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A supermolecule study of the effect of hydration on the conformational behaviour of leucine-enkephalin

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SUMMARY

A theoretical conformational study was performed on leu-enkephalin in its zwitterionic form, both in vacuo and in the presence of a number, n, of up to 13 water molecules saturating its first hydration shell. The intramolecular energy of enkephalin as well as the intermolecular enkephalin-water and water-water interaction energies were computed with the SIBFA procedure (Sum of Interactions Between Fragments Ab initio computed), which uses additive ab initio multipole systematics and analytical formulas grounded on ab initio SCF computations. Energy minimizations were performed with a polyvalent minimizer, Merlin, with which three distinct derivative and three distinct nonderivative minimizers can be activated in a sequential fashion.

Eight different candidate conformations of enkephalin were used as starting points. These conformations are either those found in distinct X-ray structures, or those proposed on the basis of theoretical computations by other authors. In the absence of hydration, they converged towards distinct folded energy-minima, the best four ones being separated by an energy gap of 8.7 kcal/mol. In marked contrast, with up to $n = 13$, the energetical separation between the six best conformers narrowed down to $\simeq$ 4 kcal/mol. They can be characterized by: (a) either a direct or a water-mediated ammonium-carboxylate interaction; b) either a close proximity (as in morphine) or a large separation between the aromatic rings of Tyr and Phe (intercenter separations of $\simeq 4.5$ Å and $\simeq$ 10.5 Å, respectively), with each of the four mutual combinations of (a) and (b) being represented.

INTRODUCTION

The endogeneous opioid pentapeptides leu-enkephalin and met-enkephalin have lent themselves to a wealth of structural studies: X-ray diffraction [1–5], NMR [6–13], fluorescence spectroscopy [14], and theoretical computations [15–23]. Conformational studies were originally stimulated by the objective of unraveling a topological analogy with morphine and derivatives [24,25]. On the

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basis of extensive subsequent structure-activity relationships, novel analogs were designed and synthesized, to target in specific fashion μ [19–26] or δ [19,27–30] receptors.

The inherent flexibility of enkephalins renders them very sensitive to environmental effects. This was exemplified by the X-ray crystal structure determinations. In these, depending upon the crystallization conditions, folded [1,4,5] or extended [2–4] structures are stabilized. A particularly noteworthy conformation of leu-enkephalin was put forward in the most recent X-ray diffraction study [5]. In it the tyrosine and phenylalanine rings are in a close orthogonal arrangement, analogous to the mutual disposition of the tyramine and cyclohexenyl rings in morphine.

Such an orthogonal disposition of the aromatic rings is observed in recurrent fashion in X-ray crystal structures of proteins [31]. On the other hand, fluorescence spectroscopy studies of enkephalin in water [14] indicate that its two aromatic rings have their centers $\simeq 10$ Å apart, rather than $\simeq 4.6$ Å in the orthogonal arrangement. Previous theoretical computations did not reveal a close approach of aromatic rings as occurring in the best energy minima [15–20,22,23]. It occurred, on the other hand, as the best constrained energy-minimum of the tetrapeptide Tyr–Gly–Gly–Phe, 4 kcal/mol above the best unconstrained minimum for this peptide [32]. A cluster analysis of enkephalin [21] revealed its possible occurrence, associated, however, with the acid form rather than the zwitterionic form of the peptide, and an extended, rather than folded, conformation of its backbone.

Proximities between the aromatic rings of Tyr and Phe residues were derived in recent theoretical studies devoted to δ enkephalin derivatives: DPDPE, a cyclic [D-Pen^2, D-Pen^5] analogue [33], and derivatives of the linear hexapeptide DTLET, Tyr-D-Thr–Gly–Phe–Leu–Thr as well as DPDPE [34]. In Ref. 33 however, a neutral nonzwitterionic form of DPDPE was considered, and the effect of solvation was taken into account only implicitly, by means of a dielectric constant of 80. In Ref. 34, the N- and C- terminal groups were respectively formylated and amidated, and solvation effects were not included. Interring separations of < 10 Å and appropriate disposition for further facilitated rapprochement were derived in Ref. 35, devoted to DTLET derivatives, bearing *O*- tertiobutyl substituents. In Ref. 35, both terminal cationic and anionic charges were halved, in an attempt to implicitly account for solvation effects. The relevant conformation has its two end groups apart, too far away for a through-one water binding (see below): such a loss of end-to-end interactions could be incurred either by the weakened charges, or the interposition of the bulky *O*-tertiobutyl groups.

These experimental and theoretical results are strongly suggestive of the occurrence of similar interring proximities in the parent compounds. They leave open the issues, however, on the one hand, of the energetical stabilities of the corresponding conformations with the zwitterionic form and full net charges on the N- and C-terminal ends, and, on the other hand, of the evolution of conformational preferences in the explicit presence of water molecules. We wish to address both in the present investigation.

The initial incentive to the present theoretical investigation was provided by the experimental crystal structure of Ref. 5. We will attempt to quantify the relative energetical stabilities of conformers with neighbouring adjacent rings, to those of conformers with alternative dispositions. The essentials of our computational effort will be devoted to the effects of hydration on the conformational preferences of enkephalin. This will be carried out within the supermolecule approach (see e.g., Refs. 36 and 37).

