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Importance of folded monomer and extended antiparallel dimer structures as enkephalin active conformation

Molecular dynamics simulations of [Met^5]enkephalin in water

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Simulations of the molecular dynamics of the [Met^5]enkephalin monomer and dimer structures in water have been carried out. The dynamic trajectories have been analyzed in terms of the distances between intra- or intermolecular polar atoms. The time-correlated conformational transitions of an extended monomer structure have been converged into a stationary state among the β-bend folded forms. However, the dynamics simulation of an extended antiparallel dimer structure has shown no noticeable conformation change. These results imply that both the β-bend monomer and the extended dimer structures exist together as the fundamental conformation of enkephalins.

Enkephalin; Molecular dynamics simulation; β-Bend conformation; Extended antiparallel dimer conformation

1. INTRODUCTION

[Met^5]-(Tyr-Gly-Gly-Phe-Met) and [Leu^5]enkephalins (Tyr-Gly-Gly-Phe-Leu) are two endogenous pentapeptides exhibiting morphine-like actions [1]. Although their physiological effects derive from their interaction with the opioid receptor, it is well known [2] that there are several subclasses such as μ-, δ- and κ-receptors. In order to effectively design new and potent opioid compounds, therefore, it is of particular importance to elucidate the active conformation for each type of receptor.

As a fundamental conformation of enkephalins, X-ray crystallographic studies [3–9] have presented the following two forms: a type I′ β-bend monomeric structure and an extended antiparallel dimer structure. The conformational comparison with μ-selective morphine [3,5] has suggested that the former structure of the enkephalin is responsible for binding with the μ-receptor. On the other hand, many chemically synthesized δ-selective opioid peptides have shown an extended antiparallel molecular aggregation [10], suggesting that the latter dimer structure of the enkephalin corresponds to a conformation suitable for binding with the δ-receptor.

Previous studies of the active conformation of the enkephalin in relation to the receptor selectivity have generally taken its monomer structure into account. It could be supposed that there is no direct evidence that the dimer structure exists at the physiological concentration. Nevertheless, our previous studies [8,10,11] have pointed to the importance of the dimer structure as a biologically active conformation. Consequently, to determine whether the dimer structure stably exists in a dilute aqueous solution could provide the evidence needed, although this is difficult to define experimentally.

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Simulation of molecular dynamics is a useful approach to examine the conformation of a flexible molecule. It can describe the molecular system in terms of its trajectory or an ensemble of conformations. This method is assumed to be an adequate representation of the distribution function, and therefore provides reliable information about the molecular motion as exemplified in the dynamic properties of nucleic acid and proteins. In this paper, dealing with the molecular dynamics simulations of both monomeric and dimeric structures of [Met^5]enkephalin, we emphasize that the dimer structure itself exists stably in water and exhibits different conformational behaviours from those of a monomer structure.

2. EXPERIMENTAL

Molecular dynamics simulations of monomeric and dimeric [Met^5]enkephalin including water and using $\epsilon = 1$ were carried out using the computer program AMBER [12]. Initial structures for an extended monomer and an extended antiparallel dimer were constructed using the X-ray crystal analysis of [Met^5]enkephalin [8]. The initial enkephalin structures were energy-refined with conjugate gradient minimization. A total of 321 (monomer) or 280 (dimer) water molecules within a sphere of a 13 Å (monomer) or 14 Å (dimer) radius were placed around the solute enkephalin conformation. These molecules were designed so as to prevent leakage from the system by a boundary force of 1 kcal/mol per Å.

The dynamics simulations were begun assuming random velocity of the atoms that followed a Maxwellian distribution at 300 K. Equilibration of the water molecules was performed in 16 ps, and the simulations were continued with a 0.4 ps^{-1} temperature coupling for 50 ps (monomer) or 60 ps (dimer) using a nonbonded cutoff of 10 Å. The time step of the dynamics was 0.8 fs, and a Verlet's integrator algorithm [13] was used to solve Newton's equations of motion by numerical integration. The simulations were carried out on a VAX8600 computer, and an IRIS3130 instrument was used to display the conformational graphs.

