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Conformation of Lysine Vasopressin: A Comparison with Oxytocin

(proton magnetic resonance/neurohypophyseal hormones/three-dimensional structure/pseudo-frequency sweep decoupling/spectral assignments)

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ABSTRACT **Starting with assignments of proton nuclear magnetic resonance previously made for oxytocin in deuterated dimethylsulfoxide at 220 MHz, we have assigned resonances for the mammalian antidiuretic hormone, lysine vasopressin. The results demonstrate that spectral assignments of neurohypophyseal hormones and their congeners can, within certain limits, be derived from each other. Comparison of the spectra of lysine vasopressin and oxytocin suggests that the gross backbone conformations of their 20-membered ring components are for the most part similar in deuterated dimethylsulfoxide, whereas the C-terminal acyclic amino-acid sequence of lysine vasopressin is more flexible than that of oxytocin.**

Based on *a priori* considerations, a certain conformational homology is to be expected among molecules with homologous amino-acid sequences; conversely, existing differences in the amino-acid sequences may introduce certain conformational variances. The incentive for much of the ongoing research in molecular endocrinology has been the question of the extent to which conformational similarity and dissimilarity between hormonal congeners may affect the profiles of their biological activity.

The two neurohypophyseal peptide hormones, lysine vasopressin (LysVP) and oxytocin (Fig. 1), are excellent examples of homologous molecules since they differ solely at two positions in their amino-acid sequence: Phe against Ile in position 3 and Lys against Leu in position 8 for LysVP and oxytocin, respectively. LysVP, the antidiuretic hormone of certain mammals (1–3), and oxytocin, the mammalian milk-ejecting principle (4), exhibit "biological crossover reactivity," i.e., at pharmacological concentrations LysVP elicits the physiological responses characteristic of oxytocin and vice versa (5, 6).

Against this background, we are extending to LysVP earlier conformational analyses of neurohypophyseal hormones involving proton magnetic resonance (PMR) and circular dichroism studies (7–11). In an earlier report we proposed a preferred solution conformation for oxytocin (12). Since then, PMR assignments for LysVP have also been made (13, 14), based on extrapolation of data from synthetic fragments. In this communication, we wish to show that PMR assignments for LysVP are readily derived from those of oxytocin (7), and that conformational differences exist between oxytocin and LysVP in deuterated dimethylsulfoxide (Me_2SO). Preliminary studies have been reported (6).

Abbreviations: Me_2SO, dimethylsulfoxide; LysVP, lysine vasopressin.

MATERIALS AND METHODS

LysVP was prepared as described by Meienhofer and Sano (15), and the various samples had pressor activities ranging from 230 to 300 U/mg when assayed on anesthetized male rats (16).

Spectra were recorded on a Varian Associates HR-220 NMR Spectrometer equipped with a 620-I computer for signal-to-noise enhancement by multiscan averaging. The temperature was controlled to within $\pm 2°$. Proton double-resonance experiments were accomplished by a "pseudo-frequency-sweep decoupling" method with an accessory that continuously decoupled a given resonance from all other resonances in the spectrum by synchronously varying the decoupling frequency as the magnetic field was swept. This technique combines the high resolution of the HR-220, normally a field-sweep spectrometer, with the greater efficiency and sensitivity normally obtained only by frequency-sweep decoupling. Spectra were measured in deuterated Me_2SO (99.8% D, Columbia Organic Chem. Co., Columbia, S.C.); tetramethylsilane was used as the internal standard.

RESULTS AND DISCUSSION

The 220 MHz PMR spectrum of LysVP in deuterated Me_2SO at 21° (Fig. 2) is essentially the same as that reported by Walter (6) and von Dreele *et al.* (13). Optimal interpretation of this spectrum depends on accurate assignment of resonances to specific hydrogen atoms. The assignment of polypeptide spectra is now a routine procedure requiring an initial estimate of the chemical shifts of side-chain absorptions. These chemical shifts may be estimated from data on unrelated small peptides or random-coil polypeptides, whose side chains experience an essentially solvated environment. However, the best information is derived from previously assigned spectra of analogous polypeptides whose side-chain hydrogens are expected to experience an environment similar to that of the particular one under study.

