Peptide Structure

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Resonance Energy Transfer Relates the Gas-Phase Structure and Pharmacological Activity of Opioid Peptides

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Abstract: *Enkephalins are efficient pain-relief drugs that bind to transmembrane opioid receptors. One key structural parameter that governs the pharmacological activity of these opioid peptides and is typically determined from condensed-phase structures is the distance between the aromatic rings of their Tyr and Phe residues. We use resonance energy transfer, detected by a combination of cold ion spectroscopy and mass spectrometry, to estimate the Tyr–Phe spacing for enkephalins in the gas phase. In contrast to the condensed-phase structures, these distances appear to differ substantially in enkephalins with different pharmacological efficiencies, suggesting that gas-phase structures might be a better pharmacophoric metric for ligand peptides.*

The biological functionality of peptide ligands is governed by the three-dimensional (3D) geometry they adopt at the binding sites of targeted proteins. These structures, which are typically determined in the condensed phase by NMR spectroscopy or X-ray crystallography, are often used to predict the pharmacological efficiency of candidate drugs. However, the prediction may become ambiguous when peptides lack rigidity and can undergo significant conformational changes upon interaction with receptors in vivo, particularly because of the hydrophobic environment of their binding pockets.[1] Isolation of biomolecules in the gas phase removes intermolecular interactions, creating a water-free environment. Thus, structural features measured in the gas-phase peptides may complement the condensed-phase data for modeling ligand–receptor interactions. Opioid peptides, which bind to transmembrane opioid receptors, have been used as efficient drugs for pain treatment for decades.[2] They all contain two aromatic rings, the spacing between which is considered one of the key structural parameters that determine the pharmacology of these drugs.[1a,3] Herein, we measure conformer-specific efficiencies of resonance energy transfer (RET) in three similar opioid peptides, leu-enkephalin (YGGFL) and two stereoisomers of [Ala^2]-leucine enkephalin (YAGFL), isolated in the gas phase. We use this data, the known gas-phase geometry of YGGFL, and our low-level structural calculations to estimate the Tyr–Phe ring spacing in the isomers and compare these distances with the pharmacological activity of the three ligands.

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Although RET in solution is a proven approach that provides an estimate of the average interchromophore distances in biomolecules,[4] researchers have only recently demonstrated the use of fluorescence[5] and, more recently, photodissociation mass spectrometry[6] for detecting RET in gas-phase ions labeled with large chromophores. Such labeling would not be appropriate for enkephalins, which experience drastic pharmacology changes with only slight structural modifications.[3a,7] Instead, the Tyr and Phe aromatic residues naturally contained in these peptides can be involved in RET.[8] We employ UV fragmentation spectroscopy–mass spectrometry (UV–MS) of ultracold ions[9] to measure the conformer-specific RET efficiencies. Recording the entire fragmentation mass spectrum at each UV wavenumber allows for more accurate measurements, and the high spectral resolution offered by cooling makes them conformer selective.

Our modified cold ion spectrometer has been described in detail elsewhere.[9,10] Briefly, we produce protonated gas-phase molecules directly from solution using nanoelectrospray ionization. The ions of interest are selected by a quadrupole mass filter and guided to a cold (6 K) octupole ion trap, where they are internally cooled by collisions with He to $T=$ 10 K.[10b] The cold ions are then fragmented by a tunable UV laser. Parent and fragment ions are detected simultaneously by an Exactive Orbitrap mass analyzer.