A number of water molecules saturating the first hydration shell will be disposed and energy-

minimized around the peptide, whilst relaxing its conformation simultaneously. We wish to mention at this point that, despite the large number of theoretical investigations devoted to enkephalin and analogues, only one was published to date, to our knowledge, in which solvation effects were taken into account by means of an explicit solvation shell model [22] rather than implicitly through the use of a dielectric screening; this study bore on the acidic form of met-enkephalin, whereas ours will be devoted to the zwitterionic form of leu-enkephalin.

For such a form, the energy balances will result from an interplay of intra- and inter-molecular effects, involving the mutual interactions of the two ionic groups, the polar backbone atoms, and the water molecules. The magnitude of the individual interaction terms can differ by significant amounts (compare, e.g., the interaction energies in formate-cation, formate-water and ammonium-water complexes [38]), and the outcome of the energy balances cannot thus be safely anticipated. If performed with reliable energy functionals, they will enable as well to confront energetically conformations stabilized by direct versus through-water ammonium-formate interactions, which to our knowledge was never investigated theoretically for oligopeptides of comparable size.

The two distinctive features of the present study are:

(1) The recourse to a refined methodology (SIBFA) [38,39], formulated and calibrated on the basis of ab initio computations, for the consistent and simultaneous computations of both the intramolecular (conformational) energies of the peptide on the one hand and the intermolecular peptide-water and water-water interaction energies on the other hand.

(2) The recourse to a polyvalent minimization algorithm (Merlin) [40], in which three distinct nonderivative minimizers and three distinct derivative minimizers can be activated in sequential fashion during a given task, and according to the behaviour of the energy functional. The recourse to this algorithm proved instrumental for unraveling new routes leading to reliable energy minima of the supersystem enkephalin-water.

In order to capitalize as much as possible on already available data, we elected to start for our energy minimizations, from well-characterized conformations of enkephalins. Although it is not deemed to be exclusive, our present set of starting conformers encompasses the following ones:

(a) A folded conformer (denoted as I subsequently) found in an X-ray diffraction study by Smith and Griffin [1] with the two aromatic rings apart.

(b) An extended conformer found in an X-ray diffraction study by Camerman et al. [2]. Two distinct routes for its solvation were used (see below); the corresponding conformers will be denoted II and III.

(c) A folded conformer (denoted as IV) found in the most recent X-ray diffraction study, due to Aubry et al. [5], with the two aromatic rings in a close orthogonal arrangement.

(d) An energy-minimized conformer of leu-enkephalin (denoted as V), due to Manavalan and Momany [20].

(e) The energy-minimized conformation of met-enkephalin, due to Li and Scheraga [22]. This conformer is denoted as VI.

(f) A conformer (denoted as VII) proposed by Maigret et al. [41], proposed to be representative for the μ-active conformers.

The fully extended conformer (VIII) was also used as an additional tentative starting point.

PROCEDURE

The variation of conformational energy of enkephalin, as well as the intermolecular enkephalin-water and water-water interaction energies, are computed with the SIBFA procedure [38,39]. Using standard bond lengths and valence angles, the oligopeptide was built of elementary constitutive fragments separated by single bonds, and the variation of intramolecular energy upon a conformational change was obtained as the variable part of the sum of the interactions between the fragments expressed as:

$$E_{conf} = E_{MTP} + E_{pol} + E_{rep} + E_{disp} + E_{tor}$$

The intermolecular interaction energies are computed as:

$$\Delta E = E_{MTP} + E_{pol} + E_{rep} + E_{CT} + E_{disp}$$

E_{MTP} and E_{pol} are the electrostatic and polarization contributions (using a dielectric constant of one) which were computed using a multipolar expansion of the ab initio SCF molecular wavefunctions of the fragments. E_{rep}, E_{CT}, E_{disp} and E_{tor} are the repulsion, charge-transfer, dispersion and torsional contributions, respectively.

The oligopeptide backbone was constructed of a succession of *N*-methylformamide molecules, split into formamides and methane in such a way as to retain the same multipolar expansion as on *N*-methylformamide: this was done while still allowing for a rotation along the $N-C_\alpha$ bonds. The side chains are built out of their elementary subfragments. Thus, e.g., tyrosine is built out of methane and phenol, leucine is built out of methane and propane, etc.

Prior energy minimizations of the oligopeptide in vacuo resorted to our standard gradient minimizers, VA13A, VA14A or E04KAF [42]. In the presence of water molecules, we elected to resort to the Merlin operating system [40], which was interfaced to the SIBFA program. This algorithm encompasses three distinct gradient minimizers: Broyden–Fletcher–Goldfarb–Shanno, Conjugate Gradient using the Pollack–Ribière method, and Davidon–Fletcher–Powell; and three distinct nongradient minimizers: Simplex, Roll, and Random, the latter two having been conceived at the University of Ioannina (see Ref. 40 for further description). These minimizers can be activated in succession along a route elected by the user for a given task.

Restarting from the above-mentioned initial structures, a two-level process was adopted:

(1) Solvation of the ammonium and carboxylate ions was first undertaken, by disposing three water molecules around the ammonium ion (one along the direction of each -NH bond) and two water molecules around the formate ion (each one in an external position to it, see e.g. Ref. 38 and Refs. therein). In one case (the Camerman et al. conformer), we also undertook the formate solvation by a third water molecule in a bridging position. A first energy minimization was carried out of the positions of the water molecules with a rigid oligopeptide conformation. In a first step, each of these water molecules was constrained in the vicinity of the solvated ionic moiety, by means of a harmonic quadratic restraint added to the energy functional; this restraint was removed in a second step. This was followed by a second minimization run, in which relaxation of the peptide occurred concomitantly with that of the positions of the water molecules.

