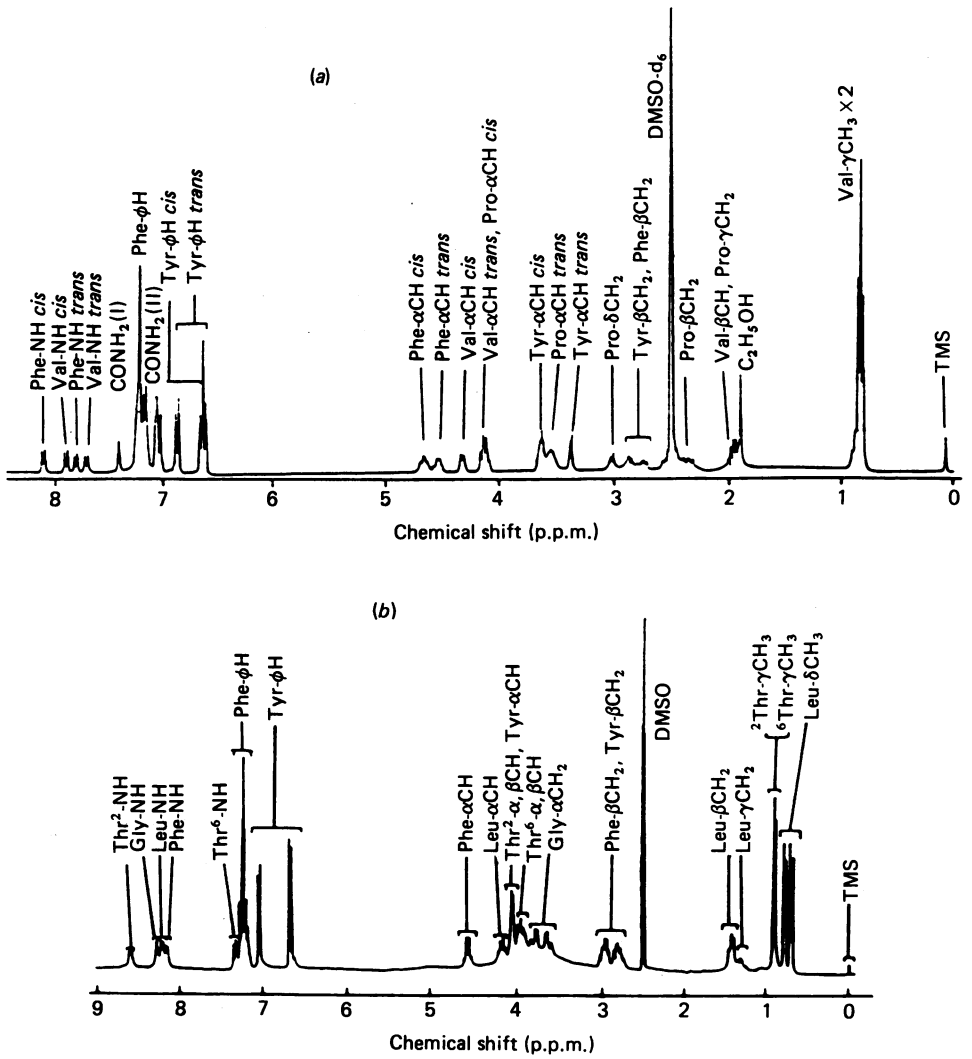
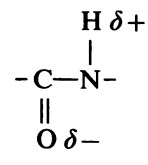


Fig. 1. 300 MHz ^{1}H n.m.r. spectra of VMC (*a*) and δ-kephalin (*b*) in $(C^2H_3)_2SO$ at 30 °C

TMS, tetramethylsilane; DMSO, dimethyl sulphoxide.

D-Thr^2 in δ-kephalin above 60 °C were not determined exactly, because of the overlapping of the signal with other protons, and because of their decreased intensities. Similarly, the NH protons of the Phe^3 (*cis*), Val^4(*cis*) and *C*-terminal amino groups in VMC above 70 °C were also not determined.

