Energy-Conformational Studies of β-Endorphins: Identification of Plausible Folded Conformers

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Abstract

β-Endorphins are 31 amino acid endogenous opioid peptides with high receptor affinity and antinociceptive acvitity. Because of their importance as neurohormones and the significant experimental effort that has been made to understand their structure activity profiles, we have begun to develop procedures that could be useful first to identify low-energy conformers of β-endorphins and ultimately their bioactive form. In the initial studies reported here, we have identified plausible initial structures of the full peptide by calculating and comparing the conformational preference of all possible extended tetrapeptide fragments of β-endorphin starting from each of the first 28 residues. Comparisons of fragment energies suggested two types of compact folded β-endorphin conformers were plausible: a helix–turn–helix and an antiparallel β-sheet conformer. These structures, as well as an extended α-helical and β-strand conformer, were assembled and total geometry optimization performed using the empirical-energy-based program AMBER. The results yield an α-helical structure as the lowest energy form consistent with recently reported NMR studies of β-endorphin. The two more compact folded structures obtained, however, are reasonable starting conformations for further planned molecular dynamics simulation studies and could yield competing low-energy structures as candidates for the bioactive form of these peptides.

Introduction

β-Endorphins, 31 amino acid fragments of a larger prohormone, are potent endogenous opioid peptides with high receptor affinity and antinociceptive activity [1]. That the sequence of β-endorphins is remarkably conserved across a variety of species is an indication that more than just the amino terminal met-enkaphalin-like portion of the peptides is important for activity. Since its discovery, about one hundred different analogs of β-endorphins have been synthesized in an attempt to determine the importance of individual residues and regions to the affinity and activity of the peptide [1–3]. These extensive structure-activity studies include replacement, omission, and addition of residues and incorporation of disulfide bridges [1–3]. In another approach, variations in residues in the 13–31 regions were made based on the hypothesis [4] that all that is required for opioid activity in β-endorphins is that this region form an amphiphilic helical structure. While the resulting analogs have shown binding affinity ranging from less than micromolar to nanomolar, and varying efficacies as analgesics, the fundamental stereoelectronic properties that determine these variations have not yet been identified.

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This lack is not surprising since the β-endorphins belong to a class of intermediate size bioactive peptides for which characterization of conformation profiles is most difficult. Such peptides pose discouraging difficulties for each of the three new disciplines: x-ray crystal structure determination, NMR studies, and theoretical energy-conformational studies, that in principle could be most useful for such studies. They are, in general, difficult to crystallize, have conformational flexibilities at room temperature, and have many possible stable structures, i.e., many local conformational minima of varying relative energies.

Recently, an NMR study of β-endorphin has been published [5]. While conformational flexibility in water did not allow analysis of the spectra, dilution with methanol did. The extensive analysis made of the NMR results, in water-methanol solution, including NOE spectra, were consistent with a predominantly α-helical structure. The NMR data do not preclude, however, a possible turn near residues 10–15. However, these studies are only a first step in addressing the question of the bioactive conformers of β-endorphins, i.e., the form in which they bind to opioid receptors. There are undoubtedly a number of lower-energy candidate structures and the environment at the opioid receptor binding site is most likely devoid of bulk solvent.

The two more formidable obstacles to energy conformation studies of peptides of the size of β-endorphin, are the existence of large numbers of stable conformers, i.e., the multiminimum problem, and the practical difficulties of constructing conformers which are good initial approximations to these minima. Human β-endorphin, for example, has 184 nonhydrogen torsion angles which render impractical the search strategies routinely used for smaller peptides such as nested rotations and "buildup" from low energy conformers of single amino acids. Thus entirely different procedures must be developed to search conformational space of these peptides for low-energy conformers.

The most common approach used to relate amino acid sequence to secondary structure in large peptides is based on statistical analysis of x-ray structure data of proteins [6]. By contrast, in the work reported here, a novel search strategy, based on comparisons of calculated optimized energies of sequential peptide fragments was developed to help identify plausible secondary structure regions for β-endorphin. These fragments were then used as guides to fold the peptide into a small number of qualitatively different conformations, i.e., into a set of tertiary structures which were then subjected to complete geometry optimizations.