(2) Saturation of the first hydration shell was then undertaken, by progressively disposing

eight water molecules around the accessible carbonyl oxygens and amide hydrogens (there is a maximum of eight such atoms in a fully extended conformation). To maintain a total number of water molecules equal to 13, seven, rather than eight molecules, were disposed in the case when there were already six waters solvating the ammonium and formate groups.

When there were no longer accessible carbonyl or amide groups, the waters were disposed around the charged residues. These preliminary locations of the water molecules were performed with the help of a graphics program interfaced to a Spectragraphics machine. To relieve the steric strains incurred by this procedure, energy minimization was performed first on the positions of these additional eight or seven water molecules, followed by a complete minimization bearing on the conformation of the oligopeptide and the positions of the 13 molecules simultaneously.

Interweaving nongradient and gradient minimizers turned out instrumental for preventing escape of individual water molecules away from the peptide due to initial steric repulsions, efficiently circumventing hydrophobic regions of the peptide and rerouting water molecules towards sites which in prior attempts were overlooked by our standard gradient minimizers. An outline of the operational strategy and sequence of minimizers selected, as well as an analysis of the performances of the minimizer, will be given in a separate article.

Because nonadditivity effects with more than two interacting entities are not properly accounted for yet in the analytical derivatives of E_{pol} and E_{CT}, as requested by the gradient minimizers, the computation of ΔE was performed without these two terms. A reduced value of the parameter F [38] in E_{disp}, namely 0.015 instead of 0.115 was adopted, leading to a slightly overestimated value of E_{disp} to compensate for the absence of these two contributions (see, e.g., Ref. 43).

Further elaborations on the present treatment will reincorporate these two terms explicitly, investigate the effects of more exhaustive hydration of enkephalin and analogues on their conformational behaviour, and implement a novel, mixed supermolecule-continuum approach for oligopeptide hydration.

RESULTS AND DISCUSSION

Isolated peptides

Standard gradient energy minimization performed in vacuo resulted in conformer V providing the most stable energy minimum. Other minima resulted from conformers I, VI and II, at 4.4, 7.4 and 8.7 kcal/mol respectively above it. Minimizations starting from IV, VII and VIII, on the other hand, remained trapped in high local energy minima, more than 40 kcal/mol above the best minimum. We did not attempt to refine these further.

Supermolecule hydration

The results of the computations in the presence of five and 13 water molecules are reported in Tables 1 and 2. Table 1 lists the starting values of the conformational angles and the converged ones (for $n = 13$); the differences, δ_C, of conformational energy of enkephalin with respect to its best conformational energy in vacuo taken as energy zero; the intermolecular interaction energy of enkephalin with the water molecules, Δ_E, and, for $n = 13$, its breakdown into the sum of water-water interaction energies, ΣW, and an enkephalin-water interaction term, ΔE_1, in which the solvent is considered as a single molecular entity; the overall energy balance $\delta E = \Delta E + \delta c$; and the difference, δ, of energy balances with respect to the best value of δE taken as energy zero. Table

TABLE I
LIST OF THE TORSIONAL ANGLES IN THE STARTING CONFORMATIONS AND AFTER ENERGY MINIMIZATION OF THE SUPERSYSTEM ENKEPHALIN-WATER, AND VALUES OF THE BINDING ENERGETICS

	Conformation I		Conformation II		Conformation III		Conformation IV		Conformation V		Conformation VI	
Starting values	φ	ψ	φ	ψ	φ	ψ	φ	ψ	φ	ψ	φ	ψ
Tyr^1		126		135		135		126	310	145	274	156
Gly^2	59	25	216	114	216	114	301	326	81	282	206	83
Gly^3	97	353	238	132	238	132	297	336	283	313	84	286
Phe^4	224	145	238	139	238	139	259	5	205	139	223	19
Leu^5	255	356	281	176	281	176	287	142	52	55	196	160
	χ_1	χ_2	χ_1	χ_2	χ_1	χ_2	χ_1	χ_2	χ_1	χ_2	χ_1	χ_2
Tyr^1	317	271					178	46	183	258	187	79
Phe^4	298	90					293	359	182	236	59	95
Leu^5	291	178					195	190	301	170		
Energy-minimized	φ	ψ	φ	ψ	φ	ψ	φ	ψ	φ	ψ	φ	ψ
Tyr^1	180	147	196	161	152	178	192	176	320	160	258	148
Gly^2	41	66	260	78	283	75	288	300	84	287	183	57
Gly^3	85	295	136	278	162	297	304	303	282	307	92	278
Phe^4	306	174	290	166	241	168	235	161	209	147	227	56
Leu^5	278	293	280	159	291	173	301	129	49	58	185	174
	χ_1	χ_2	χ_1	χ_2	χ_1	χ_2	χ_1	χ_2	χ_1	χ_2	χ_1	χ_2
Tyr^1	293	301	194	262	286	288	195	48	198	243	181	54
Phe^4	300	102	302	293	287	266	58	85	186	258	47	274
Leu^5	279	198	276	293	293	288	183	192	193	191	46	248
n = 5 waters												
δc		18.3		53.0				20.6		1.2		22.5
ΔE_{inter}		− 79.9		−100.4				− 74.8		− 67.3		− 82.9
δE		− 61.6		− 47.4				− 54.2		− 66.1		− 60.4
δ		4.5		18.7				11.9		0.0		5.7
n = 13 waters												
δc		18.3		53.0		36.0		20.6		1.2		22.5
ΔE_{inter}		−156.9		−189.9		−175.6		−160.2		−142.4		−159.4
δE		−138.6		−136.9		−139.6		−139.6		−141.2		−136.9
δ		2.6		4.3		1.6		1.6		0.0		4.3
ΔE_1		−115.7		−158.5		−141.0		−125.4		− 98.6		−141.1
ΣW		− 41.2		− 31.4		− 34.6		− 34.8		− 43.8		− 18.3