δ-Kephalin. In $(C^2H_3)_2SO$ solution, the Thr^6 NH proton shows a significantly low temperature coefficient ($d\delta/dT = 0.9 \times 10^{-3}$ p.p.m.·°C^{-1}), although the remaining protons fall within the values normally observed in the solvated peptides ($\geqslant 4.6 \times 10^{-3}$ p.p.m.·°C^{-1}). This implies that the molecular states in which the Thr^6 NH proton takes part in forming a hydrogen bond, or is in a solvent-shielded environment, exist predominantly in the solution. The tendency of the low temperature coefficient for the Thr^6 NH proton was also observed in water. The comparison between the $d\delta/dT$ values for respective NH protons in $(C^2H_3)_2SO$ and ^2H_2O solutions shows that the δ-kephalin molecule, as a whole, takes conformations sensitive to the polarity of the solvent; the $d\delta/dT$ values are all higher in ^2H_2O than $(C^2H_3)_2SO$. On the other hand, it appears important to note that only the D-Thr^2 NH proton undergoes an upfield shift by changing the solvent from $(C^2H_3)_2SO$ to water. The downfield shift of proton resonance could be caused by the decrease of the atomic charge due to the direct participation for the hydrogen bond or to the secondary effect as a result of the increase of a neighbouring oxygen atomic charge such as:



Therefore, it could be thought that the D-Thr^2 NH proton or the peptide oxygen atom of Tyr^1 participates in the interaction with the solvent or the neighbouring δ-kephalin molecules. Since the temperature coefficient of D-Thr^2 is considered too high for the direct hydrogen bond formation of the NH proton, the latter case is most probable in the $(C^2H_3)_2SO$ solution.

VMC. Characteristically, all NH protons have nearly the same $d\delta/dT$ values [$(4.0–5.5) \times 10^{-3}$ p.p.m.·°C^{-1}] in

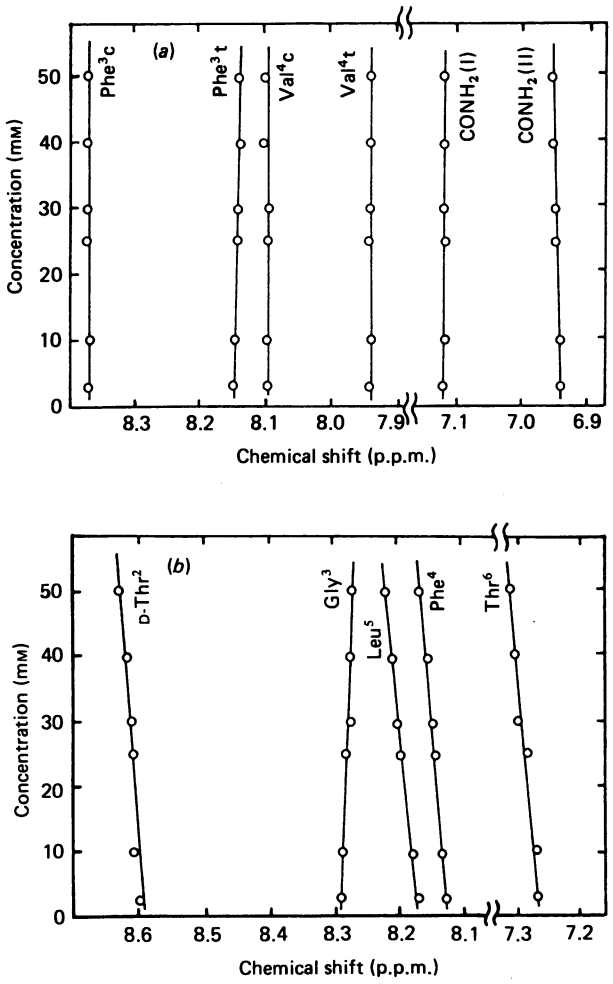


Fig. 2. Concentration dependences of chemical shifts of the amide protons of VMC (*a*) in $H_2O/^2H_2O$ (9:1, v/v) and δ-kephalin (*b*) in $(C^2H_3)_2SO$ at 30 °C

