Differential Binding of Desmopressin and Vasopressin to Neurophysin-II*

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Desmopressin is a synthetic analog of the peptide hormone vasopressin in which the N-terminal α-amino group has been removed and L-arginine in position 8 has been replaced by D-arginine. Using 1H-NMR spectroscopy, we show that desmopressin binds to neurophysin-II, whereas deamino-vasopressin does not bind. Thus, the change in configuration at Arg⁸ causes a significant difference in the binding of these hormones to neurophysin-II. We have determined the structure of desmopressin bound to neurophysin-II using two-dimensional 1H nuclear magnetic resonance-transferred nuclear Overhauser effect techniques. A common binding motif for vasopressin and desmopressin is proposed that includes a positive charge group along with the hydrophobic surface formed by the side chains of Tyr² and a β-methylene provided by Phe-3. In vasopressin, the positive charge is provided by the N-terminal NH₃⁺, whereas in desmopressin, the side chain of Arg-8 contributes the positive charge. The type II β-turn found in residues Cys¹-Pro¹-Arg⁻¹-Gly² of the bound structure of desmopressin folds the Arg⁸ side chain back toward the disulfide-bond loop, which allows the positive charged side chain of Arg⁸ to participate in binding. Such a type II β-turn is not found in deamino-vasopressin in the presence of neurophysin-II.

Neurophysin is a carrier protein for the peptide hormones vasopressin and oxytocin. The neurophysin precursor is composed of both the peptide hormone and neurophysin connected by a tri-peptide Gly-Lys-Arg linker, which is processsed into the hormone and mature neurophysin after packaging into neurosecretory granules. The processed hormone and neurophysin form a noncovalent complex that dissociates after dilution upon secretion into the blood (1). The vasopressin/oxytocin-neurophysin complexes provide good model systems for the recognition and binding of flexible peptides by proteins (1). The current view of the interaction in the hormone-neurophysin complexes suggests that the N-terminal α-amino group and residue Tyr² play central roles (1, 2). It has also been suggested that the C-terminal three-residue tail in these hormones is not involved in binding (1). This model is consistent with most known experimental results. For example, deamino-oxytocin fails to bind to neurophysin because its N-terminal α-amino group is replaced by a hydrogen (1). Also, dipeptides or tripeptides related to the N-terminal sequence of these hormones bind to neurophysin with affinities only slightly weaker than the natural hormones (3, 4). The crystal structure of the complex between a dipeptide Phe-Tyr-NH₂ with neurophysin-II largely confirms the current view of the interactions between hormones and neurophysin as deduced from spectroscopic and thermodynamic studies (5). Lippens et al. (6) solved the solution conformation of oxytocin bound to neurophysin-I by NMR transferred NOE techniques, thereby providing further insight into the structure of hormone-neurophysin complexes. More recently, the crystal structure of the oxytocin-neurophysin complex has been published, which again confirms the importance of the N-terminal NH₃⁺ group and the aromatic side chain of Tyr² for the binding of the peptide hormone oxytocin to neurophysin (7).

In the present study, transferred NOE and molecular modeling techniques have been used to study the desmopressin-neurophysin-II complex. Desmopressin is a synthetic analog of vasopressin with specific antidiuretic activity and antibleeding activity (8). It interacts specifically with the vasopressin receptor subtype V₂ (8). The specificity of its biological activity makes desmopressin one of the most important analogs of vasopressin for clinical applications (8). Desmopressin differs from vasopressin in two positions. The N-terminal α-amino group of vasopressin is replaced by a hydrogen (deamino-vasopressin), and the L-arginine at position 8 is replaced by D-arginine. Both desmopressin and deamino-vasopressin are not expected to bind to neurophysin because they lack an N-terminal α-amino group. However, using 1H-NMR transferred NOE techniques, we demonstrate herein that desmopressin does indeed bind to neurophysin-II.

