Relationship of the Crystal and Molecular Structure of Leucine-enkephalin Trihydrate to that of Morphine

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The intramolecular orthogonal contact of the Tyr and Phe aromatic rings in the solid-state folded conformation of leucine-enkephalin trihydrate confers on this peptide some resemblance to the morphine molecule.

Orginally isolated by Hughes *et al.*,[1] leucine-enkephalin (Tyr^1-Gly^2 -Gly^3-Phe^4-Leu^5) is an endogenous pentapeptide, the activity of which resembles that of morphine. Many experimental and theoretical analyses have shown that the leucine-enkephalin molecule is flexible, and capable of adopting various folded and extended conformations; this

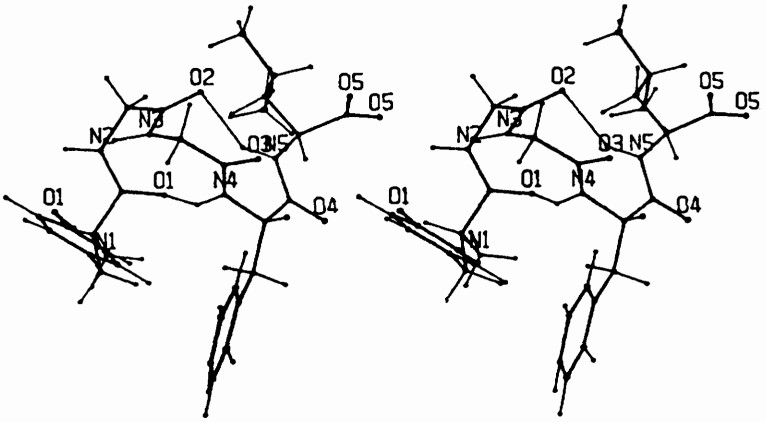

Figure 1. Stereoviews of the leucine-enkephalin molecule in the trihydrate, showing the two β-folded Gly^2-Gly^3 and Gly^3-Phe^4 sequences, and the almost orthogonal orientation of the aromatic rings. The oxygen and nitrogen atoms are indexed according to their position in the chain.

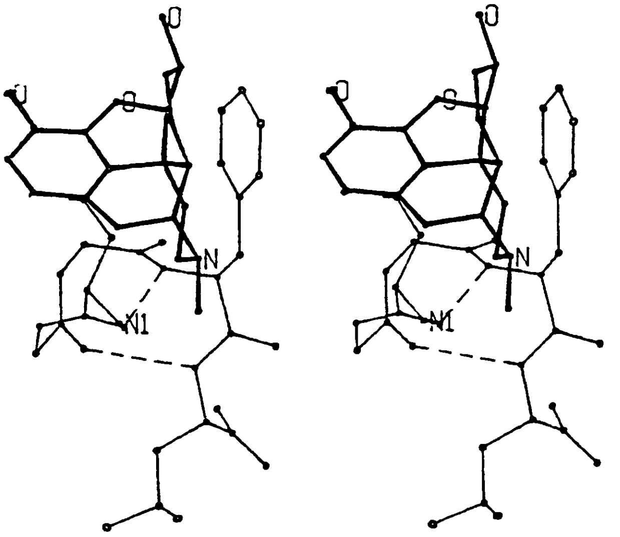

Figure 2. Comparison of the leucine-enkephalin (thin lines) and morphine (heavy lines) structures with superimposed Tyr and tyramine rings; hydrogen atoms have been omitted for clarity.

could explain the fact that it is recognized by both μ- and δ-receptor sites.[2] Some μ- or δ-specific analogues of leucine-enkephalin have been obtained by the introduction of conformational constraints,[3] but attempts to correlate closely any one of the possible conformations of leucine-enkephalin with that of the rigid morphine molecule have failed hitherto.

Four different crystal structures have been reported for leucine-enkaphalin,[4—9] and one for the [Br-4′-Phe^4]-analogue.[10] They have revealed the existence of both a βI′-folded (Gly^2-Gly^3) conformation[4,5,10] and an extended one.[6—9] A somewhat elongated conformation has also been established for leucine-enkephalin when bound to the Mcg light chain dimer in the cleft in the protein.[11] In these structures, the Tyr^1 and Phe^4 aromatic rings have no intramolecular contact, whereas their probable counterparts in morphine (the tyramine and cyclohexenyl rings) are directly linked to one another.

We have discovered a solid-state folded conformation of leucine-enkephalin trihydrate which corresponds much more closely to that of morphine.[12] Furthermore, as this trihydrate was obtained from aqueous solution, this newly-discovered conformation could well be related to the biologically active one.

The conformation of leucine-enkephalin trihydrate (Figure 1)[†] consists of two fused βIII and βI-bends in a partial 3_{10}-helix-like structure with two intramolecular hydrogen bonds [N(Phe^4) $\cdots$ O(Tyr^1) = 2.98 Å; N(Leu^5) $\cdots$ O(Gly^2) = 3.11 Å]. Each molecule is in direct contact with four neighbouring ones through eight hydrogen bonds, and interacts with eight water molecules. The NH$_3^+$ and CO$_2^-$ groups are involved in an intermolecular ionic bridge (N $\cdots$ O 2.66 Å).

There is a quasi-orthogonal contact of the Tyr^1 and Phe^4 aromatic rings, with an angle of 100° between their planes and a distance of 4.99 Å between their centres. This disposition of aromatic rings is quite frequent in crystals,[15,16] but it confers to the Tyr^1 and Phe^4 rings of leucine-enkephalin a relative disposition resembling that of the tyramine and cyclohexenyl rings in morphine (Figure 2). However, owing to the inverse configuration of the tyramine moieties,[17] the orientation of the N$^+$ atom with respect to the phenol ring is not the same (Figure 2): the Tyr^1-χ^1 torsion angle in leucine-enkephalin is 178° instead of 95° (homologous angle in morphine).

This crystal structure confirms the flexibility of the leucine-enkephalin molecule: three different solid-state conformations are now known. The large number of water molecules surrounding the peptide in the trihydrate is noteworthy, as it suggests that this folded conformation could be partly present in aqueous solution.

We thank Professor G. Ourisson for discussions. This work was supported by CEC (ST2J-0184), the CNRS, and the Greek Foundation Leonidas Zervas (scholarship to N. B.).

Received, 14th March 1988; Com. 8/01007B

[†] Crystals of leucine-enkephalin trihydrate were grown from aqueous solution: space group $P2_12_12_1$ with unit cell dimensions a = 10.967, b = 13.191, c = 21.350 Å, Z = 4. X-Ray intensity data, collected at room temperature with a Nonius CAD-4 four-circle diffractometer with Cu-K_α radiation, totalled 3418 independent reflections, of which 2551 obeyed the $I > 1.5\,\sigma(I)$ critrion. The structure was solved by a combination of direct methods with programs MULTAN-80[13] and SHELXS[14] to a final refinement factor $R = 0.047$ using anisotropic thermal parameters for non-hydrogen atoms, and isotropic thermal parameters for hydrogen atoms. Three independent water molecules were found, and all the hydrogen atoms appeared on E-map differences. Atomic co-ordinates bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Notice to Authors, Issue No. 1.

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