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Energy levels and quantum states of [Leu]enkephalin conformations based on theoretical and experimental investigations

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Abstract

This paper describes a theoretical and experimental study of [Leu]enkephalin conformations with respect to the quantum states of the atomic structure of the peptide. Results from vibrational absorption measurements and quantum calculations are used to outline a quantum picture and to assign vibrational modes to the different conformations. The energy landscape of the conformations is reported as a function of a Hamming distance in Ramachandran space. Molecular dynamics simulations reveal a pronounced stability of the so-called single-bend low-energy conformation, which supports the derived quantum picture of this peptide.

(Some figures in this article are in colour only in the electronic version)

1. Introduction

Enkephalins are known as morphine-like neurotransmitters and they are found in nociceptive pathways in the brain, in the limbic system and in the spinal cord. Enkephalins were first isolated in 1975 from pig brain [1]. This study also found that enkephalin is normally a mixture of two pentapeptides, Tyr–Gly–Gly–Phe–Leu and Tyr–Gly–Gly–Phe–Met, i.e. [Leu]enkephalin and [Met]enkephalin respectively. The ratio of [Leu]enkephalin to [Met]enkephalin varies among species, e.g. it is 4:1 in pig brain but 1:4 in cow brain [1, 2].

Enkephalin and its analogues, e.g. Tyr–Gly–Gly–Phe, bind much more strongly than morphine to their common target, the opiate receptor [3]. The extreme flexibility of enkephalin compared with morphine, a more rigid molecule, necessitates both theoretical and experimental studies aimed at locating possible electronic structures in order to elucidate structure–function relationships. However, confusion and contradiction regarding the determination of the electronic structures and corresponding conformations of this molecule prevail [4].

During the last two decades, several theoretical studies have been carried out on enkephalin [3–9]. Isogai *et al* provided conformational analysis of [Met]enkephalin [3], but

did not include any solvent effect. DeCoen performed theoretical studies on the zwitterionic form of the same molecule [5], while [Leu]enkephalin was studied by Humblet and DeCoen [6] and by Premilat and Maigret [7, 8]. Schiller reviewed several studies, and concluded that the studies agreed that various low-energy conformations of enkephalin are present in equilibrium in aqueous solution [9].

Experimentally, there have been different investigations on enkephalin including x-ray crystallographic studies on [Leu]enkephalin [10–12], on [Leu]- and [Met]enkephalin [13], NMR studies on both molecules [14–17] and vibrational circular dichroism (VCD) measurements [18]. A Raman scattering study was reported on [Leu]enkephalin crystals [19]. Recently we reported a Raman study of [Leu]- and [Met]enkephalin *in vivo* [20], where the effects of pH value on the conformational states were studied. In that work it was shown that enkephalin could be monitored in different conformational states, but due to the resolution of the NIR-Raman employed in that study it was not possible to conclude which conformation had the lowest energy.

We would like here to be clear regarding the terminology 'conformation'. We use the word conformation to mean a physically stable state of the peptide, while a 'conformational state' is any possible configuration of the peptide, e.g. it could be an excited state. Conformational states include the substates of Frauenfelder [21].

The above-mentioned x-ray studies reported four different conformations, all structured in an antiparallel β-sheet with similar backbone conformation but differing in side-chain conformation. NMR studies have shown a concentration dependence on the oligomeric state of the peptide, interpreted as evidence for a monomeric bend conformation, whereas an antiparallel β-sheet dimer appeared at higher peptide concentration [17]. Raman studies in DMSO-D6 and water suggested both a single β-bend and an extended conformation of [Leu]enkephalin, but only an extended conformation of [Met]enkephalin [22]. We recently reported on determination of [Leu]enkephalin conformations in non-polar solvents, which might act as a stabilizing environment [23]. In that work we also investigated the conformational states of [Leu]enkephalin by applying density functional theory (DFT) calculations to derive absorption spectra, which in turn were compared with vibrational absorption (VA) measurements of [Leu]enkephalin in DMSO-D6. The results suggested that [Leu]enkephalin relaxes into a single- and a double β-bend structure.

Enkephalin is one of the smallest peptides that has a biological function and yet is of a size where a detailed quantum mechanical study is feasible [23]. In the present work we present

(1) an approximate energy landscape composed of various conformers of [Leu]enkephalin as a function of Hamming distance,
(2) energy levels of quantum states of [Leu]enkephalin conformers based on the total energy of each molecule, which is also referred to as the relative energy, as derived quantum mechanically [23].

We also include molecular dynamics (MD) simulations which indicate that at least three conformational states of [Leu]enkephalin can be observed on a nanosecond timescale, and we relate this finding to the energy landscape of the peptide.