The structure of desmopressin bound to bovine neurophysin-II has been generated based on distance restraints derived from two-dimensional 1H-NMR transferred NOE data. Based on this structure, a binding motif is proposed for desmopressin that indicates a critical and novel role of the C-terminal three-residue tail of desmopressin. This binding motif suggests new probabilities for interactions within hormone-neurophysin complexes.

EXPERIMENTAL PROCEDURES

Sample Preparation—Desmopressin and deamino-vasopressin were synthesized by solid-phase peptide synthesis method and was purified by reverse-phase high-performance liquid chromatography (11). Pure bovine neurophysin-II was isolated from acetone powder prepared from bovine posterior pituitary lobes and purified using methods reported

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¶ The abbreviations used are: NOE, nuclear Overhauser effect; NOESY, nuclear Overhauser spectroscopy; ROESY, rotating frame nuclear Overhauser spectroscopy.
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RESULTS

Desmopressin Binds Weakly to Bovine Neurophysin-II—Fig. 1 compares the amide-proton region of the one-dimensional 1H-NMR spectra of desmopressin as a function of the added fraction of bovine neurophysin-II (molar ratio). These spectra show line-broadening of the amide-proton peaks of desmopressin as a function of the concentration of neurophysin-II. No significant chemical shift changes were observed. In these spectra, we assume that the free-bound peptide exchange is in the NMR fast exchange limit for both the NOE timescale and the chemical shift timescale because of the weak affinities involved (1). An estimation of the binding constant of desmopressin to neurophysin-II is about 200 M⁻¹, which is based on NMR experiments (line width versus neurophysin concentration) and competitive binding studies (data not shown). The line-broadening of amide-proton peaks indicates an interaction between the peptide and bovine neurophysin-II. The binding of desmopressin to neurophysin-II also induces new 1H-NMR transferred NOE cross-peaks. Fig. 2, A and B, compare the two-dimensional 1H-NMR transferred NOESY spectra of desmopressin in the presence of 0.1 added molar ratio of neurophysin-II with the two-dimensional 1H-NMR NOESY spectra of the peptide free in aqueous solution. Transferred NOEs develop upon binding to neurophysin-II, resulting in a larger number and more intense NOE cross-peaks at a 150-ms mixing time than those observed at a 500-ms mixing time in the absence of protein. Many of these new transferred NOE cross-peaks arise from side chain to side chain nuclei as indicated in Fig. 2.

Differences between Desmopressin and Deamino-vasopressin in the Presence of Neurophysin-II—We have also used two-dimensional 1H-NMR transferred NOE techniques to study deamino-vasopressin. The transferred NOESY spectrum of deamino-vasopressin in the presence of 0.1 added molar ratio of neurophysin-II showed no significant differences from the NOESY spectrum of the free peptide (data not shown). Fig. 2 shows that no significant differences are observed between the NOESY spectrum of the desmopressin in the absence of neurophysin-II (Fig. 2A) and the transferred NOESY spectrum of deamino-vasopressin in the presence of 0.1 added molar ratio of neurophysin-II (Fig. 2C), indicating that there is no binding. Since the J/D difference in the configuration of Arg⁶ of desmopressin and deamino-vasopressin results in such a striking difference in their binding to neurophysin-II, a conformational difference in these two peptides might be expected. Fig. 3 shows the 1H-NH regions of two-dimensional 1H-NMR NOESY spectrum of desmopressin in the absence of neurophysin-II (Fig. 3A) and the same region of transferred NOESY spectra of desmopressin (Fig. 3B) and deamino-vasopressin (Fig. 3C) in the presence of 0.1 added molar ratio of bovine neurophysin-II. Changes are observed in the NOEs that characterize the structure of residues 6–9; for example, the d₃(7, 9) cross-peak (marked by a box) is seen in Fig. 3A (weak intensity) and Fig. 3B (medium intensity) but not in Fig. 3C. In addition (data not shown), the d₅(8, 9) cross-peak is seen in the NH-NH region of NOESY (medium intensity) and transferred NOESY (strong intensity) spectra of desmopressin in the absence/presence of

