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# TOPOGRAPHICAL REQUIREMENTS FOR DELTA OPIOID LIGANDS: THE SYNTHESIS AND BIOLOGICAL PROPERTIES OF A CYCLIC ANALOGUE OF DELTORPHIN I

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**Summary:** A cyclic constrained analogue of deltorphin, [D-Cys$^2$,Cys$^5$]DT I has been proposed on the basis of an energetically favored model of a delta-selective conformation for deltorphin. The biological properties of this synthetic analogue demonstrate that incorporation of a disulfide bridge into Deltorphin I does not affect its high affinity for delta opioid receptors. The analogue shows low receptor selectivity as a result of a large increase in its affinity for mu receptors when compared to the parent deltorphin.

## Introduction

The deltorphins are two natural linear heptapeptides that were isolated from the skin of a *Phyllomedusa bicolor* frog (1). These peptides possess high affinity and selectivity for the delta opioid receptor (2), and potent antinociceptive effects (3). The presence of an aspartic acid residue in position 4 of the peptide chain of deltorphin I and the glutamic acid residue in the same position of deltorphin II is the only structural difference between these two peptides. Their sequences are depicted in Fig. 1 along with the delta-selective dermenkephalin (DRE) and the mu-selective dermorphin.

| | |
|---|---|
| **Deltorphin I: (DT I)** | **Tyr-D-Ala-Phe-Asp-Val-Val-Gly-$NH_2$** |
| **Deltorphin II: (DT II)** | **Tyr-D-Ala-Phe-Glu-Val-Val-Gly-$NH_2$** |
| **[D-Cys$^2$,Cys$^5$]Deltorphin I:** | **Tyr-D-Cys-Phe-Asp-Cys-Val-Gly-$NH_2$** |
| **Dermenkephalin: (DRE)** | **Tyr-D-Met-Phe-His-Leu-Met-Asp-$NH_2$** |
| **Dermorphin:** | **Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-$NH_2$** |

**Figure 1.** Amino acid sequences of deltorphins, their cyclic analogue, dermenkephalin, and dermorphin

Structure-activity studies on C-terminal truncated analogues of dermenkephalin (4) demonstrate that the C-terminal region is necessary for delta receptor potency and selectivity. Schiller et al. (5) suggested that a C-terminal hydrophobic tripeptide is necessary for the high delta selectivity of deltorphin analogues. SAR analysis of deltorphin analogues synthesized by Lazarus, Salvadori, et al. (6,7) pointed out that the location

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of the charged groups relative to the hydrophobic residues in the address domain of the peptide could be a critical determinant for delta receptor affinity and selectivity.

Topographical examination and comparison of opiate alkaloids and opioid peptides suggested specific topographical properties for recognition of delta type opioid receptors (8,9). We proposed that the deltorphin "delta active" conformation forms a well defined amphipilic structure (9), with similar properties in some respects to that for [D-Pen$^2$, D-Pen$^5$]enkephalin (10). A hydrophilic surface of deltorphin from the N-terminal amino group through the carboxyl of Asp$^4$ to the C-terminal amide is surrounded by the lipophilic residues of amino acids in position 2, 3, 5 and 6. Similar structural requirements were postulated for another delta selective peptide, dermenkephalin (11). Energy calculation analysis of delta selective dermenkephalin and mu selective dermorphin, as well as their hybrid peptides, suggests that the C-terminal "address" strongly influences the conformation of the N-terminal "message" (12).

The linear deltorphins appear to have flexible structures which complicates SAR analysis. An indirect approach to obtaining information on the receptor-bound bioactive conformations of opioid ligands has been to utilize constrained or conformationally restricted peptides (13,14,15). In the structural models of deltorphins (8) and dermenkephalin (11,12) constructed by different approaches, residues 2 and 5 often are in close proximity. Therefore we have synthesized and biologically characterized a constrained analogue of deltorphin I in which amino acid residues in position 2 and 5 are joined by a S-S bridge (Figure 1).

