Conformation of Dynorphin A(1–17) Bound to Dodecylphosphocholine Micelles

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The determination of the structure of medium-sized linear peptides in their biological *milieu* is an elusive but important goal. The ensemble of conformations that a peptide adopts in water or aqueous organic solvents may or may not be related to its biologically active conformation. The ability of linear peptides to adopt different conformations in different environments is likely related to their multiple functionality. For example, there is evidence that for opioid peptides which act at more than one receptor type the bioactive conformation for each receptor type is probably different. This means there may be more than one bioactive conformation. Dynorphin A(1–17) is a peptide of extraordinary importance in the mediation of analgesia at the κ-opioid receptor, and there is accumulating evidence that dynorphin A and the dynorphin peptides[1] may be involved in nonopioid activities as well.[2] Since dynorphin A is expected to induce analgesia at the κ-opioid receptor without addictive properties,[3] there is profound interest in the conformation of dynorphin A as it elicits its response at the κ-opioid receptor. In the case of dynorphin A, binding to the μ-opioid receptor may have a different effect than binding to the κ-opioid receptor. The determination of the solution structure under different conditions is clearly important in understanding what conformations are possible.

In an ideal world, one would study dynorphin A as the peptide binds to the membrane-bound κ-opioid receptor in solution. Until the κ-opioid receptor is available, conditions approximating biological reality at a receptor are necessary and informative.[4,5] This report contains the ^{1}H 2D NMR assignments and secondary structural elements of the peptide as it is bound to a perdeuterated lipid micelle.[6] Just as structural features can be probed with the conventional geometrical constraints, e.g., cyclization or lactam bridges between adjacent residues, the structural features may also be probed by a geometrical constraint imposed by the binding of the peptide to the lipid micelle. Another important reason for studying the peptide bound to a lipid micelle is that it is believed that a lipid-catalyzed conformational change may take place *in vivo*.[7] Although organic solvents are often used to simulate a membrane environment, a lipid micelle is a better approximation due to its anisotropic nature and its ability to stabilize the peptide in largely one conformation as opposed to an ensemble of conformations. This approach has been used successfully in a number of cases.[8] There have been at least two high-resolution NMR studies of a dynorphin peptide and an analog in both water and methanol.[9] In both cases the peptide assumed an ensemble of conformations, with some β-strand conformation from residues R7–R9 or R7–D15. In neither case was an α-helix observed, which was interpreted to mean that no α-helix is required for κ-opioid activity. To our knowledge, this study represents the first report of dynorphin A bound to a lipid micelle, in which it is shown that the peptide is in an α-helical conformation from residues G3 to R9. This may have profound implications for the design of powerful, nonaddictive analgesics.

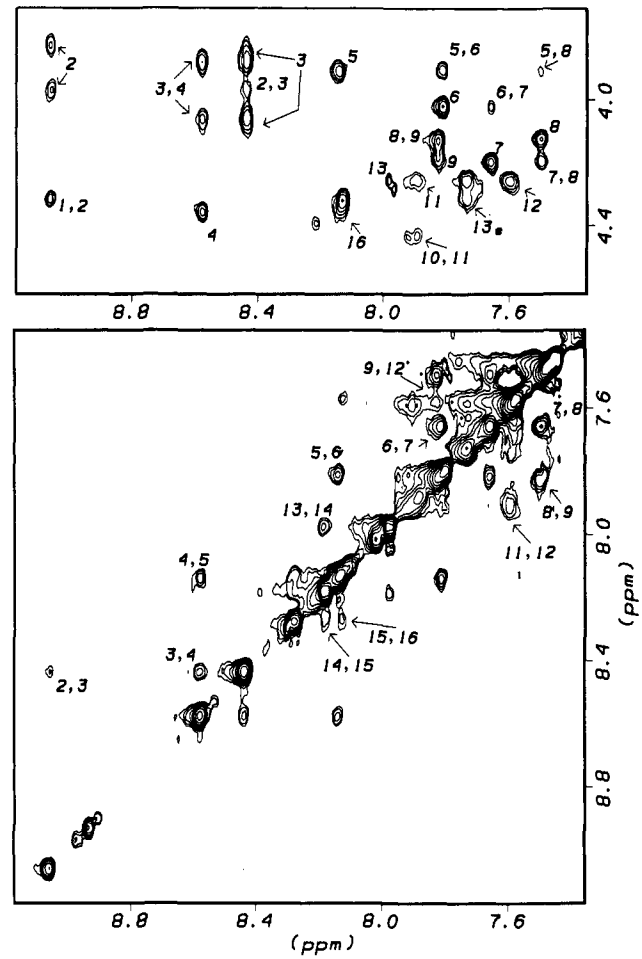

Figure 1. Portion of the ^{1}H NOESY spectrum of YGGFLRRIRPKLKWDNQ (dynorphin A) in perdeuterated dodecylphosphocholine micelles at 500 MHz, recorded at 310 K with a mixing time of 300 ms. This NOESY was used for the assignment of the (top) NH–CαH region and the (bottom) NH–NH region. In the top, the single numbers indicate the *intra*residue CαH–NH NOE, while the two numbers together (e.g., 2,3) indicate the *inter*residue CαH–NH NOE. The F4, L5 NOE is degenerate with the N16 NOE. One medium-range NOE is observed in this region, the L5–I8 NOE, verified at a mixing time of 75 ms. In the bottom, the numbers indicate the sequential NH–NH connectivity. Only the two terminal NOEs are missing, i.e., the Y1NH, G2NH NOE and the N16NH, Q17NH NOE.