By this approach, the PMR assignments of side-chain protons of LysVP (and, by extension, also of the backbone protons to which they are coupled) was accomplished in the

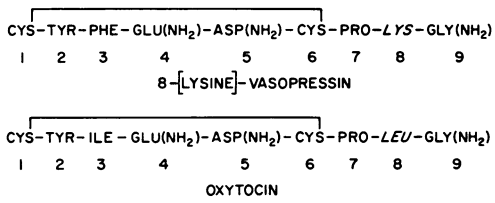

FIG. 1. Amino-acid sequence of lysine vasopressin and oxytocin. *Numbers* indicate sequence positions of individual amino-acid residues.

present study by comparison with the spectrum of oxytocin assigned by Johnson *et al.* (7). LysVP can be considered as an analog of oxytocin, possessing chemical similarities and anticipated gross conformational likeness. For assignments of Phe and Lys, which are unique to LysVP, the positions of the side-chain protons were estimated from previous determinations made by McDonald and Phillips (17) on the chemical shifts of side-chain protons of random-coil polypeptides in D_2O. Direct measurements have indicated that the chemical shifts of α- and β-proton resonances of Lys are the same in Me_2SO and water (Urry, Glickson, and Walter, unpublished), and this is also presumed to be the case with the βCH_2 peaks of Phe. Based on these estimates of chemical shifts and on intensities of the observed resonances, we have made an initial assignment of spectral resonances for LysVP (Fig. 2). Since double resonance experiments reveal the spin-coupling pattern unique to individual amino-acid residues, we used this method to test the validity of our side-chain proton assignments. Moreover, such double resonance experiments served to assign coupled αCH and peptide NH protons; the positions of the NH resonances cannot generally be estimated *a priori* because their chemical shifts depend more strongly on the amino-acid sequence and backbone conformation than do the chemical shifts of side-chain hydrogen absorptions. In analogous polypeptides, however, these difficulties are often absent, and direct comparison of αCH and peptide NH resonances can often be made. The spectral characteristics of certain amino-acid residues facilitate identification of their resonances, e.g., Gly is the only natural amino acid with a triplet NH peak, and Pro is the only natural amino-acid residue lacking a peptide NH peak.

In LysVP, the highest field peptide NH resonance at 8.02 ppm is associated with the Asn residue. This is revealed by homonuclear spin decoupling in deuterated Me_2SO, which located coupled αCH and βCH_2 absorptions at 4.44 and 2.56 ppm; the corresponding resonances of oxytocin and deamino-oxytocin exhibited similar chemical shifts (7). The characteristic peptide NH triplet of glycine appears further downfield at 8.10 ppm, and is coupled to the CH_2 peak at 3.61 ppm.

A two-proton peptide NH resonance peak located at 8.25 ppm in the spectrum of LysVP at 21° separated into well resolved resonances at 8.12 and 8.17 ppm at 40° (Fig. 2, *insert*). At 40°, the 8.12 ppm peak was decoupled from an αCH absorption at 3.89 ppm (located at 4.02 ppm at 21°); the peak at 3.89 ppm in turn was decoupled from a βCH_2 resonance at 1.47 ppm, the characteristic frequency of the βCH_2 of Lys. The γ- and δCH_2 peaks of Lys were identified at 1.36 and 1.47 ppm, respectively, whereas the ϵCH_2 of this amino acid absorbed between 2.6 and 3.3 ppm (17). The 8.17 ppm resonance at 40° was coupled to an αCH absorption at 4.13 ppm (4.17 ppm at 21°). Assignment of these resonances to Gln is indicated by decoupling of the αCH resonance from a βCH_2 at 1.69 ppm, a position similar to that of the Gln of oxytocin (7). As has been found in oxytocin and deamino-oxytocin, the γCH_2 protons of Gln in LysVP resonated at 2.11 ppm. These findings are at variance with a recent report from another laboratory (13); inspection of the temperature-dependence of chemical shifts of the peptide amide protons of LysVP reveals a reversal of the amide NH absorptions of Lys and Gln. Although in a subsequent publication (14) the assignment of the amide NH resonances has been revised, the