Figure 1 shows the UV photodissociation spectra of singly protonated Leu-, [L-Ala^2, L-Leu^5]-, and [D-Ala^2, D-Leu^5]-enkephalins (denoted below as *Enk*, *LL* and *DD*, respectively) generated by integrating the measured UV–MS spectra of the respective ions over the m/z of the most prominent b- and y- fragments (Figure S1 in the Supporting Information). The resemblance between the *Enk* and *LL* spectra suggests a likely structural similarity of these peptides. In contrast, the spectra of *DD* and *LL* differ strikingly in the position of the band origin and in the shape of the UV bands. This observation implies quite different 3D structures of the two isomers, as confirmed below by our RET measurements. The intensity in these spectra is mainly due to the strong absorption of Tyr. The peak at 37507.6 cm^{-1}, on top of the comparatively smooth absorption by Tyr in the *Enk* spectrum, has been assigned to the band origin of Phe. This assignment is based on the observed specific approximately 530 cm^{-1} vibrational progression (in-plane bending of the Phe ring)[11] and the characteristically narrow peaks in the UV spectra of Phe-containing cold peptides.[12] Similarly, we have assigned the band origins in the spectra of the two other peptides. The position of the Phe band origin is sensitive to the conformational states of peptides.[12b] Thus, the observed sharp Phe peaks very likely belong to single conformers. Figure 2

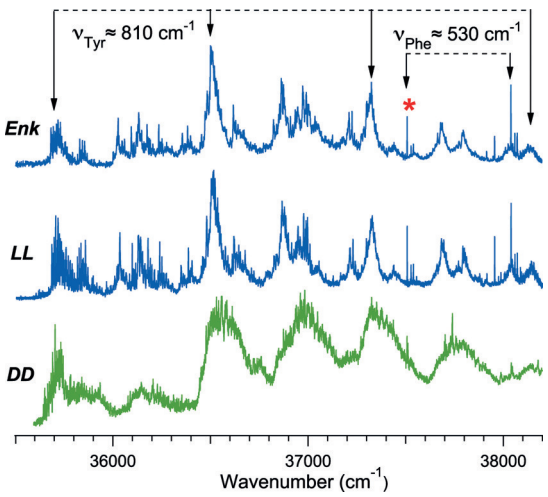

Figure 1. UV fragmentation spectra of the protonated *Enk*, *LL*, and *DD* enkephalins obtained by integrating the respective UV–MS spectra over the b- and y-fragments. The spectra are normalized to their maximum fragmentation yields for graphical clarity. The spectra of the *LL* and *DD* isomers are offset by 2.9 and 246.4 cm^{-1}, respectively, to align their Phe band origins (indicated by the asterisk). The two systems of arrows above the *Enk* spectrum indicate the vibrational progressions, specific to aromatic rings of Tyr and Phe.

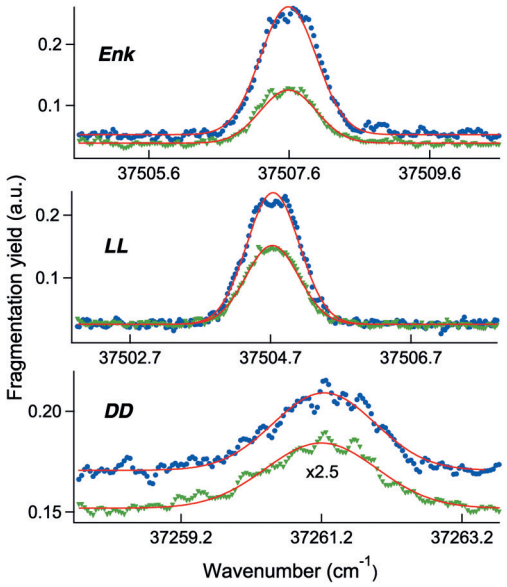

Figure 2. High-resolution UV fragmentation spectra of singly protonated *Enk*, *LL*, and *DD* enkephalins in the region of their Phe band origins. The spectra are obtained by integrating the respective UV–MS spectra over the Tyr-specific photofragments (triangles) and over the b- and y- fragments (dots) and normalized on the total number of ions and laser power. The peaks are fit by Gaussian functions (solid lines). The lowest trace is enlarged by a factor of 2.5.