The results obtained thus far indicate that the search strategy developed could be useful to construct initial conformers of bioactive peptides in general and to address aspects of the protein folding problem.

Methods and Procedures

As a guide to construction of plausible conformers of β-endorphin, 28 overlapping elongated and derivatized tetrapeptide fragments were constructed of the form: CH_3CONH–ala-(res_i-res_{i+3})ala–$CONHCH_3$, $i = 1$–28. Each of the 28 fragments were constructed in 6 idealized backbone conformations corresponding to an α-helix, a β-strand, and four β-turns; I, I′, II, and II′. The tetramers were extended to hexam-

ers by adding an alanine on each side to allow even-handed comparisons of the energies of these various secondary structures since a minimum of 6-, 5-, and 4-contiguous residues are required for favorable H-bonding in α-helical, β-strand and β-turn fragments respectively. The N-terminal and C-terminal ends were appropriately derivatized by *N*-acetyl groups and carboxy *N*-methyl to more realistically mimic the conformational behavior of the segment as part of the larger peptide chain. Facile construction of these fragments was possible using the capabilities of an interactive structure generating program called MOLECULE, described elsewhere [7], which has a library of single amino acid structures and the ability to automatically generate peptides of a chosen backbone conformation with extended side chain torsion angles. For fragments 10, 11, 12, and 13, which include the proline residue 13, two optimized proline ring geometries called PROu and PROd were used. In fragment 11, in which Pro13 is the second residue in the β-turn, only turn types I and II' are possible. For fragment 12, in which Pro13 is the first residue in the turn, only turn types II and II are possible. All of the initial structures generated for the 28 fragments were optimized in two steps, side chain angles only and then full torsion angle optimization using a quasi-Newton–Raphson energy minimization program called PEP that was developed in our laboratory. The five-term empirical energy expression in the program called ECCEP [8] formed the basis of this optimization. It contains contributions from electrostatic, hydrogen-bonded, dispersion, repulsion, and torsion angle potentials, and is described in detail elsewhere [8, 9].

In a buildup procedure similar to that used by Scheraga and co-workers [10], plausible folded structures of β-endorphin were constructed by linking energy optimized fragments corresponding to different types of secondary structures. This process was not automatic, but involved extensive use of graphics capabilities, and distance optimization to obtain interfragment side-chain conformers which eliminated major steric repulsions.

A set of 5 initial conformers generated for β-endorphin were energy optimized using the empirical energy expression contained in the program AMBER [11] described in detail elsewhere. This program allows total geometry optimization and contains a 7-term energy expression including bond angle and bond length variations in addition to the torsion angle variation and four other types of terms similar to those in the ECCEP potential. In these calculations, all atoms were explicitly included; with a nonbonding atom distance cutoff of 10 Å. A distance-dependent dielectric, $\varepsilon = r$, was used. In this program, the polar side chain residues are assumed to be ionized. To achieve charge neutrality, salt bridges were formed between oppositely charged nearby amino acid side chains and the remainder of the charged residues (Lys) were neutralized by addition of negative counterions with Van Der Waal's radii of 3 Å. This procedure resulted in salt bridge formation in the antiparallel β-sheet between Lys29–Glu31 and Lys19–Glu8 and counterions at Lys9, 24, and 28. In the β-strand, salt bridges were formed between Glu8–Lys9; Lys29–Glu31, and counterions were placed at Lys19, 24, and 28. In the α-helical structure, salt bridges were formed between Glu8–Ly9, Lys24–Glu31, and counterions placed at Lys19, 28, and 25. Finally, in the turn–helix–turn–helix–turn structure in the left-handed helix, salt bridges were formed between Gly8–Ly9, Lys28–Glu31, and counterions were placed

at Lys19, 24, and 29; while in the right-handed helix salt bridges were formed between Glu8–Lys9, Lys29–Glu31, and counterions placed at Lys19, 24, and 28.