Energies in kcal/mol, torsional angles in degrees.

TABLE 2

LIST OF THE RELEVANT INTERATOMIC DISTANCES (in Å)

	Conformation I		Conformation II		Conformation III		Conformation IV		Conformation V		Conformation VI	
Intramolecular distances	O_B^- Leu^5 – H_a^+ Tyr^1	2.08	O Tyr^1 – H_d^+ Tyr^1	2.51								
	O_B^- Tyr^1 – H_b^+ Tyr^1	2.52			O Tyr^1 – H_c^+ Tyr^1	2.24	O Phe^4 – H_a^+ Tyr^1	2.00	O Tyr^1 – H_a^+ Tyr^1	2.48	O Tyr^1 – H_c^+ Tyr^1	2.22
	O Phe^4 – H_b^+ Tyr^1	2.60			O Phe^4 – H_c^+ Tyr^1	1.75	O_A^- Leu^5 – H_b^+ Tyr^1	2.04	O Phe^4 – H_a^+ Tyr^1	1.93	O_B^- Leu^5 – H Gly^2	2.34
	O_B^- Leu^5 – H_b^+ Tyr^1	2.53			O Tyr^1 – H Gly^3	2.07	O Tyr^1 – H_c^+ Tyr^1	2.45	O_B^- Leu^5 H_b^+ Tyr^1	1.75	O Gly^2 – H Phe^4	2.54
	O Phe^4 – H_c^+ Tyr^1	1.80			O Phe^4 – H Phe^4	2.60	O Phe^4 – H Phe^4	2.51	O Tyr^1 – H Gly^3	2.20	O_A^- Leu^5 – H Leu^5	2.11
	O Gly^2 – H Phe^4	2.13	O W3 – H_a^+ Tyr^1	1.90					O Phe^4 – H Phe^4	2.34		
			O W2 – H_b^+ Tyr^1	1.86	O W3 – H_a^+ Tyr^1	1.94	O W3 – H_a^+ Tyr^1	1.98				
			O W1 – H_d^+ Tyr^1	1.91	O W1 – H_c^+ Tyr^1	1.90	O W1 – H_c^+ Tyr^1	2.13	O W11 – H_a^+ Tyr^1	2.24	O W4 – H_a^+ Tyr^1	2.02
			O W8 – H Gly^3	2.17	O W9 – H Gly^2	2.13	O W12 – H_c^+ Tyr^1	2.49	O W2 – H_b^+ Tyr^1	2.48	O W2 – H_b^+ Tyr^1	2.03
			O W5 – H Leu^5	2.44	O W10 – H Leu^5	2.17	O W10 – H Gly^3	2.49	O W1 – H_b^+ Tyr^1	1.97	O W1 – H_c^+ Tyr^1	2.08
			O W6 – HO Tyr^1	1.97	O Tyr^1 – H_1 W2	2.40	O_A^- Leu^5 – H_1 W1	2.09	O W11 – H Phe^4	2.41	O W12 – H_c^+ Tyr^1	2.35
					O Phe^4 – H_1 W2	2.30	O_A^- Leu^5 – H_1 W4	2.09	O W8 – H Leu^5	2.33	O W6 – HO Tyr^1	2.02
Intermolecular peptide-water distances	O W5 – H_a^+ Tyr^1	2.18	O Tyr^1 – H_1 W1	2.41								
	O W2 – H_b^+ Tyr^1	2.26	O Phe^4 – H_1 W2	2.00	O_A^- Leu^5 – H_1 W3	2.14	O_B^- Leu^5 – H_1 W4	2.56			O_B^- Leu^5 – H_1 W3	2.05
	O W6 – H Gly^2	2.29	O_A^- Leu^5 – H_2 W2	1.94	O_B^- Leu^5 – H_2 W3	2.45	O_B^- Leu^5 – H_2 W4	2.14	O W13 – H Leu^5	2.31	O_A^- Leu^5 – H_2 W3	2.58
	O W9 – H Gly^3	2.38	O_A^- Leu^5 – H_1 W4	2.11	O_A^- Leu^5 – H_1 W4	2.01	O_B^- Leu^5 – H_1 W5	1.96	O_B^- Leu^5 – H_2 W2	2.00	O_A^- Leu^5 – H_1 W4	1.95
	O W10 – HO Tyr^1	2.06	O_B^- Leu^5 – H_1 W4	2.57	O Phe^4 – H_1 W5	2.47	O_B^- Leu^5 – H_2 W6	2.06	O Gly^3 – H_1 W3	2.12	O_A^- Leu^5 – H_1 W5	2.19
	O Phe^4 – H_1 W1	2.30	O_B^- Leu^5 – H_2 W4	2.07	O_B^- Leu^5 – H_1 W6	2.05	O Gly^2 – H_2 W7	2.26	O_A^- Leu^5 – H_1 W4	2.00	O_B^- Leu^5 – H_1 W5	2.45
	O_A^- – H_1 W2	1.89	O_B^- Leu^5 – H_1 W5	1.97	O_A^- Leu^5 – H_2 W6	2.17	O Gly^3 – H_2 W7	2.23	O_B^- Leu^5 – H_1 W5	1.96	O Gly^3 – H_2 W6	2.05
	O_A^- – H_1 W4	2.18	O Gly^2 – H_2 W6	1.94	O Gly^2 – H_2 W7	2.00	O Gly^2 – H_2 W8	2.18	O Tyr^1 – H_1 W6	1.99	O Gly^2 – H_1 W7	2.02
	O_A^- – H_2 W4	2.59	O Gly^3 – H_2 W7	2.00	O Gly^3 – H_2 W8	2.07	O Tyr^1 – H_1 W9	1.99	O Gly^2 – H_2 W9	2.07	O Phe^4 – H_2 W9	2.09
	O_B^- – H_2 W4	2.19	O Tyr^1 – H_2 W8	2.15			O_η Tyr^1 – H_2 W10	2.57	O Gly^3 – H_2 W10	1.98	O_A^- Leu^5 – H_1 W10	2.44
	O_B^- – H_1 W5	2.03	O_A^- Leu^5 – H_1 W13	1.95					O Phe^4 – H_2 W11	2.41	O Tyr^1 – H_2 W11	2.12
	O Gly^3 – H_1 W7	1.96									O Tyr^1 – H_2 W12	2.17
	O Gly^2 – H_2 W8	2.04									O_A^- Leu^5 – H_1 W13	2.08
d_A	5.91		7.33		6.07		7.26		7.31		7.31	
d_B	3.84		5.01		4.00		4.93		4.98		4.94	
d_C	10.58		5.63		10.00		5.04		10.55		8.71	
d_D	3.22		5.29		3.96		3.36		3.08		4.22	