provides pairs of expanded views of these peaks in the three peptides. One of the spectra in each pair was generated by integrating the respective high-resolution UV–MS spectrum over the fragments that correspond to the loss of the Tyr side chain (Figure S1). This channel is specific for the UV fragmentation of Tyr-containing peptides and is absent in a thermal-like dissociation.[13] The fact that UV excitation of Phe results in this specific fragmentation suggests the presence of RET from the Phe to the Tyr chromophore. With this assessment, the conformer-specific RET efficiency can be expressed as (see Supporting Information) [Eq. (1)]

$$\eta(\nu_0) = \frac{\beta_Y(\nu_0) - \beta_Y(\nu^*)}{\beta_Y(\nu^*)} \cdot \frac{\beta_\Sigma(\nu^*)}{\beta_\Sigma(\nu_0) - \beta_\Sigma(\nu^*)} \quad (1)$$

where $\beta_Y(\nu_0)$ and $\beta_\Sigma(\nu_0)$ are Tyr-specific and thermal-like fragmentation yields, respectively, at the Phe band origin, and $\beta_Y(\nu^*)$and $\beta_\Sigma(\nu^*)$ are the respective fragmentation yields arising exclusively from absorption by Tyr. The yields have been determined from Gaussian fits of the peaks in Figure 2 under the assumption that the smooth Tyr absorption remains constant across the peaks. The application of Equation (1) then yields $\eta_{Enk} = 0.57 \pm 0.02$, $\eta_{LL} = 0.60 \pm 0.02$, and $\eta_{DD} = 0.96 \pm 0.1$ for *Enk*, *LL*, and *DD*, respectively.

At large interchromophore distances, the RET efficiency is governed by the coupling of the electronic transition dipole moments (TDMs) of the two chromophores, and it is named Förster RET (FRET) after Förster, who first described this effect [Eq. (2)][4a]

$$\eta = \frac{1}{1 + \left(R/R_0\right)^6} \quad (2)$$

Here, R is distance between two chromophores and R_0 is Förster's radius [Eq. (3)]:

$$\left(R_0^6\right)_i = C \cdot \kappa_i^2 \quad (3)$$

where $i = Enk$, LL, or DD, κ is orientation factor of TDM vectors, and C is a factor that can be explicitly expressed through the spectroscopic properties of the chromophores.[4a] The largest uncertainty in calculating R_0 often arises from a lack of information regarding κ^2 which may span from 0 to 4 and is to be measured or calculated for each conformer. In contrast, the factor C should not change significantly among peptides of similar size, composition and with the same donor–acceptor pair, such that we can hold it constant for the three enkephalins studied herein. Under this assumption, Equations (2) and (3) yield [Eq. (4)]

$$\frac{R_i^6}{\kappa_i^2} \approx \frac{R_{Enk}^6 \eta_{Enk}(1-\eta_i)}{\kappa_{Enk}^2 \eta_i(1-\eta_{Enk})} \quad (4)$$

The gas-phase structure of the lowest energy conformer of *Enk*, which was recently solved and validated by cold ion IR spectroscopy,[14] has $R_{Enk} = 11.7$ Å and ${\kappa_{Enk}}^2 = 1.27$ (see Supporting Information Section 3). Considering these numbers and the determined above FRET efficiencies, Equation (4) yields $(1.7 \pm 0.2) \times 10^6$ Å^6 and 0–2.8×10^5 Å^6 for R^6/κ^2 in *LL* and *DD* isomers, respectively. We now use these values as constraints for selecting structures of the isomers from the pools of low-energy conformers, calculated at a computationally undemanding molecular mechanics (MM) level of theory (Supporting Information Section 3). This removes 94% of the calculated *LL* structures. Although only 50% of the struc-

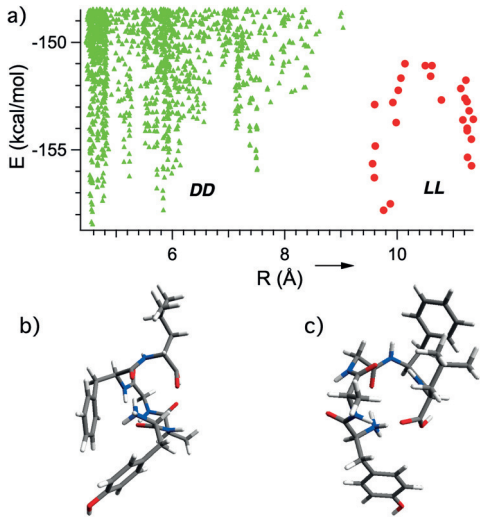

Figure 3. a) Potential energy versus Phe–Tyr distance in the remaining calculated structures, selected using the R^6/κ^2 parameters for the *DD* (triangles) and *LL* (dots) isomers. The calculated low-energy constrained structures of the b) *DD* and c) *LL* isomers.

