Conformational Studies on [3-D-Alanine]-oxytocin and [4-D-Alanine]-oxytocin in Dimethyl Sulfoxide by ^1H Nuclear Magnetic Resonance Spectroscopy. Interpretation in Terms of a β Turn in the Cyclic Moiety

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Abstract: A model for the conformation of oxytocin in Me_2SO has previously been proposed in which residues 3 and 4 occupy the corner positions of a β turn. The analogues [D-Ala^3]-oxytocin (3D-Ala) and [D-Ala^4]-oxytocin (4D-Ala) were synthesized for use in proton magnetic resonance (^1H NMR) studies designed to probe the contribution of these corner positions to the formation of a β turn. Comparison of various ^1H NMR parameters obtained at 220 MHz for backbone amide protons of 3D-Ala and 4D-Ala with those for the corresponding protons of oxytocin suggests that the backbone conformation of 3-Ala may be quite different from that of oxytocin in Me_2SO-d_6, while that of 4D-Ala may be rather similar to that of oxytocin in Me_2SO-d_6. Theoretically, the L → L sequence in positions 3 and 4 of oxytocin would allow the formation of either a type I or type II β turn, while the corresponding D → L sequence in 3D-Ala and the L → D sequence in 4D-Ala would allow the formation of only a type II′ β turn and type II β turn, respectively. The coupling constants between vicinal amide and C^α protons for residues 3 and 4 of oxytocin, 3D-Ala, and 4D-Ala in Me_2SO-d_6 are compatible with these residues being corner positions in the allowable types of β turn, while the spectral similarities between 4D-Ala and oxytocin may indicate that these two peptides can form the same type of β turn, which for 4D-Ala is a type II turn.

One of our major goals is to establish the conformation-function relationship for neurohypophyseal hormones and their analogues.[2,3] On the basis of proton magnetic resonance (^1H NMR) studies, it was proposed[4,5] that oxytocin in Me_2SO-d_6 contains a β turn[6-9] involving residues 2–5 in the 20-membered tocin-ring (cyclic) moiety formed by residues 1–6 and that this β turn is stabilized by an intramolecular hydrogen bond between the backbone CO of Tyr^2 and the backbone NH of Asn^5. Subsequently, on the basis of ^1H NMR studies combined with conformational energy calculations, Brewster et al.[10] proposed that oxytocin in Me_2SO-d_6 consists of an equilibrium mixture of three different conformers of comparable energy. While one of these three conformers corresponds to the structure proposed for oxytocin,[4,5] the other two manifest a different pattern of intramolecular hydrogen bonding and do not contain a β turn. Though conformational averaging is more prevalent than originally thought, the β turn involving residues 2–5 appears to be the major conformational feature of these residues,[4,5] and the other two structures proposed by Brewster et al.[10] do not seem to be present to any significant degree.[11]

Theory has shown that β turns in which both corner positions have an L configuration may be of either two types,[6-9] designated type I and type II (Figure 1). Examples of both of these types have been found in proteins.[9,12] In oxytocin the values of the coupling constants between the vicinal amide and C^α protons for the corner residues (Ile^3 and Gln^4) are compatible with both type I and type II β turns (see Table III below). It should be possible, however, to synthesize analogues with particular residues in the corner positions that create steric restrictions that energetically favor the formation of a specific type of β turn. Steric considerations[6,9] indicate that a type II′ β turn[13] dissimilar to that proposed for oxytocin should be favored when a residue with a D configuration is placed in the first corner position (residue 3), while a type II β turn is favored when such a residue is placed in the second corner position (residue 4); see Figure 2. We chose to synthesize [D-Ala^3]-oxytocin (3D-Ala)[14] and [D-Ala^4]-oxytocin (4D-Ala)[14] as model compounds for type II′ and type II β turns, respectively.[15] We proceeded on the hypothesis that if residues 3 and 4 do indeed form the corner positions of a β turn, then (a) it should be possible to synthesize these two analogues without too much difficulty inasmuch as D → L and L → D sequences are theoretically quite favorably disposed toward formation of β turns, (b) the values of the coupling constants between vicinal amide and C^α protons for the corner positions (residues 3 and 4) should be compatible with the predicted types of β turn, (c) the formation of β turns should permit the analogues to assume backbone conformations similar, to a first approximation, to the conformation of oxytocin, and (d) certain NMR parameters for analogues possessing a β turn similar to the β turn in oxytocin should resemble the corresponding ones for oxytocin more closely than do those for analogues possessing dissimilar β turns.

Materials and Methods

Synthesis and some biological properties of [D-Ala^3]-oxytocin (3D-Ala) and [D-Ala^4]-oxytocin (4D-Ala) used in this study have been reported.[17] Continuous wave ^1H NMR spectra of samples at a concentration of 6% (w/v) in Me_2SO-d_6 containing $(CH_3)_4Si$ as an internal standard were recorded at 220 MHz on a Varian Associates HR-220 NMR spectrometer as described previously.[18] In order to identify resonances difficult to resolve at 220 MHz, an additional experiment was performed at 360 MHz employing a Bruker HX-360 spectrometer operating in the Fourier transform mode. In the latter experiment, the sweep width was 4800 Hz, 8K data points were obtained, and the resolution was enhanced by the convolution difference technique.[19] This involved Fourier transformation of the function FID (LB 0.1 Hz) − 0.8 FID (LB 20 Hz), where FID represents the free induction decay and LB indicates exponential multiplication to yield the indicated line broadening.