## Materials and Methods

**Molecular Modelling.** Energy calculations were performed for deltorphin I (DT) in the same manner as used in calculations for dermenkephalin in (16). The same ECEPP potential field (17,18) was used for calculations. Finally 61 conformers of DT differing in peptide backbone structure and/or in rotamers of the Tyr and Phe side chains were found to satisfy the low energy criterion $\Delta E = E - Emin < 7$ kcal/mol. These final conformers of DT were considered for subsequent energy calculations carried out for [D-Cys$^2$, Cys$^5$]DT I. Only 20 conformers out of 61 met the requirement of $\Delta E < 10$ kcal/mol for [D-Cys$^2$, Cys$^5$]DT I.

**Peptide synthesis.** The synthesis of the [D-Cys$^2$, Cys$^5$]deltorphin was performed by solid phase synthetic techniques with procedures similar to those previously reported (19) using a 9500 Milligen peptide synthesizer. Purification of the peptide was performed by preparative reverse phase high pressure liquid chromatography (HPXL, Rainin, MA). The purity of the final product was assessed by analytical reverse phase liquid chromatography (RP-HPLC): capacity factor (k') = 6.1 for the following systems: Vydac 218TP104C18 reverse phase (RP) column (25x0.46 cm) with 0.1% trifluoroacetic acid/acetonitrile gradient (from 100/0 v/v to 60/40 v/v in 40 min) at a flow rate of 1.0 ml/min. The peptide was monitored at $\lambda$=230, 254, and 280 nm). Thin layer chromatography (TLC) was performed in the systems:Rf(**I**)=0.49 [butanol/acetic acid/water (4:1:1)]; Rf(**II**)=0.80 [butanol/acetic acid/pyridine/water (13:3:12:10)]; Rf(**III**)=0.79

Table I. Torsion angles of low energy conformers of DT I and [D-Cys$^2$, Cys$^5$]DT I similar to proposed model of delta-receptor bound conformers for DRE.

| | | DRE | DT I | [D-Cys$^2$,Cys$^5$]DT I | |
|---|---|---|---|---|---|
| (1) Tyr | ψ | 139 | 157 | 159 | 160 |
| | χ | -70 | -60 | -62 | -60 |
| (2) DMet/DAla/DCys | ϕ | 97 | 149 | 149 | 151 |
| | ψ | 24 | -59 | -59 | -60 |
| (3) Phe | ϕ | -98 | -73 | -65 | -65 |
| | ψ | -37 | -29 | -33 | -35 |
| | $\chi_1$ | -176 | -178 | -178 | 179 |
| (4) His/Asp | ϕ | -95 | -77 | -76 | -74 |
| | ψ | - 4 | -29 | -23 | -24 |
| (5) Leu/Val/Cys | ϕ | 67 | -77 | -81 | -79 |
| | ψ | 20 | -37 | -36 | -36 |
| (6) Met/Val | ϕ | -160 | -80 | -80 | -76 |
| | ψ | 32 | -30 | -27 | -35 |
| (7) Asp/Gly | ϕ | -89 | 82 | 84 | -73 |
| | ψ | 149 | 46 | 33 | -58 |

[2-propanol/ammonia/water (4:1:1)]; Rf(IV)=0.37 [butanol/acetic acid/ethyl acetate/water (5:1:3:1)]. Amino acid analysis: Asp:Cys:Gly:Phe:Tyr:Val 1.03:1.51:1.00:0.99:0.94:1.04 and FAB-MS: $[M+H^+]_{obs}$ = 804 ($[M+H^+]_{calc}$ = 804).

**Bioassays.** The opioid receptor binding affinity was tested in rat brain preparation by the method of replacing radiolabelled delta ([$^3$H][p-Cl-Phe$^4$]DPDPE) and mu ([$^3$H]CTOP) selective ligands (19). The receptor potency and selectivity were evaluated *in vitro* by measuring the ability of the peptides to inhibit electrically evoked contractions of the myenteric plexus longitudinal muscle preparation of the guinea pig ileum (GPI) (assay representative for the mu receptor) or of the mouse vas deferens (MVD) (assay for the delta receptor) (19).