$(C^2H_3)_2SO$ solution, which is in contrast with the case in δ-kephalin. These data would imply that respective amino acids are significantly influenced by the surrounding solvent, and therefore the conformations of VMC exist as the equilibrium state among the various conformers. On the other hand, the VMC molecule appears to take on the defined conformations in ^2H_2O solution. One of two *C*-terminal amide protons shows a low temperature dependence ($d\delta/dT = 1.7 \times 10^{-3}$ p.p.m.·°C^{-1}). This proton, named $CONH_2$(I), shifts to the downfield side by changing from $(C^2H_3)_2SO$ to water, while all of the other protons except $CONH_2$ (II) show upfield shifts with significant increases of the $d\delta/dT$ values. This observation directly indicates that there is an appreciable fraction of the conformations where the $CONH_2$(I) proton participates in hydrogen bond formation. The tendency of VMC to take on the defined conformations in the more highly polar solvent ^2H_2O than in $(C^2H_3)_2SO$ makes us also suppose that the compact forms of this molecule exist in a ^2H_2O solution. Provided that the conformation of the linear peptide could be roughly classified into the two categories of folded and extended forms, the preferred conformations of VMC in a ^2H_2O solution would correspond to the folded forms stabilized by an intramolecular hydrogen bond. This possibility was further suggested from the following concentration-dependence experiments.

Concentration dependences of NH proton chemical shifts

The concentration dependences of amide proton chemical shifts are shown in Fig. 2.

In the VMC molecule, the chemical shifts remain practically unchanged in the concentration range 3 mM–50 mM in a water solution. This result is in accordance with the lack of self-aggregation, and the molecules existing in solution can be interpreted as behaving independently. Therefore, the above-mentioned solvent- and temperature-dependences of VMC reflect the behaviour of a molecule itself, and the folded conformations with an intramolecular hydrogen bond are proposed as the prefered forms of this molecule in ^2H_2O solution.

In contrast, δ-kephalin showed significant concentration dependences in both solutions [especially in $(C^2H_3)_2SO$ solution]. As the solution is diluted, the amide proton resonances of D-Thr², Phe⁴, Leu⁵ and Thr⁶ residues shift to a higher field, while that of Gly³ shifts to a slightly lower field. It is remarkable to note that since the chemical shift changes of C_α protons (except C_α protons of Gly³) in both solutions are essentially constant, the concentration dependences of the amide-proton chemical shifts may well be taken as a direct indication of the molecular aggregations via intermolecular hydrogen bonds, by analogy with the analysis of [Met⁵]enkephalin conformation (Higashijima *et al.*, 1979). The upfield shifts of NH and C_α protons of Gly³ accompanying the increased concentration (approx. 0.02 p.p.m. upfield shift from a concentration change of 3 mM to 50 mM) could be interpreted as being the result of ring-current effects of a neighbouring aromatic ring such as a Phe⁴ residue, on these protons in the molecular aggregate. A similar phenomenon has also been reported in [Met⁵]enkephalin solution (Higashijima *et al.*, 1979). If this is the case, it could be supposed that the molecular aggregates of the δ-kephalin dipolar form are not of a linear chain type, but rather are of an anti-parallel β-structure type. This type of molecular aggregation is possibly stabilized by the electronic attraction, at either terminal of adjacent molecules, between the positively charged *N*-terminal amino group and the negatively charged *C*-terminal carboxy group, in addition to the intermolecular N—H·····O hydrogen bonds. This is also suggested from the correlation between the temperature coefficients of amide protons and the molar fraction of water in the $(C^2H_3)_2SO$ solution. The results are shown in Fig. 3. The line profiles could be roughly classified into two types of behaviour: one changes from a low temperature coefficient to a high one with the addition of water (Leu⁵ and Thr⁶); the other shows a profile in which initially the addition of water decreases the temperature coefficient, and then increases it (D-Thr², Gly³ and Phe⁴). It is worthwhile noting that the profile of the Thr⁶ NH proton shows a pattern nearly symmetrical to that of D-Thr² up to a 60% water content. This fact implies an intimate relation between both residues, and could be interpreted as follows: in the anti-parallelly arranged δ-kephalin molecules, the NH proton of the Thr⁶ residue is intermolecularly hydrogen-bonded to the carbonyl oxygen atom of Tyr¹ adjacent to the D-Thr² NH proton, as was already considered from the solvent and temperature dependences. Breaking this hydrogen bond