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2 The chemical shift assignment of desmopressin is reported in Ref. 12.
0.1 added molar ratio of neurophysin-II, but is not seen in the transferred NOESY spectrum of deamino-vasopressin in the presence of 0.1 added molar ratio of neurophysin-II. These NOE results identify a β-turn in residues Cys6-Pro7-D-Arg8-Gly9 in both free and bound desmopressin. Evidence for such a structure is not found among the same residues of deamino-vasopressin. While the NOEs d_{αN}(7,9) and d_{NN}(8,9) characterize the existence of a β-turn in these residues, the 3J_{αH-NH} coupling constant of 5.0–5.5 Hz for residue Arg8 of desmopressin indicates that this turn is a type II β-turn (9). Actually, the sequence of Pro-D-Arg at positions i+1 and i+2 is known to favor a type II β-turn (10). Interestingly, this type II β-turn was also found in the structures of desmopressin in both aqueous and trifluoroethanol solutions (11, 12).

Fig. 2 shows transferred NOEs from aromatic atoms of Tyr2 and Phe3 to the side chain β-CH2 and γ-CH2 atoms of residues of Arg8 and Gln4, as well as from β-CH2 atoms of Cys6 to the C-terminal NH2 atoms of Gly9. The NOEs from residue Arg8 to Tyr2 and Phe3 are surprising because they are not observed in either aqueous or TFE solutions or in the NOESY spectra of deamino-vasopressin in the presence of added molar ratio of neurophysin-II. Together with the transferred NOEs involving Cys6 to Gly9, these results indicate that the three residue C-terminal tail of desmopressin may be involved in binding to neurophysin-II.

Three-dimensional Structure of Desmopressin Bound to Bovine Neurophysin-II—The bound structure of desmopressin to neurophysin-II has been generated using PEPFLEX-II (12), which is a computer program for the generation of structure(s) of flexible peptides, based on transferred NOE NMR data. On the right of Fig. 4, we show 21 structures of desmopressin bound to neurophysin-II derived from 1H-NMR transferred NOE data; on the left, we show the averaged bound structure derived from these structures. There are two fused β-turns in the structure that are located between residues Phe3 and Gly9. One turn is a distorted type-I β-turn with residues Gln4 and
Asn at the i+1 and i+2 positions; the other is a type-II β-turn with residues Pro and Arg at positions i+1 and i+2, respectively. Two hydrogen bonds were found in the bound structure. One is between the amide proton of residue Phe to the carbonyl oxygen atom of residue 6, and the other is between the amide proton of residue 6 to the carbonyl oxygen of residue 9. The bound structure of desmopressin is similar to one of the minor conformations of the peptide observed in aqueous solution (12).

The most striking feature of the bound structure is the change in the conformation of the side chains of residues Tyr, Phe, Glu, and Arg that occurs upon binding to neurophysin-II. This suggests that the major interactions between desmopressin and neurophysin-II involve these side chains. Binding causes the positive charge on the side chain of Arg to be in close proximity of the side chains of Tyr or Phe and the disulfide-bonded loop (see the left structure of Fig. 4), suggesting that the C-terminal three-residue tail of desmopressin participates in the binding interactions.

This is different from structure-function studies of vasopressin and other analogs, which indicate that the three-residue tail of these peptides is not involved in binding (1). In the structures of the free peptide, the Tyr side chain is one of the least well-refined side chain, indicating its high flexibility when the peptide is in either TFE or aqueous solutions (11, 12). The flexibility of the Tyr side chain of the peptide is significantly reduced in the bound structure, indicating a strong interaction between this side chain and neurophysin-II. However, the side chain of Phe in the bound structure retains a certain flexibility, implying a looser interaction between Phe and neurophysin-II. The strong interaction of the Tyr side chain and the weak interaction of the Phe side chain are analogous to interactions of vasopressin with neurophysin-II (1). Our previous results indicated that the disulfide bond is one of the most flexible part of desmopressin; its flexibility causes several different orientations of the three-residue tail in both TFE (11) and aqueous solution (12). The flexibility of the disulfide bond remains in the bound structure of desmopressin. Unlike the disulfide bond of the peptide in the free solution structures (12), this is not coupled to different orientations of the C-terminal three-residue tail (Fig. 4).