3. RESULTS AND DISCUSSION

As a result of high flexibility of linear peptides, many conformations of the enkephalin monomer are possible. However, the conformational trajectory, monitored by the time profiles of four intramolecular atomic distances (fig.1), shows the transition from the initial extended form to the folded one (>27 ps) through an intermediate state (7–25 ps). The conformational event for each

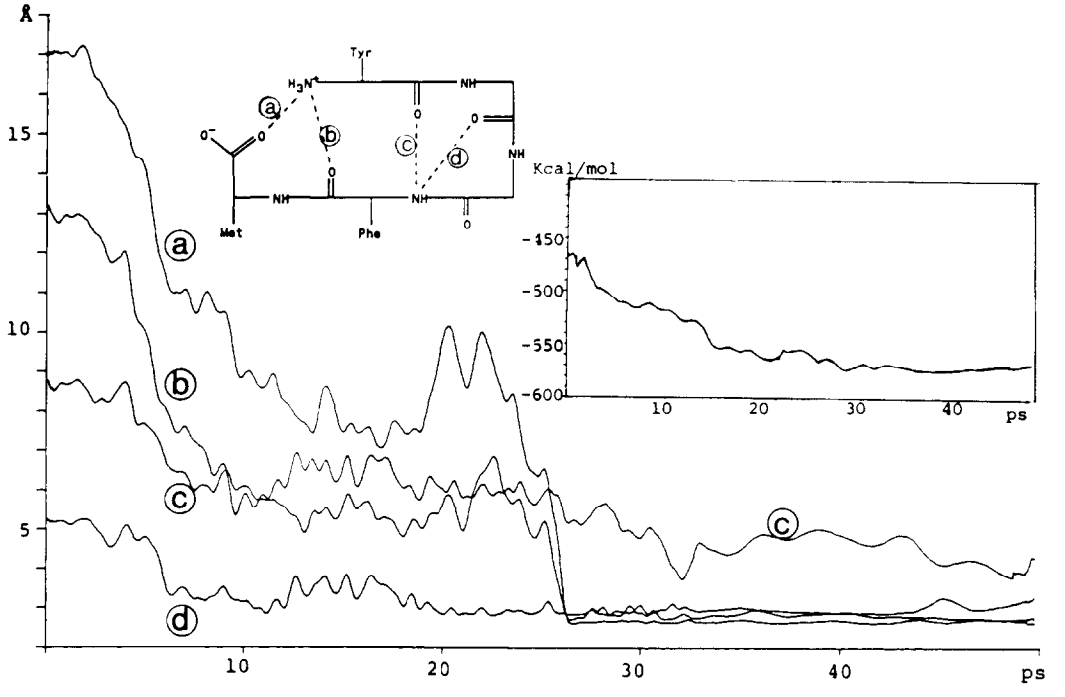

Fig.1. Time profiles of four atomic distances for the enkephalin monomer. (Inset) Time profile of the total potential energy accompanying the conformational transition.

stage is characterized by the formations of a $Gly^2(C=O)...Phe^4(NH)$ γ-bend structure (7–25 ps, fig.1d) and two further hydrogen bonds of $Tyr^1(NH_3^+)$ with $Phe^4(C=O)$ (fig.1b) and $Met^5(COO^-)$ (fig.1a), respectively. Representative photographs of each stage are shown in fig.2. The conformation of the main chain at the stationary state (>27 ps) shows no significant conformation change even during further dynamics simulation, and belongs to a typical type I′ β-bend structure, although no intramolecular hydrogen bond is formed between the $Tyr^1(C=O)$ and $Phe^4(NH)$ atoms (fig.1c). This β-bend conformation of the enkephalin monomer is in good agreement with that found in the X-ray crystal structure [3,5]. The approach between Tyr^1 and Phe^4 aromatic rings caused by taking a β-bend conformation forms a hydrophobic core at the Gly^2-Gly^3 β-bend moiety, as seen in fig.2b. This may be related to the emergence of the activity, judging from the fact that these amino acid residues are essential for opioid activity. The total potential energy change accompanying the conformational transition is shown in fig.1 (inset). It is obvious that the monomer structure of enkephalin overwhelmingly assumes an ensemble of folded forms in an aqueous solution.