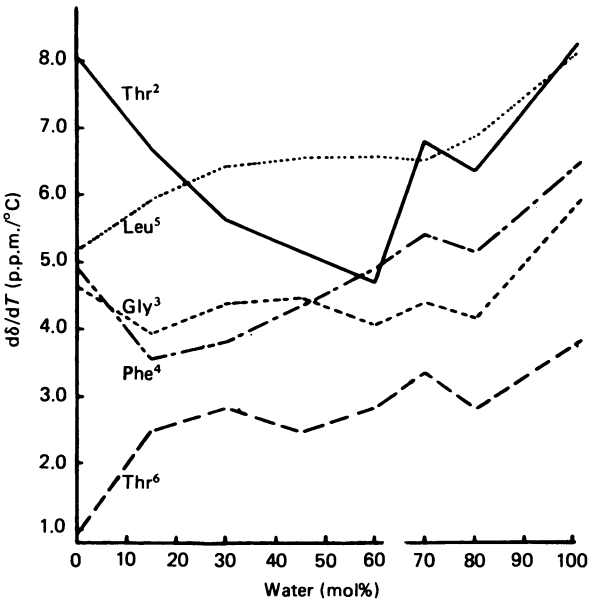


Fig. 3. Temperature coefficients of amide resonances of δ-kephalin versus molar fraction of water in $(C^2H_3)_2SO$

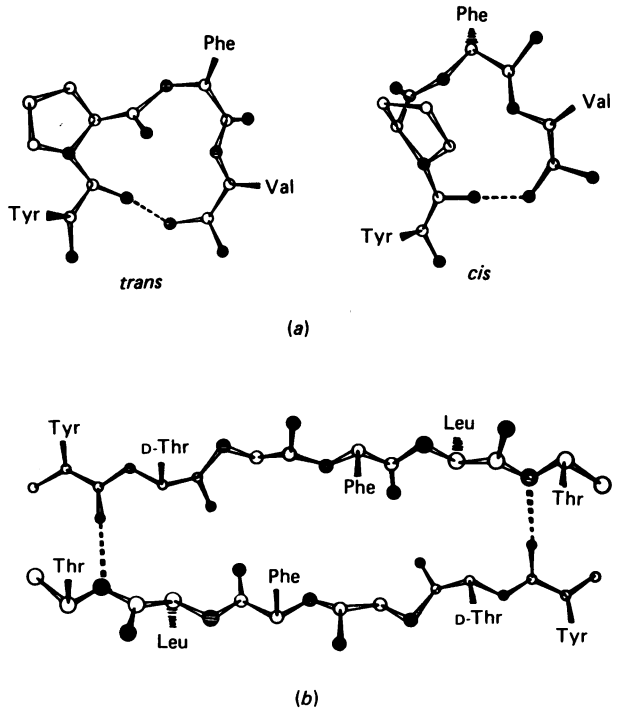


Fig. 4. Proposed solution conformations of VMC (*a*) and δ-kephalin (*b*)

Respective torsion angels of the Phe³ and Val⁴ correspond to 87° and −87° for VMC *trans* form, and −161° and −153° for VMC *cis* form, in ^2H_2O solution. The torsion angle for δ-kephalin in $(C^2H_3)_2SO$ solution corresponds to 161° (D-Thr²), −135° (Gly³), −154° (Phe⁴), −154° (Leu⁵) and −156° (Thr⁶), respectively.

by adding water causes free rotation about the Tyr¹—D-Thr² bond, and consequently the D-Thr² NH proton begins to behave like other NH protons. In other words, the $d\delta/dT$ value of the D-Thr² NH proton approaches those of Gly³ and Phe⁴ NH protons, accompanied by breaking of the intermolecular hydrogen bond.

From the solvent-composition, temperature and concentration dependences of amide protons, it could be supposed that the preferred conformations of the VMC molecule in aqueous solutions are the folded forms stabilized by an intramolecular hydrogen bond via the *C*-terminal NH_2 group. On the other hand, δ-kephalin exists as the molecular aggregates, preferentially, in which the anti-parallel dimer structure are describable as their fundamental conformations. This dimer structure would be at least stabilized by an intermolecular Thr⁶ (NH)·····(O=C)Tyr¹ hydrogen bond in $(C^2H_3)_2SO$ solution, and is to some extent retained in a ^2H_2O solution. In order to ascertain whether or not these conformations are actually possible, the following experiments were further carried out.