Results

Assignment of Proton Resonances. Comparison of the 220-MHz ^1H NMR spectrum of 3D-Ala shown in Figure 3 and that of 4D-Ala shown in Figure 4 with previously assigned spectra of oxytocin,[18,20] lysine vasopressin,[18,21] and deamino analogues of lysine vasopressin[22] provides a basis for the pre-

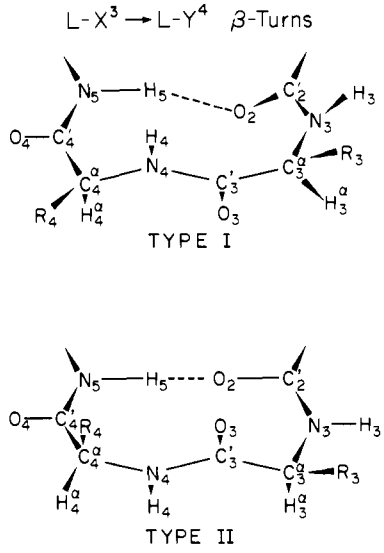

Figure 1. Type I and type II β turns with amino acid residues of the L configuration in consecutive corner positions. Numbering of residues corresponds to that of the residues proposed to be involved in the cyclic (ring) moiety of oxytocin in Me_2SO-d_6 in which positions 3 and 4 are isoleucyl and glutaminyl residues, respectively. R denotes a side chain.

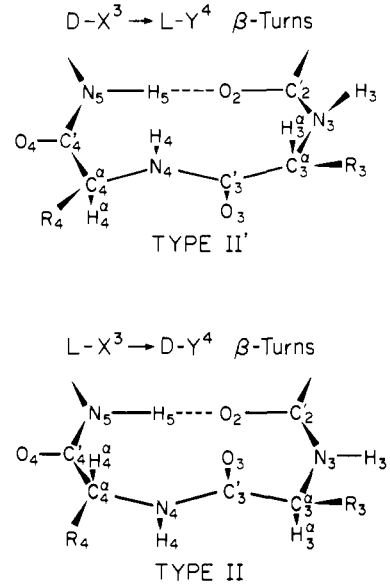

Figure 2. Type II' and type II β turns with amino residues of the D → L and L → D configurations, respectively, in consecutive corner positions. Numbering of residues for the type II' and type II β turns corresponds to that for [D-Ala³]-oxytocin (3D-Ala) and [D-Ala⁴]-oxytocin (4D-Ala), respectively. In 3D-Ala positions 3 and 4 correspond to D-alanyl and glutaminyl residues, respectively, while in 4D-Ala, to isoleucyl and D-alanyl residues, respectively.

liminary assignments of $C^{\alpha}H$ resonances. Double resonance experiments indicated in Figures 3 and 4 were used to obtain a self-consistent set of assignments and to identify resonances of backbone amide protons. The procedure was straightforward except for the following sources of ambiguity. (a) In 3D-Ala, overlap of the $C^{\alpha}H$ resonances of Tyr² and Asn⁵ at 220 MHz complicated the assignments of the corresponding backbone N*H* resonances. The resonances of these latter two protons were distinguished by the characteristic broadness and low-field position of the Tyr² N*H* resonance. Broadening of the resonance of the backbone amide proton on the residue penultimate to the N terminus results from rapid exchange of this proton, which is caused by the inductive effect of the nearby amino group.[23] The positively charged amino group also deshields the penultimate backbone amide proton and shifts its resonance to low field.[24] Similar exchange broadening of the resonance of the backbone amide proton penultimate to the NH_2 terminus has been observed in ^1H NMR spectra of other neurohypophyseal hormones and their analogues.[10,16,18,20-22,25,26] (b) In 3D-Ala, overlap of the $C^{\alpha}H$ resonances of D-Ala³ and Gln⁴ at 220 MHz prevented the unequivocal assignment of the corresponding backbone N*H* resonances. Furthermore, because the δ_{NH}'s of residues 3 and 4 are almost identical in 3D-Ala as well as quite similar in oxytocin (see δ_{NH} in Table I), they provided no rational basis for even a tentative assignment. An unambiguous assignment of these resonances as well as those of Tyr² and Asn⁵ was obtained at 360 MHz at 43 °C (Figure 5). (c) In 4D-Ala, overlap of the $C^{\alpha}H$ resonances of D-Ala⁴ and Leu⁸ complicated the assignments of the corresponding N*H* resonances. The similarity of the chemical shift of one of these two amide protons in 4D-Ala to that of Leu⁸ in oxytocin, however, provided a basis for the tentative assignment of Leu⁸ in 4D-Ala (see δ_{NH} in Table I).

Comparison of Spectral Parameters. Table I summarizes the chemical shifts of the backbone amide protons (δ_{NH}), the temperature dependences of these shifts ($\Delta\delta_{NH}/\delta T$), and the coupling constants between vicinal amide and C^{α} protons ($^3J_{NH\text{-}C^{\alpha}H}$) of oxytocin, 3D-Ala, and 4D-Ala. The chemical shifts of amide protons and the peak positions of aromatic protons of 3D-Ala and 4D-Ala, respectively, are shown as functions of temperature in Figures 6 and 7. Temperature coefficients have been employed to distinguish amide protons obstructed from contact with a solvent capable of forming hydrogen bonds (slightly positive, zero, or small negative values of $\Delta\delta_{NH}/\Delta T$) from those in contact with such a solvent.[27-36] Inaccessibility of an amide proton may result either from steric constraints or from involvement of that proton in the formation of an intramolecular hydrogen bond. The value of $^3J_{NH\text{-}C^{\alpha}H}$ reflects the dihedral angle ϕ, which defines the orientation about the N-C^{α} bond.[27,37-39] Table II summarizes the chemical shifts of the carboxamide protons of the side chains and the C-terminal group, the peak positions of the side-chain aromatic protons, and the temperature dependences of these parameters.

