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Conformation-activity relationships of *cyclo*-constrained μ/δ opioid agonists derived from the N-terminal tetrapeptide segment of dermorphin/deltorphan

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Introduction

The N-terminal tetrapeptide segments of dermorphin (Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH₂) and deltorphan (Tyr-D-Ala-Phe-Asp/Glu-Val-Val-Gly-NH₂) are agonists at the opioid receptors μ and δ , respectively. (D-Arg²,Lys⁴)-dermorphin(1–4) amide (Tyr-D-Arg-Phe-Lys-NH₂, DALDA) and [Dmt¹]DALDA (Dmt is 2',6'-dimethyl-tyrosine) are among the most potent and selective μ -agonists reported to date, both *in vitro* (the latter one having picomolar μ receptor affinity) and *in vivo*. In this communication, conformation-activity studies of cyclic tetrapeptide analogs of dermorphin/deltorphan are presented and discussed. They include the peptide Tyr-c[D-Cys-Phe-Cys]NH₂, constrained via an S^γS^γ disulfide between Cys² and Cys⁴, and its dicarba analogs, the C^γC^γ-saturated and -olefinic ones. They are potent nonselective or moderately μ -selective opioid agonists *in vitro* [1] (Table 1). With a major structural constraint imposed by the 11-membered ring spanning residues 2–4, they are expected to manifest well-defined conformations of the backbone. Given the small size and confirmed bioactivity of the peptides [1], there is a good chance that their conformations determined in solution would correspond to the receptor-bound ones. We used 2D-NMR in H₂O/D₂O supported with molecular dynamics (MD) in this study.

Results and Discussion

NMR spectra were recorded in H₂O/D₂O solution at 305 K with peptide concentrations about 3 mM, on a Varian Unity 500 Plus spectrometer with sodium 3-trimethylsilyltetraduteriumpropionate, TSP, as internal standard. The assignment of proton chemical shifts was accomplished using 2D proton spectra TOCSY (80 ms), ROESY (150 ms), and DQF-COSY. Due to problems with solubility, productive sets of 2D spectra with usable cross-peaks could be collected only for analogs **1** and **3**. Data were processed using ACDLabs [2] and XEASY [3] software. The ³JH_{NαH} coupling constants were obtained from the DQF-COSY spectra. The structures of **1** and **3** were refined using 2.9 ns productive MD runs, utilizing the Time-Averaged Constraints procedure (TAV-MD) dedicated to structure determinations from NMR of small flexible peptides [4]. Prior to the productive MD, routine operations, including parameterization of new moieties [5], TAV-constrained model-building, energy minimization, thermalization, etc., were performed. MD simulations were carried out using the AMBER, ver. 8.0 software [5]. Each set of conformations from the TAV-MD trajectories was clustered into 5 to 6 families of conformations. Analogs **2** and **4** were submitted to a similar 2.9 ns MD, non-restrained by NMR data, and with starting structures taken at random. Remarkably, the structures of these two essentially diverse MDs converged into two clearly

different types of structures: analogs **1** and **4** merged into one of these and analogs **2** and **3** into the other, as shown in Figure 1.

The aromatic rings of Tyr¹ and Phe³ in **1** and **4** are on the same side of the heterodetic ring at an interacting distance, while in **2** and **3** the two rings are located far apart from one another. The remarkable merge of analogs **1** and **4** into one and analogs **2** and **3** into the other structural type correlates with the higher μ agonist activities and μ vs. δ selectivities of the former as compared to the latter. Thus, it is tempting to hypothesize that the enhanced μ agonist potency and μ receptor selectivity of analogs **1** and **4** may be attributed to the location of the two aromatic rings in close proximity and engaged in a stacking interaction. Of course, the exocyclic Tyr¹ residue and the Phe³ side chain still enjoy considerable orientational freedom and it is possible that they may change their orientation upon binding to the receptor.

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Fig. 1.
Left: 103 refined structures of **1**; Right: 109 structures of **3**.

Table 1The analogs studied and their biological activities ^a.

$\begin{array}{c} \text{Tyr-D-Bbn}^b\text{-Phe-Bbn}^b\text{-NH}_2 \\ \qquad \qquad \qquad \\ \text{CH}_2\text{-X-X-CH}_2 \end{array}$	No	GPI (μ-selective)
		EC ₅₀ [nM]
– S – S –	1	64.7 ± 11.9
–HC=CH-(<i>cis</i>)	2	162 ± 17
–HC=CH-(<i>trans</i>)	3	436 ± 97
–CH ₂ –CH ₂ –	4	76.5 ± 5.5

^aReference [1]^bBbn – backbone.