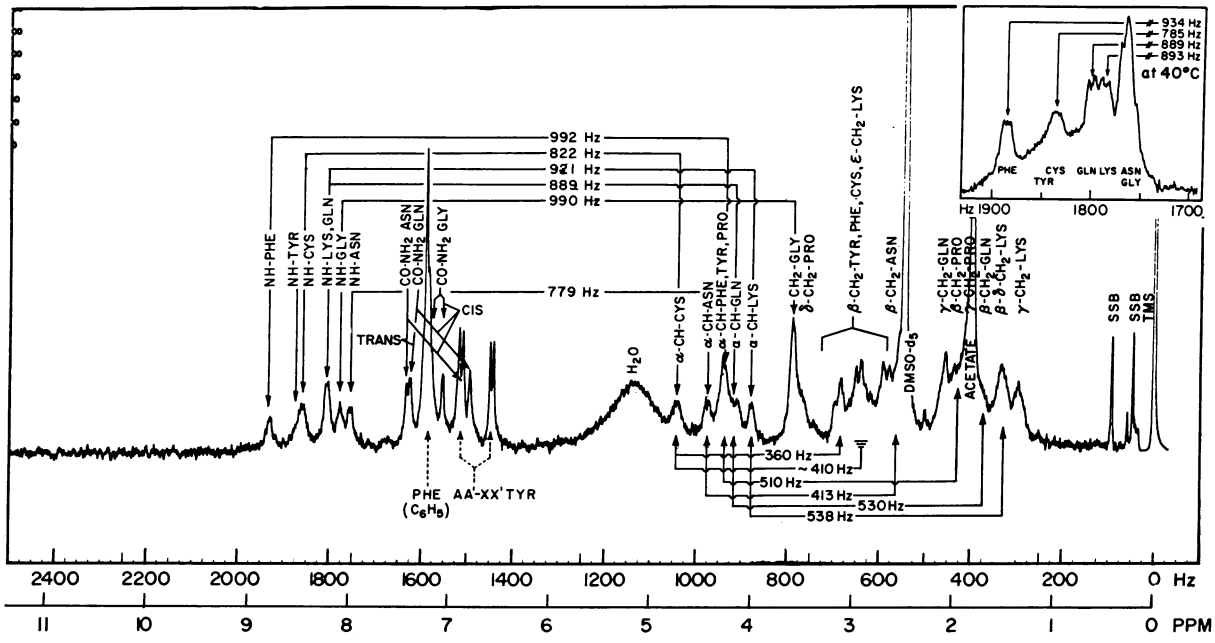

FIG. 2. Proton magnetic resonance assignment of lysine vasopressin in deuterated Me_2SO at 21°. *Bridges* connect resonances originating from vicinal protons identified by spin decoupling. *Insert* shows the region of peptide NH resonances at 40°.

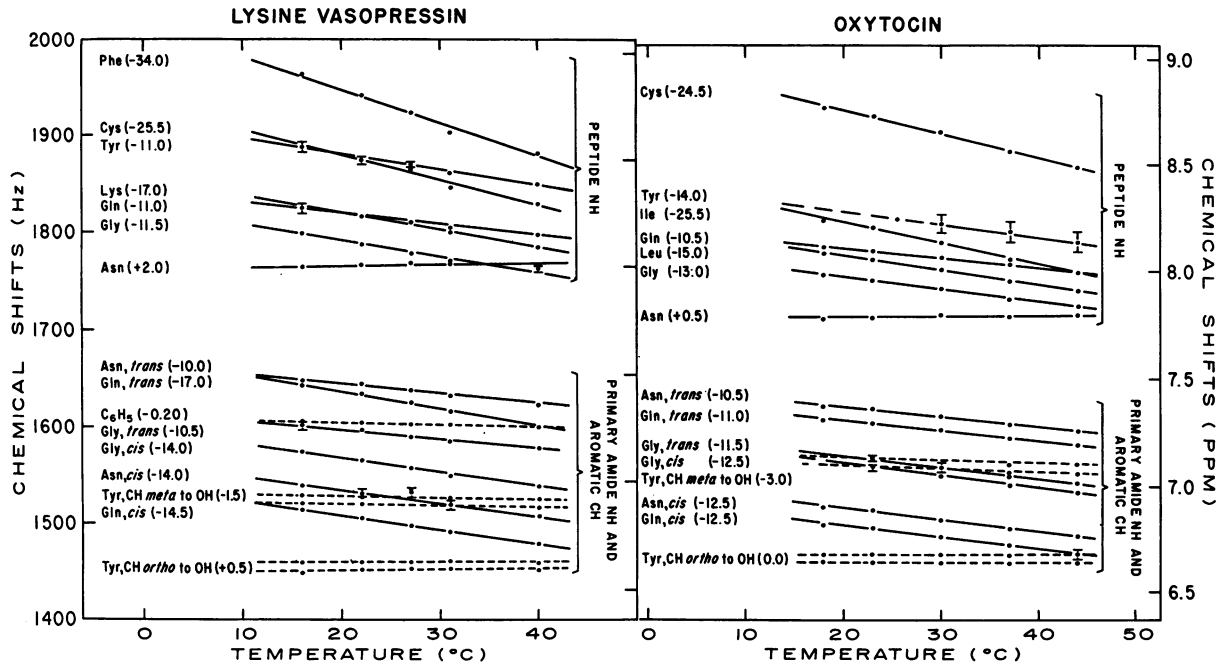

FIG. 3. Temperature dependence of chemical shifts of the low-field region [including peptide (——), carboxamide (——), and aromatic (- - -) proton resonances] of lysine vasopressin and oxytocin in deuterated Me_2SO. Slopes of the linear plots (Hz/10°) of specific proton resonances are indicated in *parentheses*.

assignments of the αCH absorptions of Lys and Gln are reversed from those that we report here (see Fig. 2).