Analyses of the vicinal $^3J_{HNC_\alpha H}$ coupling constants of VMC and δ-kephalin

The vicinal coupling constants determined from the amide signals are given in Table 2, together with the possible torsion angles (ϕ) around the C′—N—C_α—C′ bond sequence (IUPAC-IUB, 1970). For estimations of ϕ, the following equations (Bystrov, 1976) were used:

$$^3J_{HNC_\alpha H} = 9.8\cos^2\theta - 1.1\cos\theta + 0.4\sin^2\theta$$

where $\phi = |\theta - 60|$ for L-amino acids and $\phi = |\theta + 60|$ for D-amino acids;

$$\Sigma^3J_{HNC_\alpha H_2} = -9.4\cos^2\phi - 1.1\cos\phi + 14.9$$

for glycine residues.

For the following discussion the torsion angle ω (C_α—C′—N—$C_{\alpha+1}$) was treated as 180° or 0° (only for the Pro residue of VMC). The information concerning the molecular conformation including the torsion angle ψ (N—C_α—C′—N) can be obtained from the nuclear Overhauser effect measurement. Unfortunately, this straightforward strategy could not be readily transferred to these molecules, because the nuclear Overhauser effect values measured by a 300 MHz n.m.r. spectrometer vanished owing to their intermediate rotational correlation time (τ_c). Therefore the torsion angle ψ was not taken into account.

Possible conformations of VMC. Although the n.m.r. studies suggested that the VMC molecule takes predominantly the folded conformations stabilized by an intramolecular hydrogen bond, the acceptor atom for the *C*-terminal NH proton is still not identified. Therefore, various folding models were considered, based on the possible torsion angles and using space-filling (CPK) models. Although the β-turn forms are most popular for the folded peptide conformations (Rose *et al.*, 1985), the possible combinations of the respective torsion angles listed in Table 2 suggest the conformations with a 5⟶1 intramolecular hydrogen bond as the most reasonable conformations, where *C*-terminal NH is hydrogen-bonded to a carbonyl oxygen atom of Tyr¹. CPK model consideration led us to several folding forms which agree well with the listed torsion angles. A proposed conformation is illustrated in Fig. 4. This type of 5⟶1 hydrogen bond, consequently forming a 13-membered ring in the loop, appears to be stable, because similar 5⟶1 hydrogen bonds have been reported to

Table 2. $^3J_{HNC_\alpha H}$ Coupling constants from n.m.r. spectra and corresponding torsion angles for VMC and δ-kephalin in $(C^2H_3)_2SO$ and $H_2O/^2H_2O$ (9:1, v/v) solutions at 30 °C

Solvent ...	$(C^2H_3)_2SO$		$H_2O/^2H_2O$	
Amide proton	J(Hz)	φ(°)	J(Hz)	φ(°)
VMC (*cis*)				
Phe[3]	8.0	−153	6.6	−161
		−87		−79
		42		30
		78		90
Val[4]	8.6	−150	8.0	−153
		−90		−87
		52		42
		68		78
VMC (*trans*)				
Phe[3]	9.0	−147	7.0	−159
		−93		−81
				33
				87
Val[4]	9.0	−147	8.0	−153
		−93		−87
				42
				78
δ-Kephalin				
D-Thr[2]	6.8	−88	7.4	−83
		−32		−37
		79		83
		161		157
Gly[3]	11.0	−135	11.0	−135
		−54		−54
		54		54
		135		135
Phe[4]	7.8	−154	5.8	−166
		−86		−74
		40		40
		80		80
Leu[5]	7.8	−154	7.4	−157
		−86		−83
		40		37
		80		83
Thr[6]	7.6	−156	8.6	−150
		−84		−90
		38		52
		82		68

occur in other peptides (Karle, 1981; Rose *et al.*, 1985).