By the nonparametric Spearman rank-correlation test,[40] there is no significant correlation between the δ_{NH}'s of the six identical residues of 3D-Ala and oxytocin,[41] i.e., there appears to be significant scrambling of at least some of the backbone N*H* chemical shifts in going from oxytocin to 3D-Ala. At 20 °C the values of δ_{NH} for residues 2, 5, 8, and 9 of 3D-Ala are, on the average, 0.07 ppm downfield from those for the corresponding residues of oxytocin (Table I); in other words, the average change in spectral position is small for the backbone amide protons of these residues in going from oxytocin to 3D-Ala. The standard deviation (SD) for this change is 0.07 ppm, which means that the spread in data is comparable to the average change. The N*H* resonances for residues 3 and 4, the corner positions in the β turn proposed for the ring moiety, are unusually far downfield in 3D-Ala with respect to those for the corresponding residues in oxytocin. Indeed, the changes in δ_{NH} for residues 3 and 4 in 3D-Ala are approximately 2 and 4 SD, respectively, *downfield* from the average change in δ_{NH} for the aforementioned four residues. Furthermore, the change in δ_{NH} for Cys⁶ in 3D-Ala, a residue far removed from the site of amino acid substitution in position 3, is 12 SD *upfield* from this average change. There appears to be a significant decrease in the magnitude of $\Delta\delta_{NH}/\Delta T$ for Tyr² and Cys⁶, but an increase for Asn⁵; there is a decrease for residue 3, the site of amino acid substitution (Table I). On the other hand, only small changes in δ and $\Delta\delta/\Delta T$ for side-chain aromatic and

Table I. ^{1}H NMR Parameters for Backbone Amide Protons of [D-Ala^3]-Oxytocin (3D-Ala), Oxytocin, and [D-Ala^4]-Oxytocin (4D-Ala) in Me_2SO-d_6

	δ_{NH},[a] ppm			$\Delta\delta_{NH}/\Delta T$,[b] ppb/°C			$^3J_{NH-C^\alpha H}$,[c] Hz		
Residue	3D-Ala	Oxytocin[d]	4D-Ala	3D-Ala	Oxytocin[d]	4D-Ala	3D-Ala	Oxytocin[d]	4D-Ala
Tyr^2	8.35[e]	8.27 ± 0.05[e]	8.19 ± 0.05[e]	−2.7	−6.4	−6.1	6 ± 1	*f*	*f*
Ile^3 (D-Ala^3)[g]	8.46	8.23	8.43	−5.7	−11.6	−10.5	3.7 ± 0.2	3.9 ± 0.5[i]	4.8 ± 0.6
Gln^4 (D-Ala^4)[h]	8.50	8.11	8.59	−3.9	−4.8	−8.0	6.1 ± 0.2	6.6 ± 0.5	7.4 ± 0.1
Asn^5	7.96	7.79	7.90	−3.0	+0.2	−0.5	8 ± 1	6.1 ± 0.2	6.3 ± 0.2
Cys^6	7.88	8.76	8.91	−2.7	−11.1	−10.0	7.7 ± 0.3	7.5 ± 0.1	7.9 ± 0.5
Leu^8	8.09	8.07	8.11	−5.5	−6.8	−6.1	7.1 ± 0.5	7.3 ± 0.2	7.2 ± 0.1
Gly^9	7.99	7.98	7.98	−5.5	−5.9	−5.2	4.8 ± 1.0[j]	5.6 ± 0.2[j]	6 ± 1[j]

[a] Chemical shift at 20 °C downfield relative to $(CH_3)_4Si$ as an internal standard. [b] Temperature range is 15–37 °C for 3D-Ala, 18–44 °C for oxytocin, and 18–38 °C for 4D-Ala. [c] The average coupling constant and standard deviation of at least three measurements observed within the ranges used for the temperature dependence studies (see footnote *b*); no significant spectral changes are observed in these ranges. [d] From Walter et al.; see ref 18. [e] The observed chemical shift of the backbone amide protein of Tyr^2 in oxytocin and oxytocin analogues can be highly variable from one preparation to another; this shift seems quite dependent upon such factors as the contaminating water or trace amounts of acid in the sample. [f] The coupling constant cannot be extracted because the signal is too broad. [g] D-Ala^3 replaces Ile^3 in 3D-Ala. [h] D-Ala^4 replaces Gln^4 in 4D-Ala. [i] Corrected value reported here. [j] Average vicinal coupling constant.