2. Methods

Sample preparation, VA measurements, structure modelling and DFT calculations for [Leu]enkephalin are published elsewhere [23]. X-ray crystallographic structures [10, 12, 24, 25] were used as starting structures for DFT optimizations, and they are shown in figure 1, including here hydrogen atoms.

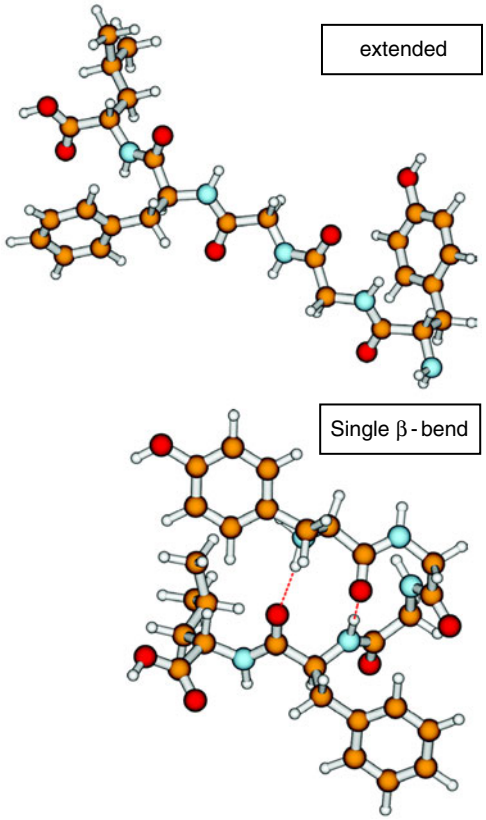

Figure 1. Extended (upper) and single β-bend (lower) x-ray structures, [24] and [10] respectively, used as starting structures for DFT optimizations [23].

The x-ray coordinates of the double-bend conformation of [Leu]enkephalin were used as starting coordinates in the MD simulation [25]. Hydrogen atoms were added using the psfgen structure-building module distributed with the molecular dynamics program NAMD [26]. The zwitterionic form of the peptide, which is present in aqueous solution at pH 7, was solvated in a rectangular water box of dimensions $50 \times 55 \times 55$ Å^3. The resulting system was energy minimized prior to the MD simulation. The TIP3P [27] water model was used along with the Charmm22 parameter set for the peptide [28]. The MD program NAMD [26] was used for an MD simulation conducted for 10 ns at constant temperature (300 K) and pressure (1 atm). The PME method was used for calculation of electrostatic forces [29]. Full periodic boundary conditions were imposed.

3. Results and discussion

The DFT calculations identified eight distinct extended (e) and ten distinct single β-bend (s) as well as two double β-bend (d) conformations. The d conformations are not considered here as they are of much higher energy than the lowest-energy conformations. Consequently, we group the conformations into two basic classes, the extended (e) and the single β-bend (s). In the following, we give a tentative picture of the energy landscape of the e and s conformations of [Leu]enkephalin, and their quantum energy states. Estimation of all the potential barriers between the 18 identified conformational e and s states is beyond the scope of the present work.

Table 1. Relative energies in kcal mol^{-1} of the different conformations of [Leu]enkephalin calculated quantum mechanically (DFT, B3LYP/6-31G*) [23].

Folded	DFT	Extended	DFT
s1	7.09	e1	6.94
s2	6.22	e2	10.25
s3	6.22	e3	7.84
s4	0.22	e4	8.45
s5	2.37	e5	11.35
s6	5.36	e6	7.82
s7	0.99	e7	11.59
s8	4.03	e8	15.80
s9	0.99	e9	10.73
s10	0.00	e10	12.87
d1	7.27	e11	14.96
d2	6.70	e12	14.89

3.1. Energy landscape of [Leu]enkephalin

Relative energies of all [Leu]enkephalin structures obtained in [23] are shown in table 1. We define the energy of the s10 conformation to be 0.00 kcal mol^{-1}, as this conformation has the lowest energy at the B3LYP/6-31G* level of theory.

Since we are able to classify the conformational states of [Leu]enkephalin, it is possible to draw an energy landscape. The classification of the conformational states is based on the DFT calculations [23]. For the purpose of classification we use the dihedral angles [31] that provide a metric in the Ramachandran space of conformations. As a pentapeptide, enkephalin has: $5 \times 2 - 2$ dihedral angle parameters describing the backbone structure; 2 for each residue minus the 2 termini. For enkephalin, having for each residue i the dihedral angles (ϕ_i, ψ_i), a distance between two conformational states can be defined as a Hamming distance, which is the shortest path between structures A and A$'$ in Ramachandran space. Thus, we define the Hamming distance Δ, as follows:

$$\Delta = \sum_i \left\{ \left(\phi_i \pm \phi_i'\right)^2_{\min} + \left(\psi_i \mp \psi_i'\right)^2_{\min} \right\}^{1/2}.$$

Accordingly, this formula defines a metric in which various conformational states can be positioned, e.g. in an energy landscape.