tures have been removed for *DD*, this constraint reduces the maximum possible value of R_{DD} from about 13 to around 8 Å. Figure 3a shows plots of the potential energy versus R in the remaining structures. The distribution of *LL* structures exhibits two families with $R \approx 9.9$ and 11.2 Å, which yield the overall average distance $R_{LL} = 10.6 \pm 0.7$ Å. The distribution of the calculated *DD* structures exhibits three distinct conformational families with $R \approx 5$, 6 and 7.5 Å. The large number of conformers likely leads to broadening of the Tyr bands in the spectra of *DD*. Averaging over all of the *DD* structures in Figure 3a yields $R_{DD} = 5.7 \pm 1$ Å. At such short distances, the Dexter[4b] rather than the Förster approximation of RET may become more appropriate for the quantification of R. Regardless of the particular number, the interchromophore distance in *DD* is clearly significantly shorter than in *LL* and *Enk*. A comparison of the linewidths of the peaks in Figure 2 further supports this conclusion. The band origin of *DD* (Figure 2) is noticeably broader than that of *LL* (full width at half maximum height (FWHM) of 1.14 and 0.81 cm^{-1}, respectively). The broadening may originate from i) the larger rotational constants of *DD* and/or ii) from the shorter lifetime of the excited state of Phe in *DD* than in *LL*. The case (i) is consistent with the calculated rotational constants for the structures in Figure 3b and c. The case (ii) implies a higher rate of RET in *DD* than in *LL* and is consistent with the highest RET efficiency measured in *DD*.

Among the three peptides examined herein, the *DD* isomer, called DADLE, is by far the most potent and selective δ-opioid receptor agonist.[2c,7] The question remains as to whether the highest efficiency of DADLE correlates with its Phe–Tyr spacing. The available X-ray diffraction data suggest 5 to 13.9 Å (depending on the conformer) and 9.3 Å for this spacing in crystallized Leu-enkephaline and DADLE, respectively.[3a] These values of R exhibit no apparent correlation with the drug efficiencies. Similar uncertainty remains in correlating structures and pharmacological activity for many other opioid peptides.[1a,3b] In the gas phase, the short Phe–Tyr spacing constrains the structures of DADLE to the "double bend" family, whereas *LL* and *Enk* exhibit a similar "single bend" structural motif (Figure 3c and Ref. [14]). Therefore, there is a qualitative difference between the gas-phase structures of the two drugs, which differ significantly in their efficiencies. Although the interchromophore distance is not the only important pharmacophoric parameter, the peptides in vivo are, likely, zwitterions and the final validation of the calculated structures must be completed by a method such as conformer-selective IR spectroscopy, the question arises as to whether this distance, when determined in the gas phase, is a better pharmacological metric for these receptor ligands than the same distance in crystal structures. Certainly, additional gas-phase data are required before drawing a firm conclusion. Herein we have demonstrated how the Phe–Tyr distance can be estimated in the gas-phase enkephalins using FRET and MM calculations. Most of the opioid peptides contain the Phe–Tyr pair, allowing for the extension of our study to other ligands of this family, as well as to other peptides with these chromophores.

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[1] a) B. G. Nielsen, M. O. Jensen, H. G. Bohr, *Biopolymers* **2003**, *71*, 577–592; b) S. Granier, A. Manglik, A. C. Kruse, T. S. Kobilka, F. S. Thian, W. I. Weis, B. K. Kobilka, *Nature* **2012**, *485*, 400–404.