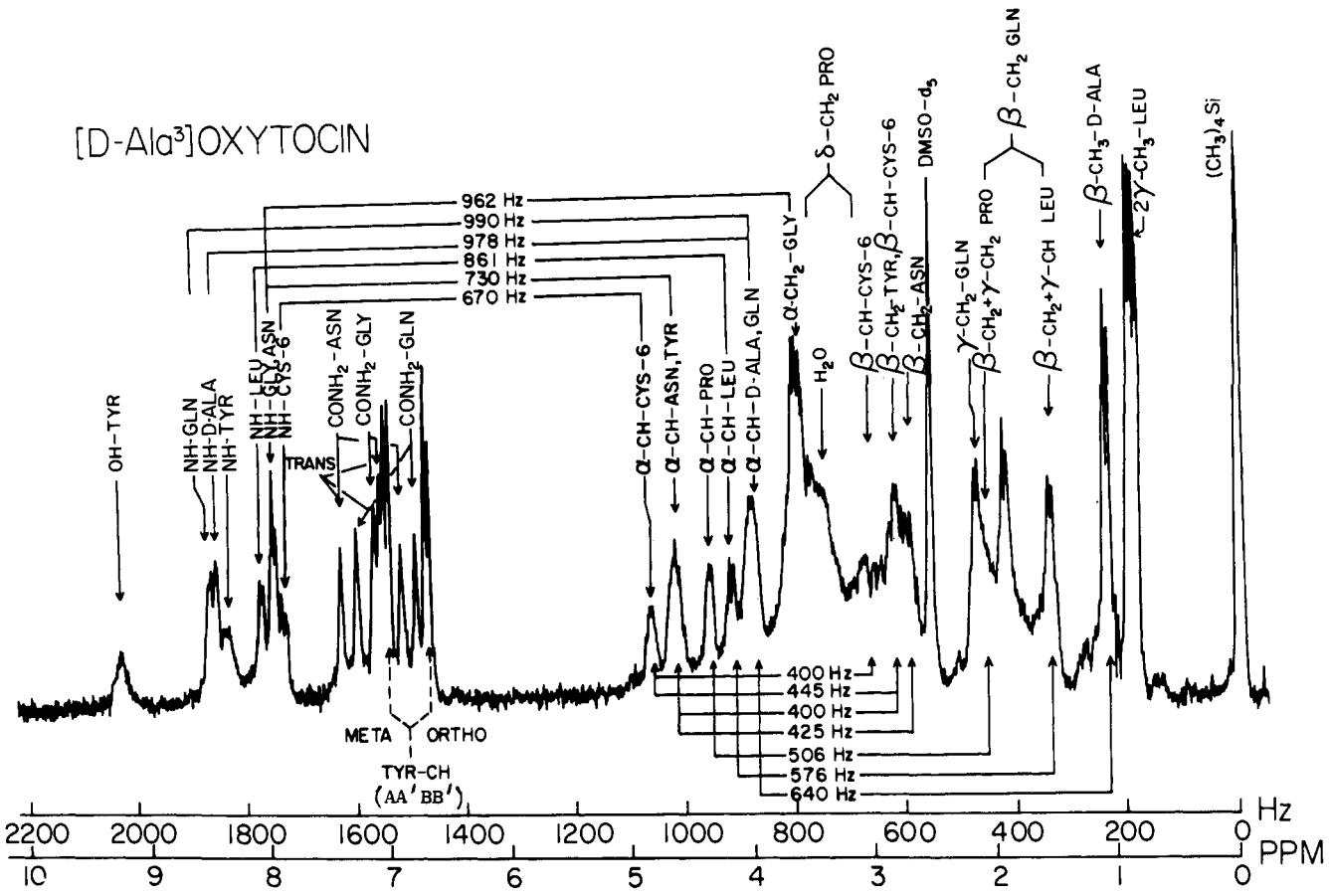

Figure 3. The assigned 220-MHz ^{1}H NMR spectrum of [D-Ala^3]-oxytocin (3D-Ala) in Me_2SO-d_6 at 20 °C. The concentration is 6% (w/v). Me_2SO-d_5 indicates the resonance from [$^1H_1$,$^2H_5$]dimethyl sulfoxide. Couplings detected by double-resonance experiments are denoted by bridges, and separations are given in hertz. Backbone amide protons are designated by N*H*; side-chain and C-terminal carboxamide protons by CONH_2; and side-chain aromatic protons of the tyrosyl residue that form an AA'BB' spin system, by meta and ortho. The meta and ortho resonances correspond predominantly to the two δ and two ϵ protons, respectively. The downfield and upfield resonances of each pair of carboxamide protons correspond to the protons that are trans and cis, respectively, to the vicinal carbonyl oxygen atom.[44]

carboxamide protons and for C-terminal carboxamide protons are observed (Table II).

In contrast, using the Spearman rank-correlation test, there is a significant correlation between the δ_{NH}'s of six identical residues of 4D-Ala and oxytocin,[42] i.e., there appears to be little scrambling of these shifts in going from oxytocin to 4D-Ala. At 20 °C, the values of δ_{NH} for residues 2, 5, 6, 8, and 9 of 4D-Ala are, on the average, 0.04 ppm downfield from those for the corresponding residues of oxytocin (Table I), while the SD for this change is 0.09 ppm. The N*H* resonances for residues 3 and 4 in 4D-Ala are approximately 2 and 5 SD, respectively, downfield from the average change in δ_{NH} for the aforementioned five residues. There appears to be no significant change in any of the values of $\Delta\delta_{NH}/\Delta T$, except for a possible increase in the magnitude of $\Delta\delta_{NH}/\Delta T$ for residue 4, the site of the amino acid substitution (Table I). Only small changes in δ and

Table II. ^{1}H NMR Parameters for Side-Chain Aromatic, Side-Chain Carboxamide, and C-Terminal Carboxamide Protons of [D-Ala^3]-Oxytocin (3D-Ala), Oxytocin, and [D-Ala^4]-Oxytocin (4D-Ala) in Me_2SO-d_6[a]

Proton	δ, ppm			Δδ/ΔT, ppb/°C		
	3D-Ala	Oxytocin[b]	4D-Ala	3D-Ala	Oxytocin[b]	4D-Ala
Tyr^2 meta 1[c]	7.01	7.12	7.15	0	−1.1	−0.7
2[c]	6.97	7.09	7.12	0	−1.4	−0.9
Tyr^2 ortho 1[c]	6.68	6.67	6.67	0	0	0
2[c]	6.64	6.64	6.64	0	0	0
Gln^4 cis	6.80	6.80		−5.7	−5.7	
trans	7.27	7.29		−4.1	−5.0	
Asn^5 cis	6.92	6.88	6.80	−5.5	−5.7	−4.3
trans	7.40	7.35	7.27	−4.6	−4.8	−4.8
Gly(NH$_2$)^9 cis	7.09	7.09	7.10 ± 0.02	−5.2	−5.7	−3.2 (−6.1)[d]
trans	7.12	7.12	7.10 ± 0.02	−4.1	−5.2	−6.1 (−3.2)[d]

[a] Footnotes *a* and *b* of Table I also apply to this table. [b] From Walter et al.; see ref 18. [c] Peak positions rather than chemical shifts are reported. [d] The C-terminal carboxamide N*H* resonances of Gly(NH$_2$)^9 cannot be distinguished from each other.