Judging from the distribution of the backbone dihedral angles, for each residue there are usually preferred combinations (pairs) of ϕ–ψ values, i.e. two for Tyr, two for Leu and two for Phe, but no preferred combinations of the dihedral angles for the two Gly residues in [Leu]enkephalin.

Based on the data in table 1 and the corresponding dihedral angles [23], we derive a conformational energy landscape, plotted in figure 2, as a function of Hamming distance between the s and e conformations of [Leu]enkephalin. This picture could also represent peptide folding dynamics in going from the extended unfolded structure to the single-bend folded structure.

3.2. Quantum states of two [Leu]enkephalin conformers

Based on a comparison between VA measurements and DFT calculations, it is possible to picture the various vibrational quantum states in the e and the s conformations (figure 3).

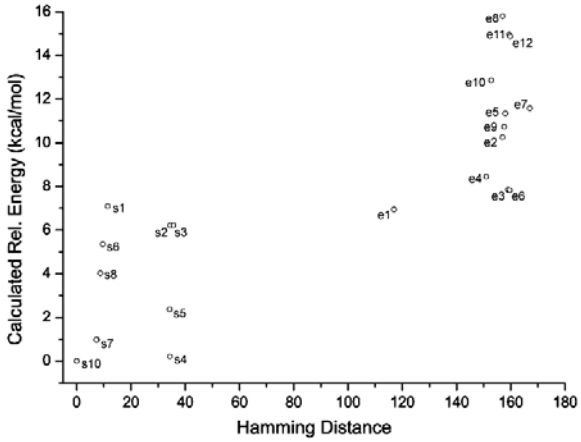

Figure 2. Energy landscape of the calculated relative energies in kcal mol^{-1} as a function of Hamming distance for e and s conformations of [Leu]enkephalin.

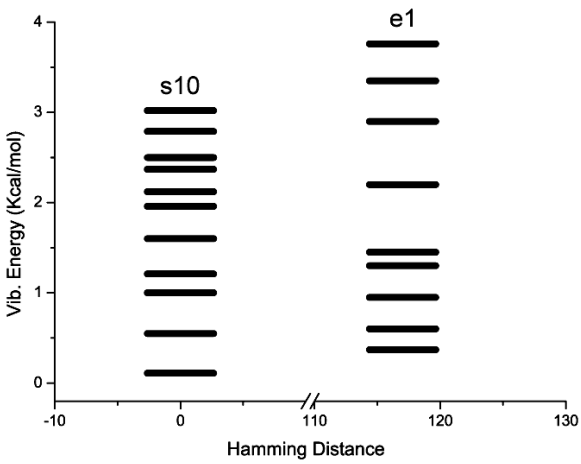

Figure 3. Vibrational energy substates of two [Leu]enkephalin conformations; single-bend s10 (left) and extended e1 (right) in kcal mol^{-1} as a function of Hamming distance.

We present the conformations e1 and s10 since they have the lowest total energy in the e and s classes, respectively [23] (cf table 1).

Assignment of vibrational quantum states is possible since we previously derived the VA spectra from the DFT optimized structures and compared these with the measured VA spectrum of [Leu]enkephalin in DMSO-D6 [23]. We estimate the barrier for the conformational transition between the e and the s conformations to be about 12 kcal mol^{-1}. Interestingly, this corresponds roughly to the energy of three to four hydrogen bonds formed between the *C*- and *N*-termini of the s conformation, as found in the MD simulations (figures 4 and 5).

MD simulations, carried out using a double-bend structure as the starting configuration, showed that a single-bend structure forms after 3–4 ns and remains stable up to 10 ns. This is indicated by the end-to-end distance, r_{ee}, monitored as a function of simulation time in figure 5. Clearly, once the molecule establishes three to four hydrogen bonds, the single-bend backbone structure is energetically very favourable. Furthermore, it is clear from the