Results and Discussion

The optimized energies of the 28 extended tetrameric fragments of β-endorphin in the different backbone conformations are summarized in Table I relative to that of the alpha helical form. As shown in this table, a β-turn conformation is preferred for the enkephalin portion of the peptide. These results are consistent with our own [12] and other previously reported energy-conformation studies of both tetra and pentapeptides and NMR studies of metenkephalin [13, 14] in which evidence for both a gly–gly and a gly–phe β-bends have been reported. For fragments 10, 11, 12, and 13, which include the proline 13 residue, one proline ring geometry, Pro–D, definitely favored an optimized alpha helical structure with some deviations from ideal backbone angles. No turns were possible with this proline ring geometry. For the other proline ring geometry however, β1-type turns, again deviating from ideal, were favored for fragments 10, 11, and 12, identifying a crucial turn region in the middle of the β-endorphin sequence which could allow a highly folded structure. A third turn region involving C-terminal residues 28–31 was also suggested by these results, though an α-helix is somewhat favored.

In addition to identification of possible turn regions, the results suggest that the remaining contiguous region of the peptide, i.e., residues 5–10, and residues 15–27 are in modified alpha helical rather than β-strand conformations. Thus the most plausible folded conformer of β-endorphins, is predicted to be of the helix–turn–helix pattern with possible additional turns at both the N-terminal and C-terminal end and an internal turn beginning at residue 11 or 12. Two highly folded structures of this type were constructed, one with a right-handed and the other a left-handed helix, to explore the effect of the sense of the helical portions of the conformation and energy. A totally helical structure was also constructed as a possible variation of these compact folded structures.

While β-strands, with only a few exceptions, were not energetically favorable secondary structures for fragments, nevertheless, the possibility existed that a fully extended β-strand could be a low-energy conformer of the full peptide since β-strands are likely to have less hindered interfragment interactions than α-helices. It is also possible that a highly folded β-sheet structure, which can be formed from antiparallel β-strands by interstrand H-bonding could be energetically favorable if the energy gained from interstrand interactions outweighs the favorable α-helical versus β-strand contiguous domain energies. These possibilities were explored by including among the initial conformers to be optimized an extended β-strand and an antiparallel β-sheet conformer with turns at residues 1–4, 11–14, and 23–26.

The results of total geometry optimization of the five types of conformers of β-endorphins using the AMBER program are summarized in Table II. These optimized conformations are shown in Figure 1; their backbone conformations in Figure 2, and the corresponding backbone torsion angles are listed in Tables III–VI. As seen in Tables III and IV, fairly regular α-helical [Fig. 1(a)], and β-strand [Fig. 1(b)] structures are retained after optimization. This is not true for the more folded struc-

TABLE I. Optimized energies[a] of β_h-endorphin fragments CH_3CONH–ala[Res_i–$Res_{(i+3)}$]-ala–$CONHCH_3$.

i	Fragment	$\Delta E_{\beta s}$	$\Delta\beta_T$
1	Tyr	3	– 4(II')[b]
2	Gly	7	–11(I)
3	Gly	21	17(II)
4	Phe	20	19(I)
5	Met	17	11(I)
6	Thr	14	8(I')
7	Ser	16	5(I)
8	Glu	17	– 4(I)
9	Lys	23	25(II)
10	SerU[c]	–1	– 9(I)
	SerD	2	HIND[f]
11	GlnU[c,e]	–6	– 2(I)
	GlnD	1	HIND
12	ThrU[c,e]	0	– 1(I)
	ThrD	2	HIND
13	ProU[c]	7	4(I)
	ProD	7	HIND
14	Leu	21	23(I)[b]
15	Val	14	15(II)
16	Thr	12	9(I)
17	Leu	15	13(I)
18	Phe	14	9(II)
19	Lys	16	9(I)
20	Asn	15	17(I)
21	Ala	16	14(I)
22	Ile	16	16(II)
23	Ile	14	12(I)
24	Lys	14	11(II)
25	Asn	14	9(II)
26	Ala	15	7(I)
27	Tyr	15	9(I)
28	Lys-[g]	3	2(II)

[a] ΔE in kcal/mol relative to energy of α-helix.

[b] ( ) = optimized turn with lowest energy of I, I', II, II'.

[c] Two proline ring geometries called U and D were used in these fragments.

[d] Only β-turn types I and II' possible with Pro13.

[e] Only β-turn types I and II possible with Pro13.

[f] Turns sterically hindered.

[g] The C-terminal fragment is Lys–Lys–Gly–Gln.

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