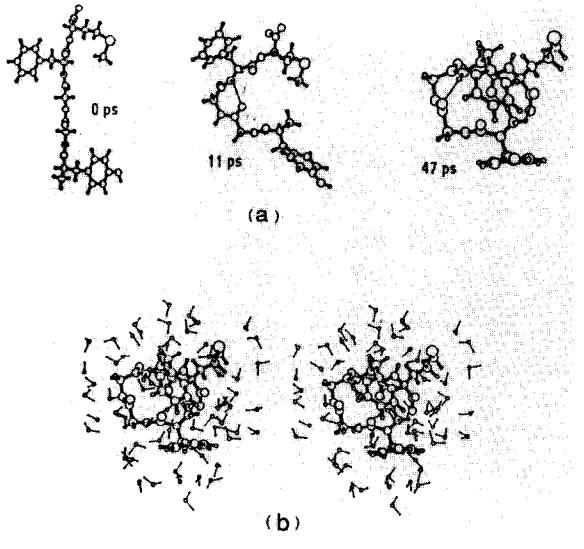

Fig.2. (a) Photographs of monomeric enkephalin conformations at 0, 11 and 47 ps. Thin lines indicate the possible intramolecular hydrogen bonds. (b) Molecular distributions of inner waters within 4 Å from any atom of enkephalin at 47 ps simulation.

Different experimental results have suggested that extended and folded conformations of enkephalin occur in equilibrium within a solution rather than in a single preferred conformation [14]. The dynamics simulation of the extended antiparallel dimer structure in water, as the biological representative of an enkephalin extended conformation, has been carried out. The conformational trajectory was monitored by time profiles of four distances of $Tyr^1_A(NH_3^+)...Met^5_A(COO^-)$ (fig.3a), $Tyr^1_B(NH_3^+)...Met^5_B(COO^-)$ (fig.3b), $Tyr^1_A(NH_3^+)...Met^5_B(COO^-)$ (fig.3c) and $Tyr^1_B(NH_3^+)...Met^5_A(COO^-)$ (fig.3d) atomic pairs, where the subscript letters, A and B, each denote an enkephalin molecule in the dimer structure. The results, along with the total potential energy change as a function of simulation time, are shown in fig.3. The starting structure (0 ps) and the photographs taken at 30 ps and 60 ps are shown in fig.4. Interestingly, the extended antiparallel dimer structure is essentially maintained throughout the dynamics simulation from 0 to 60 ps, although each molecule in the dimer itself shows a considerable amount of elasticity (see fig.3a and b). Each molecule in the dimer is tightly linked with its neighbour by two hydrogen bonds between the terminal NH_3^+ and COO^- groups (see fig.3c and d). These hydrogen bonds have also been observed by the ^1H NMR analysis of the δ-receptor selective δ-

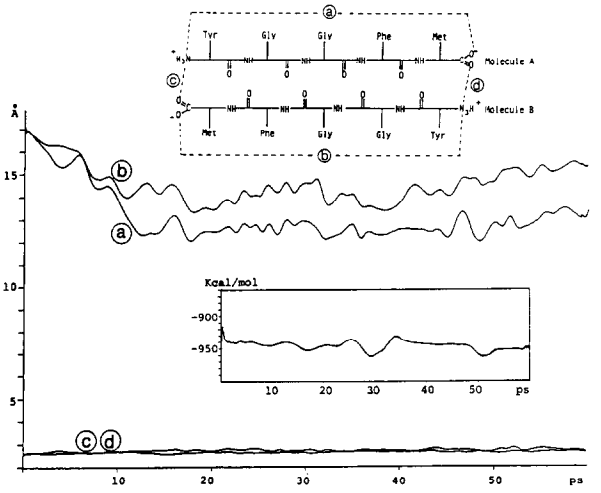

Fig.3. Time profiles of four atomic distances for the enkephalin dimer. (Inset) Time profile of the total potential energy accompanying the conformational transition.