Figure 1 shows two regions of the NOESY of the micelle-bound peptide.[10] The experimental details are contained in the

(1) "Dynorphin A(1–17)" refers to the full endogenous ligand, the 17-mer. It will henceforth be called "dynorphin A". "Dynorphin peptides" refers to the 17-mer and its natural deletion analogues.

(2) For a discussion of the nonopioid effects of dynorphin A and related peptides, see, for example: Shukla, V. K; Lemaire, S. *J. Psychiatr. Neurosci.* **1992**, *17*, 107–119.

(3) Millan, M. J. *Trends Pharmacol. Sci.* **1990**, *11*, 70–76.

(4) Organic solvents have been used to induce secondary structure formation in many cases; in other cases dodecylphosphocholine micelles have been used as well to simulate a membrane; see, for example: Karslake, C.; Piotto, M. E.; Pak, Y. K.; Weiner, H.; Gorenstein, D. G. *Biochemistry* **1990**, *29*, 9872–9878.

(5) While this manuscript was in preparation, we became aware that the κ- and δ- opioid receptors from mouse brain were purified and cloned. See: Yasuda, K.; Raynor, K.; Kong, H.; Breder, C. D.; Takeda, J.; Reisine, T.; Bell, G.I. Cloning and functional comparison of kappa and delta opioid receptors from mouse brain. *Proc. Natl. Acad. Sci. U.S.A.*, in press.

(6) The lipid micelle is perdeuterated dodecylphosphocholine, $CD_3(CD_2)_{11}OPO_2O(CD_2)_2N(CD_3)_3$. The critical micelle concentration is *ca.* 1 mM; our samples contained a lipid:peptide ratio of 25:1 with the peptide at 8 mM. It has been previously shown that this lipid forms homogeneous peptide–micelle complexes at the high concentrations required for NMR. See Lauterwein et al. (Lauterwein, J.; Bosch, C.; Brown, L. R.; Wuthrich, K. *Biochim. Biophys. Acta* **1979**, *556*, 244–264) and ref 8 for a discussion of the behavior of this lipid micelle.

(7) Schwyzer, R. *Biopolymers* **1991**, *31*, 785–792 and references within.

(8) Ikura, T.; Go, N.; Inagaki, F. *Proteins: Struct., Funct., Genet.* **1991**, *9*, 81–89 and references within. See also ref 4.

(9) (a) Lancaster, C. R. D.; Mishra, P. K.; Hughes, D. W.; St. Pierre, S. A.; Bothner-By, A. A.; Epand, R. M. *Biochemistry* **1991**, *30*, 4715–4726. (b) Vaughn, J. B.; Taylor, J. W.; *Biochim. Biophys. Acta* **1989**, *999*, 135–146.

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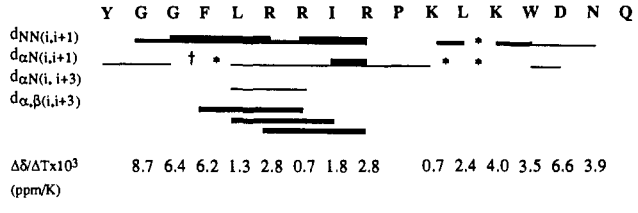
Table I. Secondary Structure Table and Amide Hydrogen Chemical Shift Temperature Coefficients for the Micelle-Bound Dynorphin[a]



[a] The pattern and intensity of NOEs is indicative of an α-helix from residues G3 to R9. (†) One medium NOE, one weak NOE from G3 to F4, evident at the 75-ms mixing time. (*) Obscured NOE due to overlap. The thick lines represent strong NOEs, the medium lines represent medium NOEs, and the thin lines represent weak NOEs.