2 lists, for each converged minimum, the sum of hydrogen-bonding interactions, occurring both within the peptide backbone and between the peptide and the water molecules.

It also lists the following interatomic distances between selected centers of enkephalin: d_A, distance between the ammonium nitrogen of Tyr^1 and its phenolic oxygen; d_B, distance between the ammonium nitrogen and the center of the phenol nucleus; d_C, distance between the centers of the two aromatic rings. Values for these distances matching those between corresponding centers in morphine [24,25] could be crucial to ensure a topological analogy between the two molecules. With the latter one, the values of d_A, d_B, and d_C are 7.0, 4.5 and 5.0 Å, respectively. In order to outline the extent of folding of the investigated conformers, the end-to-end distance d_D between the ammonium nitrogen and the carboxylate carbon atom is also listed in Table 2. Conformers VII and VIII are not listed in Tables 1 and 2, because energy minimization led to significantly higher energy minima than I–VI.

A representation of the energy-minimized structures of conformers I–VI in the presence of 13 water molecules is given in Figs. 1 to 6.

The results of Table 1 indicate that:

(a) The converged conformers I–VI are energetically separated by very large conformational energy differences of up to 52 kcal/mol between II and V.

(b) Solvation of enkephalin by five water molecules results in a significant reduction of the energy gap entailed by δ_C, down to 18.7 between the two extreme values of δE. This results from an interplay between the intra- and intermolecular interactions, the latter most favouring the least stable conformer.

(c) Upon saturating the first hydration shell by up to 13 water molecules, a further, dramatic reduction of the energy gap has occurred, down to 4.3 kcal/mol between the extreme values of δE. Two conformers, III and IV, are now only 1.6 kcal/mol above the global minimum, and another one, I, is only 2.6 kcal/mol above it. This is due to a further, more contrasted, interplay of intra- and intermolecular effects.

(d) The value of δ_C did not evolve upon passing from $n = 5$ to $n = 13$.

(e) Within the intermolecular term ΔE, for $n = 13$, the individual contributions of ΣW and ΔE_I can vary by significant amounts (compare, e.g., I and VI).

Conformers I–VI can be characterized by:

(a) Either a direct ammonium-carboxylate interaction, as in I, IV and V, or a through-water one, as in II, III or VI.

(b) Either a remote disposition of the aromatic rings of Tyr^1 and Phe^4, as in I, III, V and VI, or a proximal one, in an orthogonal arrangement as in II and IV.

Each of the four possible mutual combinations of (a) and (b) is represented within the set of conformers I-VI.

With respect to the starting crystal conformations, the conformational changes occurring in I-IV take place along a limited number of backbone dihedral angles. The torsional angle variations there can be very large, although compensatory to some extent. They enable for the partial folding of the structure and are assisted by intermolecular hydrogen bonds with water molecules. Thus, in I, they occur along φ_3, ψ_4 and ψ_5. In II and III, they occur along φ_3, ψ_3 and ψ_4. In IV, they occur along ψ_1 and ψ_4.