**DISCUSSION**

In this study, a large excess of peptide over neurophysin-II was used for the transferred NOE experiments to minimize the NMR signals from neurophysin-II. Such an excess may lead to nonspecific binding (13). However, our results indicate that the interaction between desmopressin and neurophysin-II is specific compared to the control experiment, which demonstrated that deamino-vasopressin does not bind to neurophysin-II under the same experimental conditions.

The current view of the binding mode between peptide hormones and neurophysin does not predict the binding of desmopressin to neurophysin (1), because it lacks the N-terminal α-amino group, which plays a crucial role in the binding of natural hormones vasopressin and oxytocin to neurophysin (1). This group forms a strong salt bridge with the side chain carboxyl group of residue Glu in neurophysin and hydrogen bonds to neurophysin backbone carbonyls of Glu, Leu, and Ser (5). Removal of this N-terminal NH$_3^+$ causes the loss of the binding activity of oxytocin (1), and a similar result is also seen in this paper for deamino-vasopressin. However, the acetamidation of this amino group does not change binding affinity or the binding-induced dimerization of the hormone-neurophysin complex (14). This indicates that a longer and more bulky positive charge on the acetamidated group can also form a strong salt bridge. Both desmopressin and deamino-vasopressin lack this N-terminal α-amino group. However, our NMR data indicate that although desmopressin binds to neurophysin-II, deamino-vasopressin does not. The only positive charge in desmopressin/deamino-vasopressin is that from the i-arginine group, which plays a crucial role in the binding of natural hormones vasopressin and oxytocin to neurophysin (1).
neurophysin-II may not be as close as that of the α-amino group in vasopressin. Also, the side chain of Arg⁸ is bulkier, even than the acetimidated amino group, and may necessitate conformational adjustments by the protein. These factors may influence the strength of the interaction between desmopressin and neurophysin-II and may, therefore, be responsible for a binding affinity difference between desmopressin and vasopressin.

Another important interaction between the natural hormone and neurophysin-II is that the aromatic side chain of Tyr² binds in a tight pocket with its hydroxyl group hydrogen bonded with neurophysin (1). The bound structure of desmopressin supports this interaction, although we cannot determine whether the hydroxyl group of the Tyr² side chain is hydrogen bonded with neurophysin-II. Although the side chain of Tyr² in bound oxytocin is the most flexible side chain of the peptide in its free solution structures (11, 12), the binding to neurophysin-II significantly reduces its flexibility. The bound structure also indicates a certain mobility for the aromatic side chain of Phe³. This is also consistent with earlier studies of hormone-neurophysin complexes, which indicated that the side chain of Phe³ binds to the protein principally via the β-methylene position (1). Furthermore, thermodynamic studies indicate hydrogen-bonding between the hormone backbone of residue 2 and 3 to neurophysin (5, 15), which was supported by ¹⁵N NMR spectroscopy study (16). In the crystal structure of the complex of Phe-Tyr-NH₂ with neurophysin-II, the terminal amide NH of the peptide is hydrogen-bonded to the protein (5). This is analogous to the amide proton of Phe³ in our bound structure, which is directed to the inside of the 20-membered ring moiety. Similar observations were noted with the bound structure of oxytocin to neurophysin-I (6).