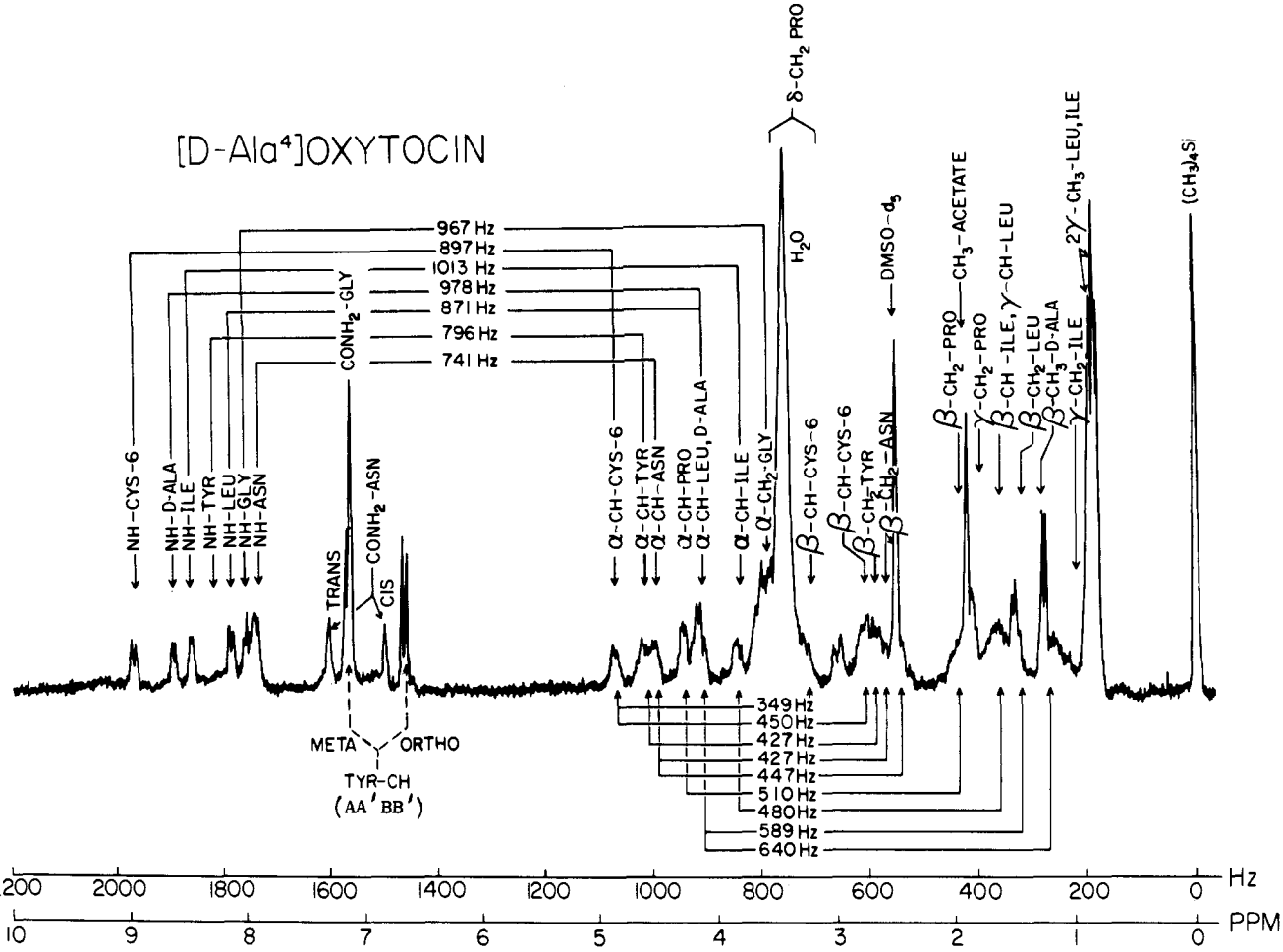

Figure 4. The assigned 220-MHz ^{1}H NMR spectrum of [D-Ala^4]-oxytocin (4D-Ala) in Me_2SO-d_6 at 20 °C. See the caption of Figure 3 for additional comments. Note the ambiguity in the assignments of the N*H* resonances for D-Ala^4 and Leu^8 because of overlap of the C$^\alpha$ proton resonances for these two residues; see the text for justification of the tentative assignments shown in this figure.

$\Delta\delta/\Delta T$ for side-chain aromatic and carboxamide protons and for C-terminal carboxamide protons are observed (Table II).