The peptide NH peak at 8.47 ppm in the spectrum of LysVP was coupled to an αCH at 4.76 ppm, whose anomalous low-field position—also observed in oxytocin and deamino-oxytocin (4.77 and 4.78 ppm, respectively) (7)—identifies it as originating from the cysteine residue at position 6 of the hormone. Furthermore, upon complete replacement of labile hydrogens by deuterium in 50% D_2O/deuterated Me_2SO (v/v) at 20°, the αCH was decoupled from βCH_2 resonances at 3.13 ppm and about 2.85 ppm. These are the two-proton resonances that are associated with the nonequivalent β-methylene hydrogens of the cysteine residue at position 6 (6). As with oxytocin, the αCH peak of Cys-1 was not observed in LysVP. At 4.29 ppm, we observed a peak of three-proton intensity. The αCH resonance that did not couple to a peptide NH resonance must originate from Pro. This assignment was further supported by spin decoupling of the αCH proton from a βCH_2 absorption at 2.00 ppm, which is known to be the position of the β-methylene peak of Pro of oxytocin.

TABLE 1. *Chemical shifts and αCH–NH coupling constants of the peptide NH resonances of oxytocin and lysine vasopressin*

Sequence position	Amino-acid residue: Lys-VP	Amino-acid residue: Oxytocin	Coupling constant* (αCH–NH, in Hz): LysVP†	Coupling constant* (αCH–NH, in Hz): Oxytocin‡	Chemical shift (at 20°, in Hz): Lys-VP	Chemical shift (at 20°, in Hz): Oxytocin
2	Tyr	Tyr	Broad	Broad	1876	1820 ± 10§
3	Phe	Ile	8 ± 1	4.4 ± 0.5	1942	1811
4	Gln	Gln	6 ± 1	6.6 ± 0.5	1815	1784
5	Asn	Asn	7 ± 1	6.1 ± 0.2	1760	1713
6	Cys	Cys	6 ± 1	7.5 ± 0.1	1875	1927
8	Lys	Leu	7 ± 1	7.3 ± 0.2	1815	1776
9	Gly	Gly	5 ± 0.5	5.6 ± 0.2	1787	1755

* The coupling constants remained constant within experimental error between 20° and 40°.

† Value from a single spectrum.

‡ Values are the average of readings from at least three spectra.

§ Value obtained by extrapolation from elevated temperatures, see Fig. 3.

Double irradiation at a frequency of 4.29 ppm decoupled the only two unassigned peptide NH resonances at 8.51 and 8.78 that, therefore, must be associated with Tyr and Phe. In contrast to von Dreele *et al.* (13), we were unable to distinguish between the Tyr and Phe NH resonances on the basis of spin decoupling because double irradiation of the overlapping αCH resonances at 4.29 ppm in Me_2SO of Tyr and Phe invariably decoupled both NH resonances. We assigned the well-resolved lowest field resonance at 8.78 ppm to the peptide NH proton of Phe, and to Tyr the very broad resonance that was a shoulder on the low-field side of the Cys-6 NH absorption. This assignment was derived from analogous broadening of the Tyr NH absorption in oxytocin and several of its analogs (7, 11). Also in line with the current assignment was the similar and characteristic behavior of the Tyr NH resonances in terms of broadening and resonance displacements after the addition of H_2O to deuterated Me_2SO solvent.

We had previously assigned the primary amide protons of Asn, Gln, and Gly-NH_2 (6) in LysVP (as indicated in Fig. 2) by similarities to oxytocin with respect to peak positions and temperature-dependence in deuterated Me_2SO (6). The PMR spectra of synthetic LysVP intermediates, by the approach described for oxytocin (7), have secured these identifications. According to Anet and Bourn (19), the lower-field resonance

of each primary carboxamide two-line pattern corresponds to the *trans* proton, and the higher-field resonance corresponds to the *cis* proton. The assignments of the primary carboxamide resonances in LysVP were confirmed by von Dreele *et al.* (14). The positions of aromatic C*H* protons of Tyr and Phe in LysVP have also been identified (6, 14).