With respect to the starting theoretical conformations V and VI, the conformational changes were found to have either negligible (as in V), or limited ($< 40°$) amplitudes, as in VI, in which

case they were distributed throughout the whole backbone. The set of backbone torsional angles of V is also similar to the one put forward by Premilat and Maigret [18].

The water molecules solvating I–VI are structured into two or three clusters. The largest ones encompass from five up to nine molecules, organized in a wide area around the buttoning salt bridge.

In spite of the considerable flexibility they confer to zwitterionic enkephalin, the water molecules do not provoke a reversal of the overall conformational preference for V at the benefit of alternative conformers. In V, the two aromatic rings have an intercenter separation of 10.5 Å. This distance is consistent with the corresponding one of 10 ± 1 Å derived by fluorescence spectroscopy in water solution for a met-enkephalin derivative [14].

Conformer IV, retaining a disposition of its aromatic rings conform to that of the starting crystal structure of [5], analogous itself to that of the tyramine and cyclohexenyl rings of morphine, is endowed with an energetical stability ($\delta = 1.6$ kcal/mol) close to that of the best conformer V with rings apart.

Whereas in IV the distances d_A, d_B and d_C match very closely the corresponding ones in morphine, another, albeit less perfect, match occurs with conformer II, yet with a very different set of torsional angles and a significantly larger end-to-end distance (5.29 Å as compared to 3.36 Å with IV). When the two aromatic rings are apart, on the other hand, as in conformers I, III, V and VI, a wider spread of the values of d_A, d_B and d_C can be evidenced.

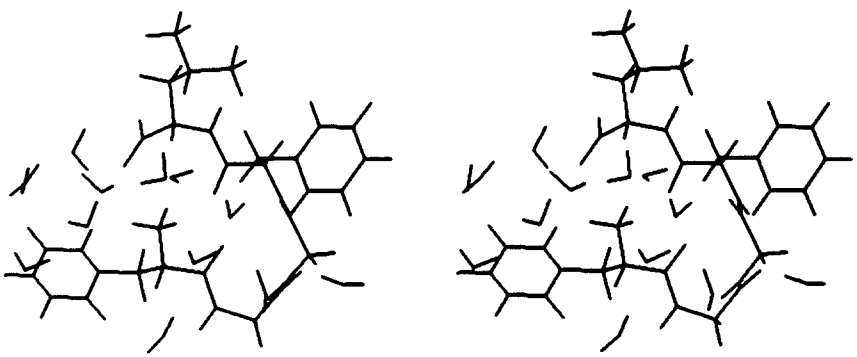
Some distinct intramolecular H-bonds are encountered in recurrent fashion throughout I–VI. Particularly privileged is the one involving the carbonyl oxygen of Phe^4 and the ammonium group of Tyr^1, as in conformers I, III, IV and V. An H-bond between the carbonyl oxygen of Tyr^1 and the amide hydrogen of Gly^3 occurs twice, in $C7_{ax}$ and $C7_{eq}$ arrangements in conformers III and V, respectively. An H-bond between the carbonyl oxygen of Gly^2 and the amide hydrogen of Phe^4 occurs twice, in $C7_{eq}$ arrangements in conformers I and VI.

An H-bond between one carboxylate oxygen of Leu^5 and the amide hydrogen of Gly^2 is found in VI. Finally, several intramolecular interactions within a given residue can be found. The most persistent ones occur between the carbonyl and ammonium groups of Tyr^1, as in conformers II–VI, and between the carbonyl oxygen and amide hydrogen of Phe^4, as in conformers III–V.

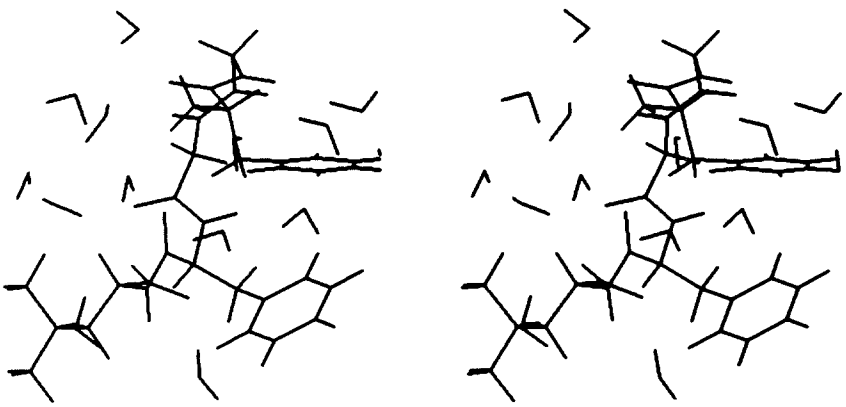
The intramolecular H-bond between the carbonyl oxygen of Phe^4 and the ammonium group of Tyr^1 was found in the crystal structures of leu-enkephalin [1,4] and the theoretical study of Manavalan and Momany [20]. The existence of an intramolecular H-bond between the carbonyl oxygen of Tyr^1 and the amide hydrogen of Gly^3 was indicated in an earlier NMR investigation by Khaled et al. [9], and appears also from the theoretical study of Manavalan and Momany [20]. Its occurrence was also proposed in the μ-active conformation of the derivative Tyr–cyclo(N^γ–DA_2bu–Gly–Phe–Leu) [44]. A joint 2D NMR and theoretical investigation devoted to hexapeptide enkephalin derivatives [35], indicated its existence with derivatives Tyr–D-Ser–Gly–Phe–Leu–Thr, Tyr–D-Thr–Gly–Phe–Leu–Thr, as well as Tyr–D-Ser(OtBu)–Gly–Phe–Leu–Thr. An intramolecular H–bond between the carbonyl oxygen of Gly^2 and the amide hydrogen of Phe^4 was indicated for derivative Tyr–D-Thr(OtBu)–Gly–Phe–Leu–Thr. Let us also mention that a molecular dynamics investigation devoted to Tyr–c[DA_2bu–Gly–Phe–Leu] showed the stabilization of C7 structures [44], as well as theoretical investigations devoted to morphiceptin analogues [45].