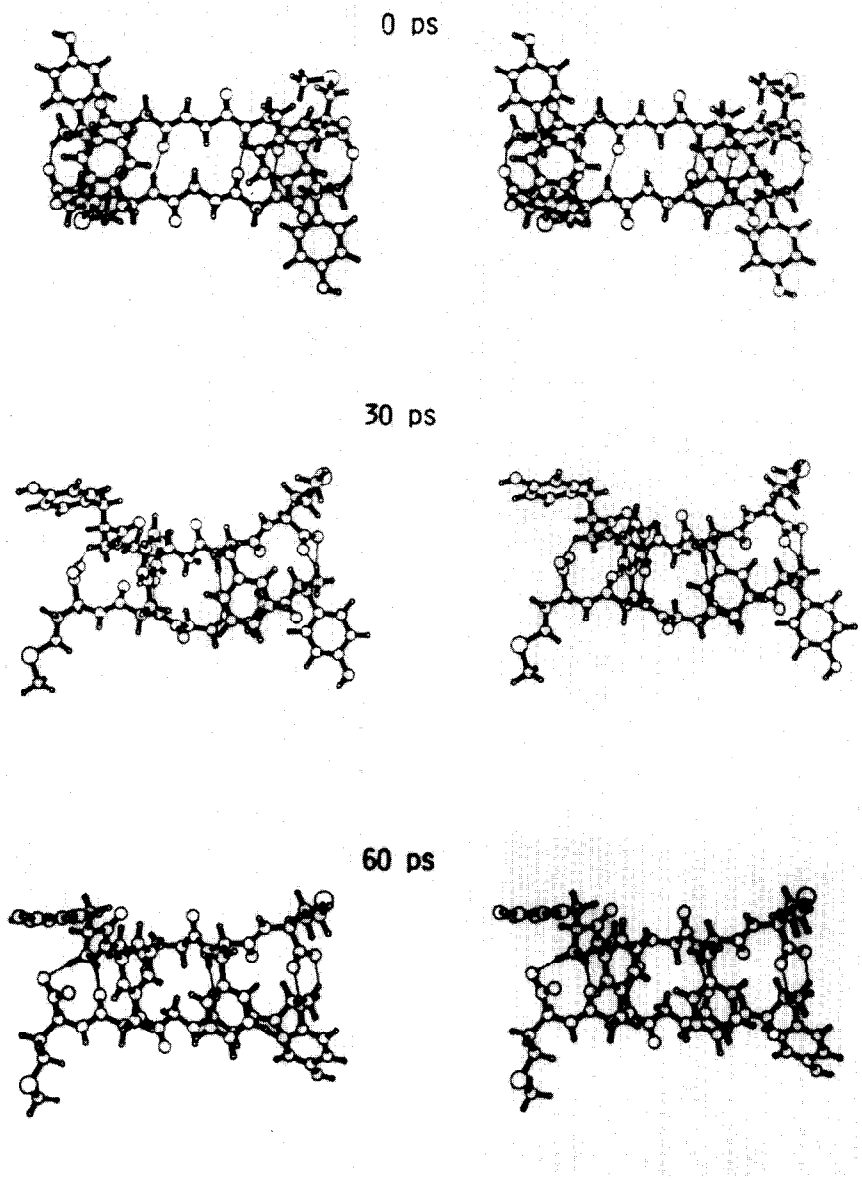

Fig.4. Stereoscopic photographs of dimeric enkephalin conformations at 0, 30 and 60 ps. Thin lines indicate the possible intermolecular hydrogen bonds. Water molecules are omitted for the sake of clarity.

enkephalin (Tyr-D-Thr-Gly-Phe-Leu-Thr) [10]. The total potential energy change accompanying the conformational transition is very small and, therefore, it would be reasonable to consider that the dimer structure observed in crystal structures of enkephalins [4,6–9] can be smoothly transformed from the elastic form in the solution state without large loss of energy.

The mean directions (MD) and their angular standard deviations (ASD) [15] of all side-chain

Table 1

Torsional variations (degree) of enkephalin side-chains

Torsion	Tyr^1		Phe^4		Met^5		
	χ^1	χ^2	χ^1	χ^2	χ^1	χ^2	χ^3
Monomer							
MD	56.9	−96.2	61.2	93.6	58.3	−176.3	37.5
ASD	21.3	21.8	16.9	23.8	18.6	24.0	84.5
Dimer molecule A							
MD	−47.3	−64.0	56.2	91.3	65.3	−176.1	−132.9
ASD	53.2	32.2	14.9	23.7	15.5	21.2	96.5
Dimer molecule B							
MD	58.5	−91.3	71.5	−58.9	−66.9	179.0	179.4
ASD	22.1	18.7	15.7	24.9	15.3	31.2	17.0

torsion angles for monomer and dimer structures are listed in table 1. They show relatively large ASDs around their MDs, many of which correspond to the energetically stable conformations. This implies that the side-chain torsions rotate considerably within their preferable regions and thus are relatively flexible.

In conclusion, the present simulation of molecular dynamics provides important insight into the dimer structure of enkephalins. These structures never dissociate from each other, even when they exist in a dilute aqueous solution. The fundamental conformations of enkephalins were determined as the β-bend monomer form and the extended antiparallel dimer structure. Accordingly, this result can account for the conflicting conclusions presented in previous solution studies on the stable conformation of enkephalins [14]. This paper further indicates a biological implication for the discussion on enkephalin monomer and dimer structures with respect to the opioid receptor multiplicity [4,5,8,11,16,17].

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