figure caption. The observed cross peaks are indicative of a folded, largely structured peptide bound to the lipid micelle.[11] The NOEs are large and negative, and the chemical shifts of the backbone NH and CαH hydrogens are shifted downfield relative to both average random coil chemical shift values and the observed amide hydrogen chemical shift values of the peptide in water in the absence of lipid.[12] The relative intensities of the NOEs were used to construct Table I, and the secondary structure elements were deduced according to standard methodology[13] as discussed below. The classification of the strength of the NOEs was based on short mixing time data.[14] Table I shows the short-range NOEs characteristic of an α-helix from residue G3 to R9, namely, strong $d_{NN(i,i+1)}$ NOEs and weak $d_{\alpha N(i,i+1)}$ NOEs. The medium-range NOEs, $d_{\alpha\beta(i,i+3)}$, are observed for the F4, R7; L5, I8; and R6, R9 pairs. One medium range $d_{\alpha N(i,i+3)}$ NOE is observed from L5 to I8. Both types of medium-range NOEs strongly support the helix secondary structure interpretation. The R9 CαH to P10 CδH NOE was substituted for the normally observed sequential $d_{\alpha N(i,i+1)}$ NOE for R9 to P10, which indicates a *trans* proline. If the region K11 to N16 were in a β-strand, we would expect the relative intensities of the sequential NOEs to be reversed from that of a helix, i.e., weak $d_{NN(i,i+1)}$ NOEs and strong $d_{\alpha N(i,i+1)}$ NOEs. This is not observed. In fact, the relative intensities of the NOEs in the C-terminal region indicate some conformational averaging, with a greater-than-unfolded propensity to sample the helical region of space. This is based on the observation that several $d_{NN(i,i+1)}$ NOEs are of medium intensity and the corresponding $d_{\alpha N(i,i+1)}$ NOEs are of weak intensity.[15] We are currently looking at structural models of the entire peptide which are consistent with the NMR data. The complete assignment of all the protons in the peptide and the details of the three-dimensional structures which are consistent with the NMR data will be reported elsewhere.[12]

Table I also contains the amide proton temperature coefficients. For comparison, the amide temperature coefficients of dynorphin A in water are all greater than *ca.* 6 ppb/K.[12] It is not known whether the lowered amide temperature coefficients in the helical region are due primarily to a hydrogen bond from an amide at *n* to the carbonyl at *n* −3 or they are due to the amide hydrogen being shielded from solvent by the lipid micelle, resulting in hydrophobic protection.[16] It is likely that both factors contribute. The low temperature coefficients in the region K10–W14 show an interesting upward trend and may reflect hydrophobic interactions between these residues and the micelle. We are currently investigating this issue and the related issue of where the peptide resides with respect to the lipid micelle.

We conclude that the opioid peptide dynorphin A binds to lipid micelle in a conformation which is helical from residues G3 to R9. Whether this conformation is similar to the bioactive conformation is not known. Alanine analogs of dynorphin are being studied in this laboratory to determine if the alanine substitution correlates with increased helicity in the case of the analog which is more active in the opioid binding assay, and less helicity in the case of the analogs which are less active than the native peptide in the opioid binding assay.[17] Such studies may help clarify the issue of whether helicity is required for dynorphin's activity.

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(10) The spectra were recorded on a GE Omega spectrometer at 310 K in hypercomplex mode with a mixing time of 300 ms, in 90% H_2O/10%D_2O, at pH 3.2. A typical data set contained 2K × 512 data points and was processed using FELIX from Dr. Dennis Hare.

(11) The criteria for deducing that the peptide is in largely a nonrandom conformation are described in the following: Dyson, H. J.; Wright, P. E. *Ann. Rev. Biochem. Biophys.* **1991**, *20*, 519–538.

(12) D. A. Kallick, to be submitted. The line widths of the resonances also increase proportionately in the presence of lipid.

(13) Wuthrich, K. *NMR of Proteins and Nucleic Acids;* John Wiley and Sons, Inc.: New York, 1986.

(14) Since the intensities of NOE cross peaks may only be related to distance when the two-spin approximation is valid, additional NOESYs were obtained back-to-back at mix times of 75, 100, and 150 ms to determine the validity of the two-spin approximation in deducing intensities of cross peaks. It was found that, using a calibration distance spatially close to the spin pair in question, the distances calculated at mix times of 75 and 100 ms for some spins did not differ at all, while for other spins they differed by only several percent. Some distances calculated with a mix time of 150 ms deviated significantly from those at 75 ms and were not used. The NOESY at a mix time of 75 ms was thus used to calculate intensities, although we use these in a qualitative sense only, as "strong", "medium", or "weak" for the purposes of this paper.

(15) One way to estimate the types of conformations contributing to an ensemble is to calculate the ratio of intensities of sequential NOEs: Osterhout, J. J.; Baldwin, R. L.; York, E. J.; Stewart, J. M.; Dyson, H. J.; Wright, P. E. *Biochemistry* **1989**, *28*, 7059–7064.

(16) In myohemerythrin, the combination of hydrophobic sequestering of amides and hydrogen bond formation has been suggested to be responsible for at least one observed low amide temperature coefficient: Dyson, H. J.; Merutka, G.; Waltho, J. P.; Lerner, R. A.; Wright, P. E. *J. Mol. Biol.* **1992**, *226*, 795–817.

(17) The opioid receptor activities of dynorphin A(1–13) and its 1–11 alanine analogs are evaluated in the following: Turcotte, A.; Lalonde, J.-M.; St. Pierre, S.; Lemaire, S. *Int. J. Pept. Protein Res.* **1984**, *23*, 361–367. Dynorphin A(1–17) and eight of its deletion analogs, including dynorphin A(1–13), are evaluated in the following: Sanchez-Blazquez, P.; Garzon, J.; Lee, N. M. *Eur. J. Pharmacol.* **1984**, *98*, 389–396.