The above assignments serve as a point of departure for a conformational analysis of LysVP. In Fig. 3, we compare the temperature-dependence of low-field resonances of LysVP and oxytocin, and the slopes associated with the individual proton resonances are indicated. A significant feature of the data is the greater temperature-dependence of N*H* resonances as compared to C*H* absorptions; the aliphatic C*H* resonances and the aromatic C*H* peaks varied only slightly. This argues that conformational changes and alterations in vibrational, rotational, and electronic energy levels do not contribute much to the observed spectral changes, although small localized conformational or energy perturbations cannot be excluded. Thermal disruption of hydrogen bonds, therefore, appears to be the dominant cause of the high-field shift in all but one of the N*H* resonances, namely, the peptide N*H* of Asn in LysVP (Fig. 3). It is to be expected that the greater lability of hydrogen bonds formed by peptide residues exposed to the solvent, as compared to those of peptide residues not accessible to solvent (whose hydrogen bonds may even be thermally stabilized by lyophobic interactions), would be reflected in a greater temperature-dependence. This criterion for distinguishing "internal" from "external" hydrogen bonds has been used in defining the structures of cyclic hexapeptides (20, 21), gramicidin S-A, and valinomycin (22, 23)—structures that were in general agreement with those derived independently by PMR (24, 25), tritium exchange (26), and x-ray (27–29) determinations. Similarly, temperature-dependencies of chemical shifts (8), deuterium exchange rates (11), and αC*H*–N*H* coupling constants (7, 8) have been the basis for defining the solution conformation of oxytocin (8, 12). Recent x-ray studies by Rudko *et al.* (30) of *S*-benzyl-L-cysteinyl-L-prolyl-L-leucyl-glycinamide and of its virtually isomorphous seleno analog support in detail the conformation proposed by Urry and Walter (12) for the acyclic, terminal tetrapeptide sequence of oxytocin in deuterated Me_2SO solution. LysVP retains the low temperature-dependence of the Asn peptide resonance, which is indicative of a β-turn (23) or β-fold (31, 32) involving a hydrogen bond between the Tyr C=O (termed residue i of the β-turn) and the Asn peptide N*H* (residue $i + 3$). A β-turn is further characterized by a peptide N*H* absorption of residue $i + 3$, which is high-field shifted because of shielding by the N*H*-CO moiety of residues $i + 1$ and $i + 2$ (Phe and Gln in LysVP); that is, the peptide N*H* proton of Asn is close to the face of the planar Phe–Gln peptide bond and is subjected to the magnetic anisotropy of this moiety.

The αC*H*–N*H* coupling constants are a measure of the dihedral angle defined by the αC*H*–N*H* moieties (23, 33–35). With the exception of Phe against Ile, there was good agreement in the values of the αC*H*–N*H* coupling constants between the residues at corresponding sequence positions in LysVP and oxytocin (Table 1). The small αC*H*–N*H* coupling constant of Ile, and the large coupling constant of Gln in oxytocin, are consistent with expectations for residues $i + 1$ and $i + 2$ in the β-turn. However, in LysVP the αC*H*–N*H* coupling constant of Phe was larger than would be anticipated; the anomalies in coupling constant and chemical shift of the N*H* resonance of Phe appear to be associated in some way with an interaction between Phe and Tyr. The upfield shift of the aromatic *meta* C*H* proton resonances of Tyr in LysVP as compared to that of oxytocin (Table 1) may also reflect such an interaction. Perhaps this effect is similar to those described for these hormones in water by Deslauriers and Smith (36). If the cystine residue that closes the 20-membered ring component of LysVP possesses a right-handedness, as was proposed for oxytocin (9, 10), we can conclude that the gross backbone conformation of the ring component of LysVP in Me_2SO is similar to that of oxytocin despite some distortions.

As regards the acyclic, terminal peptide sequence of LysVP, the proline αC*H* resonance at 4.29 ppm favors a *trans* Cys–Pro bond. We had reached the same conclusion for oxytocin (12). Such a *trans* conformation is in line with studies on *trans* prolyl-containing peptides in deuterated Me_2SO (37). The αC*H*–N*H* coupling constants in LysVP of Lys and Gly and their peptide N*H* temperature coefficients were essentially the same as those of Leu and Gly in oxytocin; however, there are discrepancies between the chemical shifts of the peptide N*H* resonances of LysVP and those of oxytocin, including those at the acyclic dipeptide (Table 1). The greater lyophilic quality of the Lys side chain of LysVP as compared to the lyophobic Leu side chain of oxytocin, is expected to render the acyclic, terminal tripeptide moiety of LysVP more flexible. Coulombic interaction between the charged ϵ-amino group of Lys with a second cationic site on LysVP would also favor a looser structure for LysVP than for oxytocin.

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