We did not observe, on the other hand, intramolecular H–bonds between the carbonyl oxygen of Gly^2 and the amide hydrogen of Phe^5, the existence of which was proposed in some earlier in-

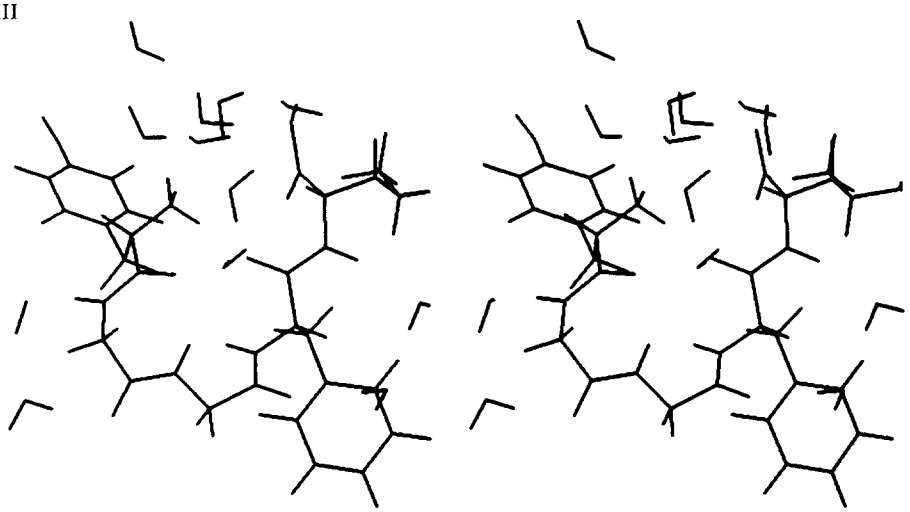
I

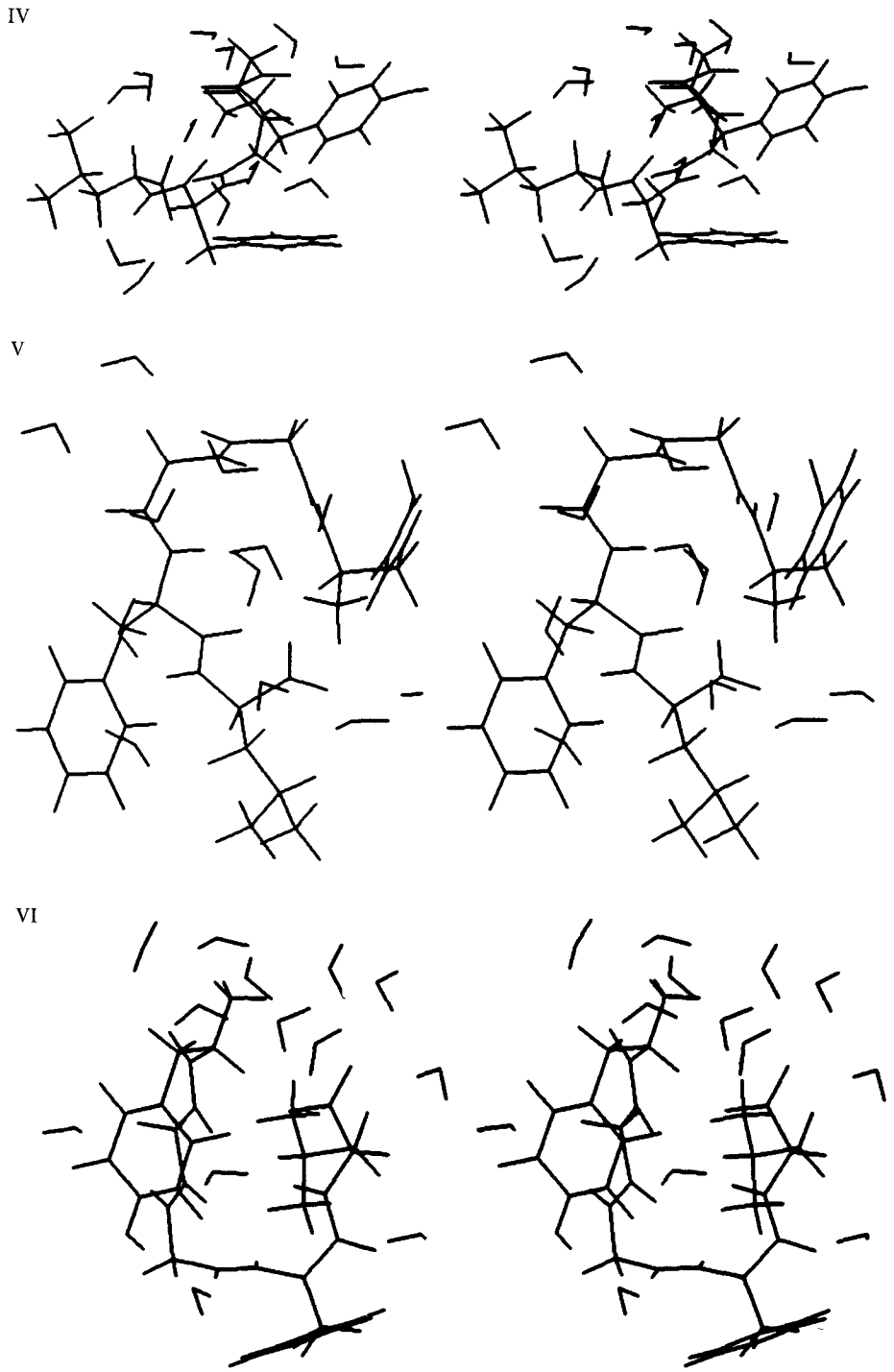


II



III





Figs. 1-6. Representation of the energy-minimized structures of the complexes of conformers I-VI in the presence of 13 water molecules.

vestigation within an overall folded conformation (see, e.g., Ref. 6). The existence of such an H-bond was also put forward on the basis of ^{1}H NMR measurements of transferred nuclear Overhauser effects for a membrane-bound conformation of active enkephalin analogues [46]; the latter conformation, however, is an unfolded one, with the ammonium and carboxylate groups remote, too far for a one-water-mediated interaction. Binding of the analogues to the polar heads of phosphatidylserine and/or phosphatidylcholine making up the model membranes may be responsible for such an extension. Explicit studies of peptide-polar head interactions (see e.g., Ref. 47) would be necessary to account for such an effect. We did not observe either evidence for an intramolecular H-bond between the carbonyl oxygen of Tyr^1 and the amide hydrogen of Phe^4 as observed in the crystal structures of Refs. 1 and 4. The shortest corresponding CO...HN distances we found were 2.7, 3.3 and 3.2 Å in conformers I, III and V, respectively. In our energy-minimized structures, such an H-bond is lost at the profit of the interaction involving the charged ends. A more exhaustive sampling of candidate starting conformers may be necessary to further elucidate these points.

To widen the scope of our supermolecule approach, we are presently implementing a 'continuum' model [48] to simulate the effect of solvation beyond the first shell, and compare the results with those involving a large number (>40) of 'discrete' water molecules. Such an approach should provide a viable alternative to the much more computationally expensive Monte-Carlo [49] or molecular dynamics [50] ones. Its application to enkephalin and pharmacologically relevant analogues will be reported separately.

CONCLUSIONS