Possible conformations of δ-kephalin. The n.m.r. data suggested that anti-parallel dimer structures via intermolecular Thr[6] (NH)·····(O=C)Tyr[1] hydrogen bonds are the preferred δ-kephalin conformations. Taking this insight into account, it could be possible to build several extended models of δ-kephalin, which agree well with the possible angles given in Table 2. One of them is also shown in Fig. 4. These types of anti-parallel, extended, dimer conformations have been frequently observed in the crystal structures of the linear and zwitterionic peptides with terminal NH_3^+ and COO^- groups such as [Met[5]]- (Griffin *et al.*, 1986; Mastropaolo *et al.*, 1986; Doi *et al.*, 1987) and [Leu[5]]enkephalins (Camerman *et al.*, 1983).

The ultimate goal of our research on opioid peptides is to arrive at an understanding of the active conformation of the peptides suitable for the binding of the μ-, δ-, and other opioid receptors. The n.m.r. studies have shown the conformational difference between the predominant forms of the μ-selective VMC and δ-selective δ-kephalin molecules in the solution state. It must also be borne in mind that VMC and δ-kephalin molecules prefer to take definite conformations in ^2H_2O and $(C^2H_3)_2SO$ solutions, respectively. The folded conformations for the μ-selective ligand and the dimeric anti-parallel ones for the basic structure of the δ-selective ligand, proposed from the n.m.r. data of VMC and δ-kephalin, respectively, are consistent with the speculation based on the X-ray crystallographic results (Doi *et al.*, 1984, 1987; Ishida *et al.*, 1984); the dimeric extended form is suitable for the δ-receptor, and the folded form is suitable for the μ-receptor. Nevertheless, it is especially important to further elucidate whether or not this conformational difference reflects the substrate specificity for μ- and δ-opioid receptors, because opposite results have also been reported (Camerman *et al.*, 1983; Renugopalakrishnan *et al.*, 1985) and its conformational relation to the biological activity is not fully established

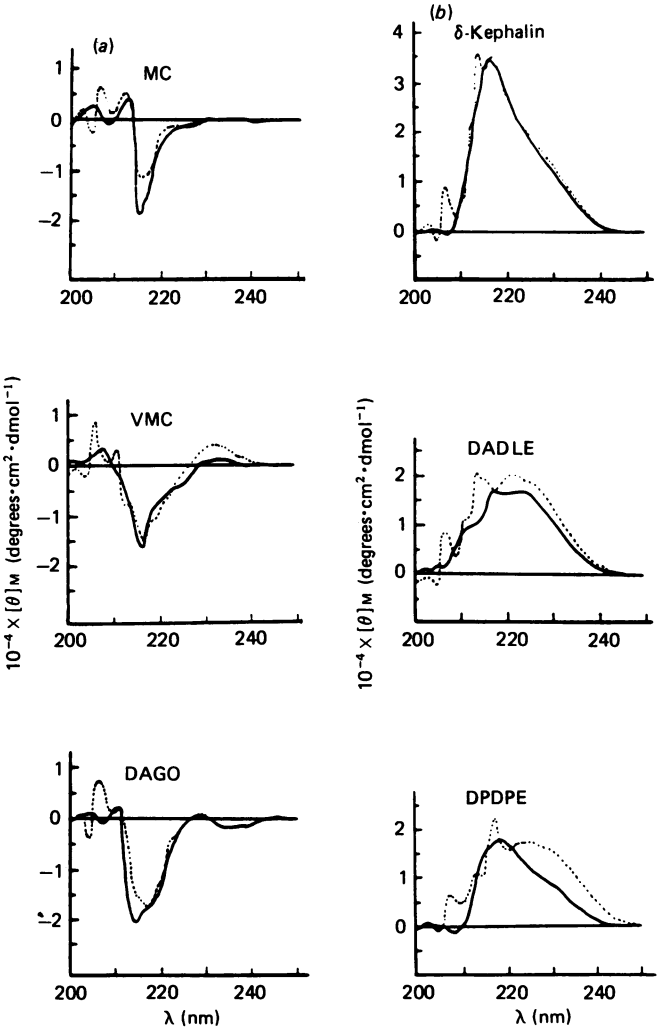


Fig. 5. C.d. spectra of μ-selective (*a*) and δ-selective (*b*) opioid peptides

Solid lines represent the spectra in 20 mM-Tris/HCl buffer (pH 7.4) and broken lines represent the same spectra in the presence of lysophosphatidylcholine.

at present. In order to ovecome this dilemma, the following c.d. measurements were carried out.