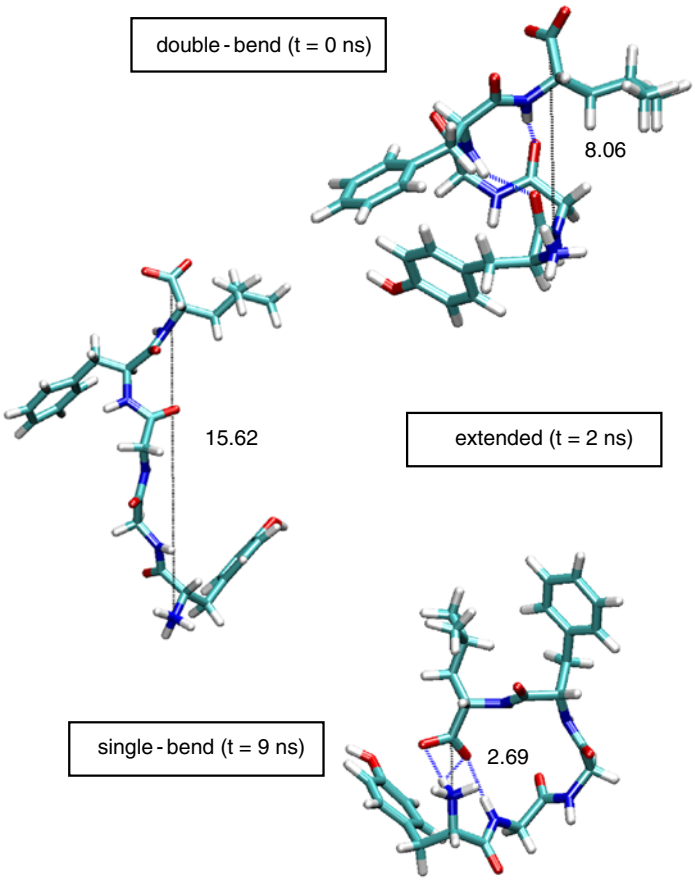

Figure 4. Snapshots of double-bend, single-bend and extended conformations of [Leu]enkephalin taken from the MD simulations at $t = 0$, 2, and 9 ns. The snapshots were rendered with VMD [30]. The double bend structure, used as the starting conformation in the simulation, exhibits an intermediate value, 8.1 Å, of the end-to-end distance, r_{ee} (annotated dashed line) as measured between Tyr1:N and Leu5:C, see also figure 5. The displayed extended and single-bend conformations represent maximal, 15.6, and minimal, 2.7 Å, values for r_{ee}, respectively. Hydrogen bonds present in the two bend conformations are shown with (unannotated) dashed lines.

simulation that [Leu]enkephalin goes through several different conformations, including an extended conformation, before relaxing to a final single-bend conformation, as demonstrated in figures 4 and 5.

4. Conclusion

From VA measurements and DFT calculations we have derived an approximate energy landscape of two types of [Leu]enkephalin conformations; a single-bend conformation and an extended conformation, as a function of Hamming distance. This quantum picture can also represent peptide folding dynamics in going from the double bend conformation, through the extended, to the single-bend conformation. These conformational transitions can occur on a nanosecond timescale as found by MD simulations.

Assignment of vibrational quantum states to these two conformations is also possible since we previously derived VA spectra from DFT optimized structures and compared them with

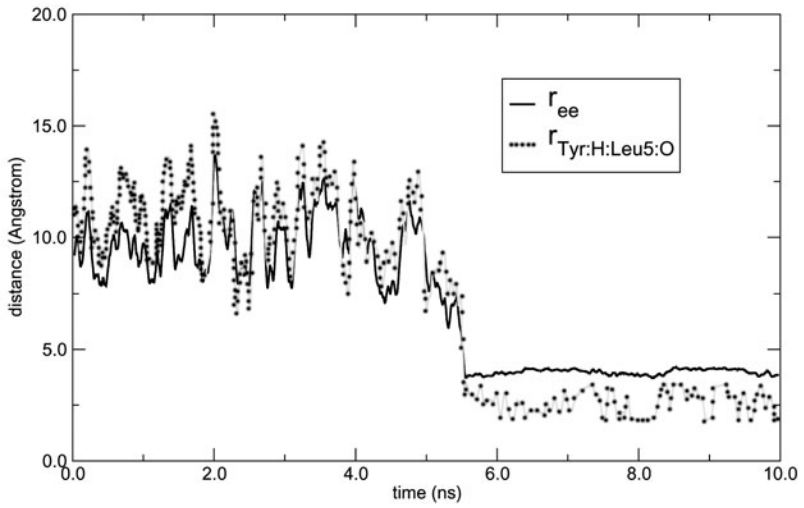

Figure 5. End to end distance, r_{ee} (solid line) monitored as a function of time during MD simulation of [Leu]enkephalin in water. The length of one of the hydrogen bonds, $r_{\mathrm{Tyr-H:Leu5:O}}$, formed between the termini is also displayed (dotted line).

the measured VA spectrum of [Leu]enkephalin in DMSO-D6. This allows here an estimation of the barrier of the conformational transition between the lowest-energy conformations e1 and s10 in the e and s conformational classes respectively. We estimate this barrier to be about 12 kcal mol^{-1}, corresponding to the energy of three to four hydrogen bonds as formed between the termini of the s conformation, in accord with results from MD simulations, where thermal fluctuations on a nanosecond scale can bring [Leu]enkephalin into a folded, single-bend conformation, significantly stabilized by the hydrogen bonds formed between the termini.

Acknowledgments

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