The fact that the disulfide bond retains its flexibility in the bound structure of desmopressin suggests that it may not interact with neurophysin-II. This is interesting because earlier studies demonstrated that the disulfide bond of the peptide hormones binds to an apolar region of neurophysin (1). This suggests that there are differences in the binding between desmopressin and vasopressin/oxytocin to neurophysin. The flexibility of the disulfide bond in the free solution structure causes different orientations of the three-residue tail, as we discussed previously (11, 12); however, the flexibility of the disulfide bond in the bound structure of desmopressin does not cause different orientations of the three-residue tail, as shown in Fig. 4. This difference also supports the concept that the three-residue tail of desmopressin is involved in binding to neurophysin, because the conformation of the three-residue tail is fixed by the interaction between the side chain of Arg⁸ and neurophysin-II.

Taken together, the results in the present study indicate both similarities and differences in the binding of desmopressin to neurophysin-II when compared with that of the natural hormones vasopressin and oxytocin. Fig. 5 compares the bound structure of desmopressin with the crystal structure (wet-form) of deamino-oxytocin (Protein Data Bank code 1XY1). Note that Rose et al. (7) reported recently that the conformation of the critical residues Cys¹ and Tyr² of this structure is similar to those of Cys¹ and Tyr² observed in the oxytocin-neurophysin complex. To mimic the bound form of oxytocin, the crystal structure of deamino-oxytocin was modified to include a NH₃⁺ group in the N-terminal. Fig. 5 shows oxytocin and desmopressin in a manner where a positive charge and the aromatic side chain of Tyr² have similar orientations. The positive charge is from the N-terminal NH₃⁺ in oxytocin and from the side chain of D-Arg⁸ in desmopressin. The type-II β-turn in the three-residue tail brings the side chain of Arg⁸ to be close to the side chain of Tyr² in desmopressin. The side chain of Tyr² orients toward to the N-terminal NH₃⁺ in oxytocin, which was confirmed by the crystal structure of oxytocin bound to neurophysin (7). In an analogous way, the positive charge of D-Arg⁸ also orients toward the Tyr² side chain in desmopressin. The orientation of the Tyr² side chain of the desmopressin bound structure is different from that of the structure of bound oxytocin in solution by Lippens et al. (6). In the structure of bound oxytocin in solution, the Tyr² side chain points away from Cys¹ and orients toward the Ile³ backbone; however, the Tyr² side chain of desmopressin orients toward the positive charge of Arg⁸. The orientation of the side chain of residue 3 between oxytocin (crystal structure/wet-form) and desmopressin is different. Although the side chain of Ile³ points away from the side chain of Tyr² in oxytocin, the side chain of Phe³ orients
toward the side chain of Tyr² in desmopressin. A similar result was also observed when the free crystal structure (wet-form) of deamino-oxytocin was compared with the crystal structure of the bound form of oxytocin (7). The Ile³ side chain in the bound form of oxytocin orients toward the Tyr² side chain, as clearly shown in Fig. 5 of Ref. 7.

The similarity between bound oxytocin and desmopressin suggests a possible common binding motif for all neurophysyal hormones, consisting of a positive charge, one apolar aromatic side chain, and a β-methylene group. The positive charge is from N-terminal NH₃⁺ in vasopressin/oxytocin and from the positive side chain of D-Arg in desmopressin. The apolar aromatic side chain is from Tyr², and the β-methylene is from Phe³. This common binding motif suggests that desmopressin may bind to neurophysin-II at its major hormone binding site. If desmopressin adopts an upside-down binding motif compared with that of oxytocin and vasopressin, the positive charge on D-Arg⁸ can be directed toward the Tyr² side chain by the type-II β-turn in the C-terminal three-residue tail, thus forming the binding motif. Unlike oxytocin and vasopressin, the disulfide bond in desmopressin is away from this binding motif and is not involved in binding, therefore retaining its flexibility as shown in Fig. 4. This proposed new binding mode of desmopressin may give new insights into the mechanism of ligand-protein recognition in this system and suggests an unexpected adaptability of the protein binding site to accommodate novel ligands.

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