The present investigation exemplifies the conformational flexibility imparted on leu-enkephalin in its zwitterionic form, by water molecules saturating its first hydration shell. Out of nine starting conformers, four conformers are separated by energy differences, δ, of 2.3 kcal/mol and less, and two additional conformers are 4.3 kcal/mol above the global minimum. Such reduced values of δ can result from a strongly contrasted interplay of intra- and intermolecular interactions. This is the most conspicuous with conformer II ($\delta = 4.3$ kcal/mol), having the largest end-to-end distance ($d_D = 5.3$Å), a highly unfavourable conformational energy term ($\delta_C = 53$ kcal/mol), but the distinctively strongest intermolecular energy term ΔE. To our knowledge, such dramatic compensations of intra- and intermolecular contributions were never put forward before for zwitterionic peptides of this size and with a reduced number of water molecules.

Conformers I–VI are stabilized by electrostatic interactions between the terminal ammonium and carboxylate ends. These can be either direct or water-mediated. The most stable conformer computed in vacuo V, is closely similar to the one derived by other authors [18, 20]. It remains the preferred conformer in the presence of water although at the cost of a considerably diminished energy gap with respect to the two second best conformers ($\delta = 1.6$ kcal/mol). In V, the interring separation is of $\simeq 10.5$ Å, consistent with the results of fluorescence transfer measurements on the Trp^4, Met^5 derivative in water [14]. Second best in energy is a conformer (denoted IV) closely similar to the one obtained in the X-ray crystal structure of Aubry et al. [5], with an interring separation of 4.5 Å. Both this and conformer II ($\delta = 4.3$ kcal/mol) have values of their d_A, d_B and d_C distances matching closely those of morphine, although with a set of different backbone and side-chain torsional angles, and different end-to-end distances. As mentioned above, such close inter-

aromatic ring separations in a mutually orthogonal disposition were recently put forward in a series of novel, high affinity δ-enkephalin derivatives [33,34].

In addition to the buttoning ammonium-carboxylate interaction proper, and despite the variability in torsional angles and competition by water molecules, some intramolecular interresidue H-bonds were encountered in recurring fashion throughout I–VI: these are the interactions between the carbonyl of Phe^4 and the ammonium of Tyr^1, as well as those between the carbonyl of Tyr^1 and the amide of Gly^3, or between the carbonyl of Gly^2 and the amide of Phe^4, which both occur in C7 arrangements.

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