Discussion

Possible β Turns Involving Residues 2–5. The relatively low values of the coupling constants between vicinal amide and C$^\alpha$ protons ($^3J_{NH\text{-}C^\alpha H}$) for residue 3 in oxytocin, [D-Ala^3]-oxytocin (3D-Ala), and [D-Ala^4]-oxytocin (4D-Ala) are not consistent with extensive conformational averaging about the N-C$^\alpha$ bond of this residue (ϕ_3). A small value of $^3J_{NH\text{-}C^\alpha H}$ (in general, <5 Hz) is compatible with a residue being in the first corner position of a β turn, and therefore the hypothesis that residues 3 and 4 in the aforementioned three peptides form the corner positions of such a β turn is tenable and worth subjecting to further experimental exploration. In general, however, the value of $^3J_{NH\text{-}C^\alpha H}$ provides no criterion for distinguishing type I and II β turns inasmuch as ϕ for the first corner position is

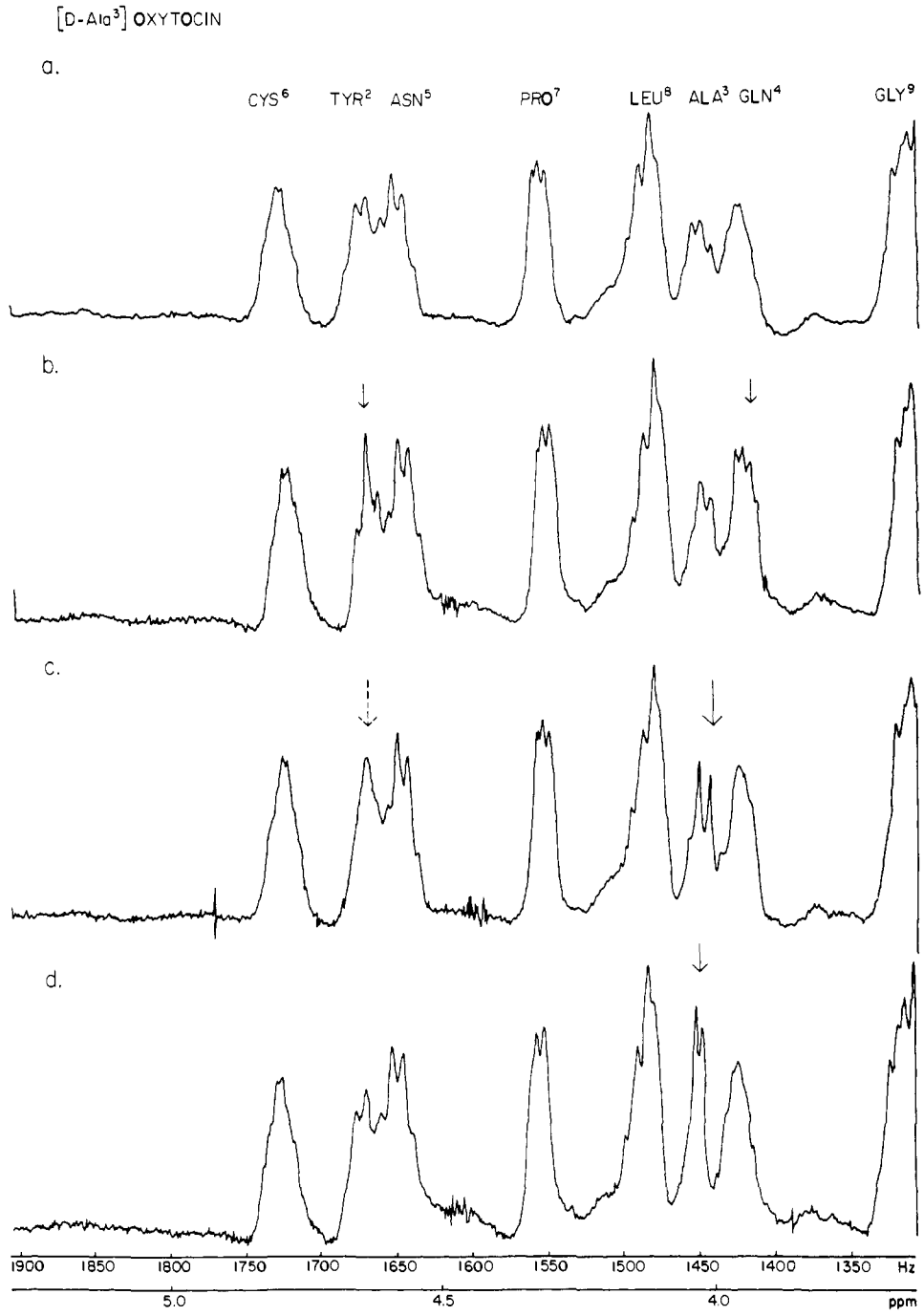

Figure 5. Double resonance experiments of [D-Ala³]-oxytocin (3D-Ala) at 360 MHz showing the $C^{\alpha}H$ region of the spectrum (43 °C): (a) no double irradiation, (b) the lowest field NH (Gln⁴) irradiated, (c) the next lowest field NH (D-Ala³) irradiated, and (d) the D-Ala³ CH_3 irradiated. Arrows indicate the $C^{\alpha}H$ resonances which are decoupled. The broad Tyr² NH, which overlaps strongly with the D-Ala³ NH and weakly with the Gln⁴ NH, is completely decoupled when the former resonance is irradiated, and is only partially decoupled when the latter peak is saturated. These experiments confirm the Tyr², D-Ala³, Gln⁴, and Asn⁵ assignments presented in the text.

approximately the same for both of these types (see Figure 1); a similar statement applies to type I' and type II' β turns.

Table III shows the possible β turns with residues 3 and 4 as corner positions that are compatible with the observed values of $^3J_{NH\text{-}C^{\alpha}H}$ for these residues in oxytocin, 3D-Ala, and 4D-Ala. Any β turn that might be formed by residues 2–5 could be stabilized by an intramolecular hydrogen bond between the backbone C=O of residue 2 and the backbone NH of residue 5. The extremely small magnitude of the temperature dependences of the chemical shifts of the backbone NH's ($|\Delta\delta_{NH}/\Delta T|$) of Asn⁵ in oxytocin and 4D-Ala suggests that these protons are obstructed from contact with solvent (Table I). The relatively small value of $\Delta\delta_{NH}/\Delta T$ of Asn⁵ in 3D-Ala also suggests that this proton is somewhat inaccessible to the solvent, but to a lesser degree than the corresponding proton in either oxytocin or 4D-Ala.