[2] a) J. A. H. Lord, A. A. Waterfield, J. Hughes, H. W. Kosterlitz, *Nature* **1977**, *267*, 495–499; b) M. Waldhoer, S. E. Bartlett, J. L. Whistler, *Annu. Rev. Biochem.* **2004**, *73*, 953–990; c) A. Janecka, J. Fichna, T. Janecki, *Curr. Top. Med. Chem.* **2004**, *4*, 1–17.

[3] a) J. R. Deschamps, C. George, J. L. Flippen-Anderson, *Biopolymers* **1996**, *40*, 121–139; b) J. R. Deschamps, J. L. Flippen-Anderson, C. George, *Biopolymers* **2002**, *66*, 287–293.

[4] a) T. Förster, *Discuss. Faraday Soc.* **1959**, *27*, 7–17; b) D. L. Dexter, J. H. Schulman, *J. Chem. Phys.* **1954**, *22*, 1063–1070.

[5] a) A. S. Danell, J. H. Parks, *Int. J. Mass Spectrom.* **2003**, *229*, 35–45; b) F. O. Talbot, A. Rullo, H. Yao, R. A. Jockusch, *J. Am. Chem. Soc.* **2010**, *132*, 16156–16164; c) V. Frankevich, V. Chagovets, F. Widjaja, K. Barylyuk, Z. Yang, R. Zenobi, *Phys. Chem. Chem. Phys.* **2014**, *16*, 8911–8920.

[6] a) S. Daly, F. d. r. Poussigue, A.-L. Simon, L. MacAleese, F. Bertorelle, F. Chirot, R. Antoine, P. Dugourd, *Anal. Chem.* **2014**, *86*, 8798–8804; b) N. G. Hendricks, N. M. Lareau, S. M. Stow, J. A. McLean, R. R. Julian, *J. Am. Chem. Soc.* **2014**, *136*, 13363–13370; c) A. Kulesza, S. Daly, L. MacAleese, R. Antoine, P. Dugourd, *J. Chem. Phys.* **2015**, *143*, 025101.

[7] a) H. W. Kosterlitz, J. A. H. Lord, S. J. Paterson, A. A. Waterfield, *Br. J. Pharmacol.* **1980**, *68*, 333 – 342; b) J. S. Morley, *Annu. Rev. Pharmacol.* **1980**, *20*, 81 – 110.
[8] G. D. Scholes, *Annu. Rev. Phys. Chem.* **2003**, *54*, 57 – 87.
[9] V. Kopysov, A. Makarov, O. V. Boyarkin, *Anal. Chem.* **2015**, *87*, 4607 – 4611.
[10] a) S. R. Mercier, O. V. Boyarkin, A. Kamariotis, M. Guglielmi, I. Tavernelli, M. Cascella, U. Rothlisberger, T. R. Rizzo, *J. Am. Chem. Soc.* **2006**, *128*, 16938 – 16943; b) O. V. Boyarkin, V. Kopysov, *Rev. Sci. Instrum.* **2014**, *85*, 033105.
[11] G. Féraud, M. Broquier, C. Dedonder, C. Jouvet, G. Grégoire, S. Soorkia, *J. Phys. Chem. A* **2015**, *119*, 5914 – 5924.
[12] a) J. A. Stearns, S. Mercier, C. Seaiby, M. Guidi, O. V. Boyarkin, T. R. Rizzo, *J. Am. Chem. Soc.* **2007**, *129*, 11814 – 11820; b) N. S. Nagornova, T. R. Rizzo, O. V. Boyarkin, *J. Am. Chem. Soc.* **2010**, *132*, 4040 – 4041.
[13] a) T. Tabarin, R. Antoine, M. Broyer, P. Dugourd, *Rapid Commun. Mass Spectrom.* **2005**, *19*, 2883 – 2892; b) V. Kopysov, N. S. Nagomova, O. V. Boyarkin, *J. Am. Chem. Soc.* **2014**, *136*, 9288 – 9291.
[14] N. L. Burke, J. G. Redwine, J. C. Dean, S. A. McLuckey, T. S. Zwier, *Int. J. Mass Spectrom.* **2015**, *378*, 196 – 205.

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