C.d. spectral patterns of μ- and δ-selective opioid peptides

In order to make clear the difference between the peptide conformations, it is useful to measure their c.d. curves. Fig. 5 shows the c.d. spectra of μ-selective DAGO, MC and VMC molecules and δ-selective DADLE, DPDPE and δ-kephalin molecules measured in 20 mM-Tris/HCl buffer (pH 7.4 at 25 °C) alone and containing lysophosphatidylcholine (peptide/lipid = 1/20, M/M). Their c.d. parameters are also given in Table 3, along with the receptor selectivities of the opioid peptides used. The c.d. spectra of MC and VMC in ^2H_2O solution have already been reported (Sakaguchi *et al.*, 1986). Surprisingly, all c.d. patterns for the μ-selective peptides show negative bands in the range of 213–230 nm, while all the δ-selective peptides exhibit characteristic positive c.d. bands in the range of 210–240 nm. Interestingly, the c.d. curves of respective μ- and δ-selective peptides are similar to one another, and the curves are little affected by the coexistence of lysophosphatidylcholine, which was used for investigating the conformations of peptides in the binding state with receptors or membranes, although the interaction of both molecules was suggested from the solution thermal analyses (the significant changes of enthalpy accompanying the phase transition of *lyso*-phosphatidylcholine were observed in the presence of these peptides). These c.d. results imply that respective μ- or δ-selective peptides take on a few stable conformations which are similar to one another. It is important to note that the c.d. spectra at pH 5.5 (corresponding to the same condition as the n.m.r. measurement) or pH 3.0 were similar to those in Fig. 5, implying the general forms of these c.d. spectra being independent of pH.

Sudha & Balaram (1981) have analysed the conformation of Boc-Gly-Aib-Phe-Met-NH_2 (where Boc is *tert*-butyloxycarbonyl and Aib is aminoisobutyrate) as a 3_{10} helix structure consisting of two consecutive β-turn foldings based on its c.d. and n.m.r. data, which exhibits a conformational similarity to the proposed form for the

Table 3. C.d. paramaters of μ- and δ-selective opioid peptides in 20 mM-Tris/HCl buffer and 20 mM-Tris/HCl buffer + lysophosphatidylcholine

GPI, Guinea-pig ileum; MVD, mouse vas deferens; lyso-PC, lysophosphatidylcholine. Receptor selectivity values were estimated from the pharmaceutical data (Fournie-Zaluski *et al.*, 1981; Gacel *et al.*, 1981; Mosberg *et al.*, 1983; Sakaguchi *et al.*, 1986).

Peptides	Receptor selectivity [IC_{50}GPI(μ)/IC_{50}MVD (δ)]	λ (nm)(($10^{-4} \times [\theta]_M$)	
		20 mM-Tris/HCl	20 mM-Tris/HCl + lyso-PC
μ-Selective			
DAGO	0.15	215(−2.05)	218(−1.82)
MC	0.014	215(−1.91)	215(−1.14)
VMC	0.074	217(−2.05)	216(−1.45)
δ-Selective			
DADLE	87.2	217 (1.77) 223 (1.77)	213(2.14) 221(2.09)
δ-Kephalin	3067.0	216 (3.50)	214(3.64)
DPDPE	3164.0	217.5(1.82)	217.5(1.82)

VMC molecule (Fig. 4). Since its c.d. curve is almost the same as those observed in the μ-selective peptides, it could be considered that the preferred conformations of these μ-selective peptides are the folded forms in the solution state.

On the other hand, δ-selective peptides exhibit the positive c.d. curves, in contrast with those of μ-selective ones. Although it is at present unclear whether or not these c.d. curves reflect the dimeric extended conformations of δ-selective opioid peptides, it may be worthwhile to say that the extended forms of these peptides, including DPDPE, a cyclic peptide, can also be built by CPK modellings.

In conclusion, these c.d. data clearly show that the preferable solution conformations of μ-selective peptides are different from those of δ-selective ones. This insight appears to be very important for analysing the substrate specificities of μ- and δ-opioid receptors.

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