Differences between 3D-Ala and Oxytocin. There are striking differences between certain corresponding ^{1}H NMR parameters for the amide protons of oxytocin and 3D-Ala (Table I). For example, there is a large, unexplained upfield shift of almost 1 ppm of the backbone NH resonance of Cys⁶ in 3D-Ala relative to the corresponding resonance of oxytocin. Furthermore, as can be judged from the magnitude of $\Delta\delta_{NH}/\Delta T$, the backbone amide protons of Tyr² and Cys⁶ appear to be significantly more obstructed from contact with the solvent in 3D-Ala than in oxytocin. Thus, it may well be that the back-

Table III. Possible β Turns with Residues 3 and 4 as Corner Positions in [D-Ala^3]-Oxytocin (3D-Ala), Oxytocin, and [D-Ala^4]-Oxytocin (4D-Ala) in Me_2SO-d_6 at 20 °C

		First corner position					Second corner position				
				ϕ_3,[c] deg					ϕ_4,[c] deg		
Peptide	Type of β turn[a]	Residue	$^3J_{NH-C^\alpha H}$,[b] Hz	Exptl[d]	Theor.[e]	Overlap[f]	Residue	$^3J_{NH-C^\alpha H}$,[b] Hz	Exptl[d]	Theor.[e]	Overlap[f]
3D-Ala	II_{DL}'	D-Ala^3	3.7 ± 0.2	+60 → +70	+45 → +75	+60 → +70	Gln^4	6.1 ± 0.2	−86 → −74	−155 → −45	−86 → −74
Oxytocin	I_{LL}	Ile^3	3.9 ± 0.5	−73 → −59	−65 → −35	−65 → −59	Gln^4	6.6 ± 0.5	−91 → −76	−135 → −85	−91 → −85
Oxytocin	II_{LL}	Ile^3	3.9 ± 0.5	−73 → −59	−65 → −45	−65 → −59	Gln^4	6.6 ± 0.5	+26 → +50	+35 → +65	+35 → +50
4D-Ala	II_{LD}	Ile^3	4.8 ± 0.6	−79 → −65	−75 → −45	−75 → −65	D-Ala^4	7.4 ± 0.1	+83 → +94	+45 → +155	+83 → +94
									(+146 → +157)[g]		(+146 → +155)

[a] For β turn nomenclature see ref 6 and 9. [b] Coupling constants are taken from Table I. [c] Recent convention is used to express ϕ.[43] [d] The *experimental* range of ϕ is calculated from the Karplus relationship given in Figure 3 of Bystrov et al.[37] Observed values of $^3J_{NH-C^\alpha H}$ are multiplied by 1.09, the correction factor for the electronegativities of the substituents on the C^α atom, before being used in the Karplus relationship. The range of ϕ accounts for both the variation in the observed value of $^3J_{NH-C^\alpha H}$ and the uncertainty in the Karplus relationship. In general, up to four different values of ϕ correspond to each value of $^3J_{NH-C^\alpha H}$. Only values of ϕ compatible with β turns are given here. [e] The theoretical range of ϕ is taken from Table 1 of Chandrasekaran et al.[9] Because 10° intervals are used in the theoretical calculations, the lower and upper boundaries reported here are decreased and increased, respectively, by 5° from those in the original table. [f] The *overlap* of ϕ corresponds to the common interval of the *experimental* and *theoretical* ranges. [g] Two ranges of ϕ are compatible with the second corner position of a type II_{LD} β turn.

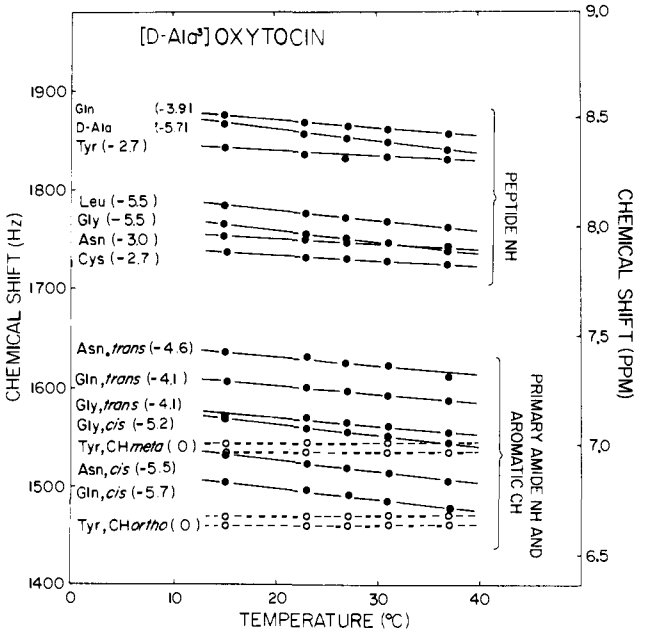

Figure 6. The temperature dependences of the chemical shifts of protons that resonate in the amide-aromatic region of the 220-MHz spectrum of [D-Ala^3]-oxytocin (3D-Ala) in Me_2SO-d_6 over the range 15–37 °C. Peak positions rather than chemical shifts are shown for the resonances of the aromatic protons. Values shown within parentheses are the dependences in ppb/°C; over the temperature range used 0.1 ppb/°C corresponds to 0.5 Hz. See the caption of Figure 3 for a description of the designations cis, meta, ortho, and trans.