## Results and Discussion

The short linear opioid peptides are very flexible and the methods which have been applied to obtain low energy conformation up to now have resulted in a number of different models. One of our models for the delta-receptor bound conformer of dermenkephalin and DPDPE was based on the similar spacial

Table II. Pharmacological characteristic of deltorphin I and its cyclo analogue.

| | | DT I | [D-Cys$^2$,Cys$^5$]DT I |
|---|---|---|---|
| Receptor binding | μ [$^3$H][CTOP] | 2,140±700 | 5.15±0.55 |
| $IC_{50}$ (nM) | δ [$^3$H][p-Cl-Phe$^4$]DPDPE | 0.60±0.03 | 0.87±0.04 |
| Smooth muscle | GPI | 2,890±250 | 2.98±0.37 |
| $IC_{50}$ (nM) | MVD | 0.11±0.05 | 0.23±0.04 |

arrangements of their α- amino groups, the Cγ- and the Cζ-atoms of the Tyr and Phe side chains, and the Cα-atom of the residue in position 2 for low energy conformers of these delta-selective peptides (12,16). Comparison of the low energy conformers of DT to the previous model reveals a pronounced similarity of one of the low energy structures of DT to the proposed conformer for DRE with rms<1.0Å for the atomic centers mentioned above, using the algorithm described in (20). This similarity can be seen in Fig. 2 and in Table I. It is also seen from Fig. 2 that the side chains of the D-Ala(2) and Val(5) residues are fairly close to each other in the low energy conformer of the linear DRE which suggests the possibility of linking the

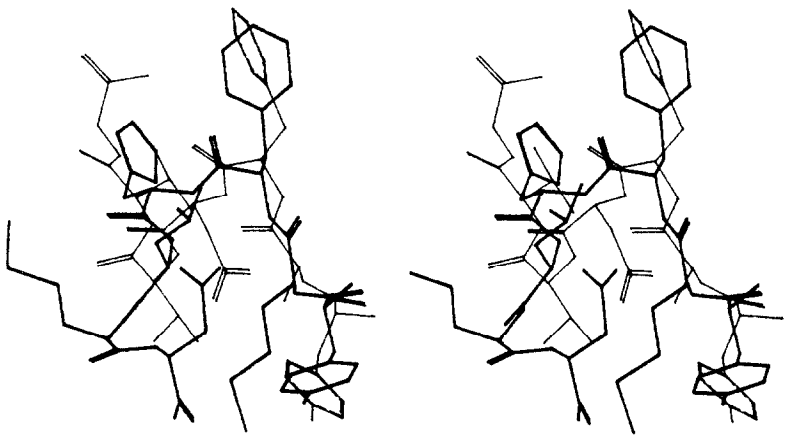

**Figure 2.** Geometrical comparison of low energy conformer of DT I (thin line) to similar low energy conformer of DRE (bold line). All hydrogen atoms are omitted.

positions 2 and 5 in DT by a disulfide bridge while preserving the chirality of the corresponding Cα-atoms. Energy calculations for [D-Cys$^2$, Cys$^5$]DT analogue further confirmed this suggestion showing that two of its low energy conformers are very close (rms<0.25 Å) to the DT conformer described in Table I.

The [D-Cys$^2$,Cys$^5$]DT analogue is the most potent cyclic analogue of deltorphin described so far (5). These findings provide supporting evidence for the model for delta-receptor bound conformation proposed in (12,16). It also can explain the significant loss of activity for the cyclic DT analogues previously reported, obtained by linking the positions 2 and 4, and 2 and 7 in the peptide chain (5). The cyclic analogue lost its receptor selectivity as a result of a large increase in affinity for mu-receptors. This is interesting in that whereas conformational restriction often increases receptor selectivity if the restriction is preferred by one receptor relative to another. In this case, the restriction is compatible with δ receptor interaction since little change in binding or biological activity occurs in the δ receptor assay. However, there is a large increase in μ binding and biological activity indicating that the constraint enhances μ receptor recognition. The results suggest that the cyclic analogue posseses low energy conformations different from those in the linear deltorphin. This also supports the suggestion (12,16) that the N-terminal "message" in DT analogues could serve as a delta or mu-"address" depending on the ability of functionally important groups like the α-$NH_3$ amino group and the aromatic moieties of Tyr and Phe residues to acquire the proper topology. This suggestion does not exclude the possible participation of the C-terminal fragment of the molecule in direct interaction with mu- or delta-receptors (21). It is noteworthy also that several low energy conformers of DT appeared to be similar (rms<1.0 Å) to some low energy structures of the mu-selective dermorphin, reported for this peptide previously (12,16).

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