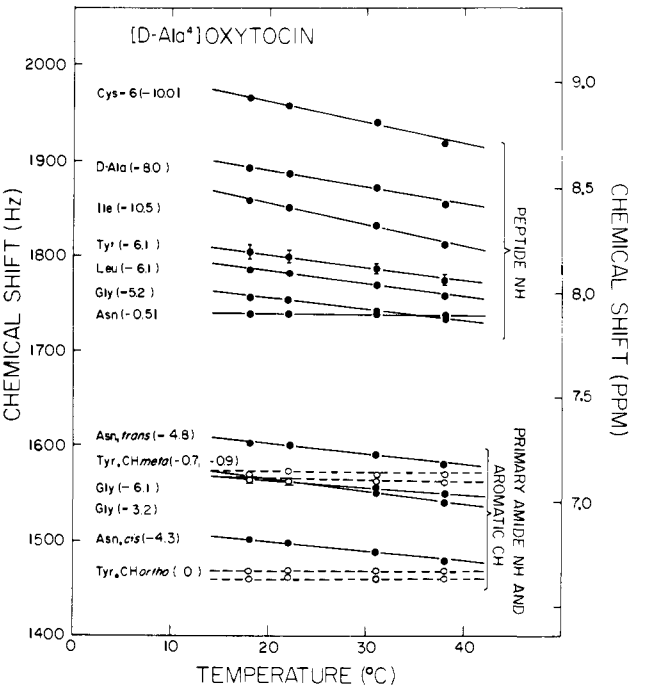

Figure 7. The temperature dependences of the chemical shifts of protons that resonate in the amide-aromatic region of the 220-MHz spectrum of [D-Ala^4]-oxytocin (4D-Ala) in Me_2SO-d_6 over the range 18–38 °C. See the caption of Figure 6 for additional comments.

bone conformations of these two peptides are quite different.

Theoretically, residues 3 and 4 of 3D-Ala, but not those of oxytocin, could occupy the corner positions of a type II′ β turn;[6-9] those of oxytocin, however, could occupy a type I β turn, which is similar to a type II′ β turn with respect to the orientation of the C=O group in the peptide moiety that links the corner residues, but quite different with respect to the conformations about certain bonds along the backbone in the region of the β turn (cf. type I in Figure 1 with type II′ in Figure 2). Thus, the hypothesis that residues 2–5 of oxytocin form a β turn leads to the prediction that the corresponding residues of 3D-Ala cannot form a similar type of β turn and to the expectation that the predicted conformational differences might be reflected in spectral differences; in other words, the observed spectral differences are not particularly surprising if the aforementioned hypothesis is correct.

Similarities between 4D-Ala and Oxytocin. The similarity of corresponding ^1H NMR parameters of the backbone amide protons of oxytocin and 4D-Ala (Table I) suggests that the

backbone conformations of these peptides in Me_2SO-d_6 may be quite similar. Theory predicts that residues 3 and 4 of 4D-Ala could occupy the corner positions of a type II but not a type I β turn, while those of oxytocin could occupy the corner positions of either of these two types.[6,9] Although the similarity of spectral parameters between 4D-Ala and oxytocin may indicate that these two peptides possess the same type of β turn in the cyclic moiety, we believe that further studies are needed, particularly of residues 3 and 4 in oxytocin per se, before a definite conclusion can be reached about the type of β turn in the latter peptide.

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(13) A type II' β turn is a mirror image of a type II β turn and is similar to a type I β turn with respect to the orientation of the backbone carbonyl oxygen atom of the residue in the first corner position.[6,9]
(14) Abbreviations used are: 3D-Ala, [D-Ala^3]-oxytocin; 4D-Ala, [D-Ala^4]-oxytocin; δ_{NH}, chemical shift of a backbone amide proton; $\Delta\delta_{NH}/\Delta T$, temperature coefficient of δ_{NH}; $^3J_{NH\text{-}C^\alpha H}$, coupling constant between vicinal amide and C^α protons; SD, standard deviation. Optically active amino acid residues are of the L configuration unless otherwise noted.
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(41) The appropriate chemical shifts for the residue at the site of substitution (residue 3) are not included in the test because of the possibility that a difference in δ_{NH} between 3D-Ala and oxytocin for this residue might reflect the difference in primary structure rather than a possible difference in conformation. Spearman's coefficient of rank correlation (r_s) is 0.086, and therefore the hypothesis that there is no correlation between backbone N*H* chemical shifts in 3D-Ala and oxytocin cannot be rejected because, with six pairs of data, the hypothesis can be rejected at the 0.05 level of significance only when $r_s > 0.886$. Acceptance of the hypothesis does not mean that it is necessarily correct, i.e., there may indeed be some degree of correlation between some of the chemical shifts. The acceptance does indicate, however, that there is a significant degree of scrambling of some of the chemical shifts in going from oxytocin to 3D-Ala. Inclusion of data for residue 3 does not change the conclusion of the test.
(42) The appropriate chemical shifts for the residue at the site of substitution (residue 4) are not included in the test. Spearman's coefficient (r_s) is 0.943, and therefore the hypothesis that there is no correlation between backbone N*H* chemical shifts in 4D-Ala and oxytocin can be rejected at the 0.02 level of significance because the critical value of r_s for rejection at this level happens to be 0.943, i.e., the probability is approximately 1 in 50 that the correspondence between the chemical shifts in these two peptides could have occurred by chance. Inclusion of data for residue 4 does not change the conclusion of the test, but the level of rejection in this case is between 0.02 and 0.05.
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