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Solution Conformation of Thymopoietin$_{32-36}$: A Proton Nuclear Magnetic Resonance Study†

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ABSTRACT: The aqueous solution conformation of Arg-Lys-Asp-Val-Tyr (TP5), corresponding to positions 32–36 of the thymic hormone thymopoietin has been investigated by proton nuclear magnetic resonance (NMR). This pentapeptide fragment retains the biological activity of the parent protein, viz., induction of selective differentiation of T lymphocytes. All the observed NH and CH resonances of TP5 have been assigned, and the solution conformation of this peptide has been investigated by analysis of chemical shift variations with pH, vicinal NH–C$^{\alpha}$H coupling constant data, and amide hydrogen exchange rates. The latter were measured in H_2O by using a combination technique consisting of the transfer of solvent saturation and saturation recovery NMR experiments. The data are compatible with the assumption of a highly motile dynamic equilibrium among different conformations for TP5. A comparison of the amide hydrogen exchange rates of the pentapeptide with that of solvated model compounds shows that Val^4-NH is significantly shielded from the solvent. In addition, the chemical shift variations with pH suggest that the guanidino-N$^{\epsilon}$H of arginine is associated with one of the carboxylate groups. These observations provide specific boundary conditions for the construction of molecular models of the conformation(s) of TP5 in aqueous solution.

Thymopoietin is a polypeptide hormone of the thymus. The 49 amino acid sequence of this hormone has been determined (Schlesinger & Goldstein, 1975). Thymopoietin has a number of biological activities. Initially, it was detected and isolated by its effects on neuromuscular transmission (Goldstein & Hofmann, 1969; Goldstein, 1974). Subsequently, thymopoietin was shown to induce early T cell differentiation (Basch & Goldstein, 1974), and this action was quite selective in that thymopoietin inhibited early B cell differentiations (Scheid et al., 1978). In addition to generating lymphocyte populations by induction of early T cell differentiation, thymopoietin also appears to regulate more mature populations (Sunshine et al., 1978; Weksler et al., 1978). Two synthetic fragments were shown to be biologically active—first a tridecapeptide corresponding to positions 29–41 (Schlesinger et al., 1975) and, subsequently, a pentapeptide corresponding to positions 32–36 (Goldstein et al., 1979). Thus, the synthetic pentapeptide arginyllysylaspartylvalyltyrosine (TP5) appears to correspond to a biologically active site of the parent thymopoietin molecule. Information concerning its solution conformation would be of interest in relation to the activity of the parent hormone.

Retention of the thymopoietic activity by TP5 suggests that this fragment may be capable of assuming a conformation similar to the active site of thymopoietin. We have, therefore, concentrated on defining the conformation–activity relationship in this smaller molecule which is more amenable to study by physicochemical techniques. Toward this end, we have employed NMR[1] to characterize the free solution conformation

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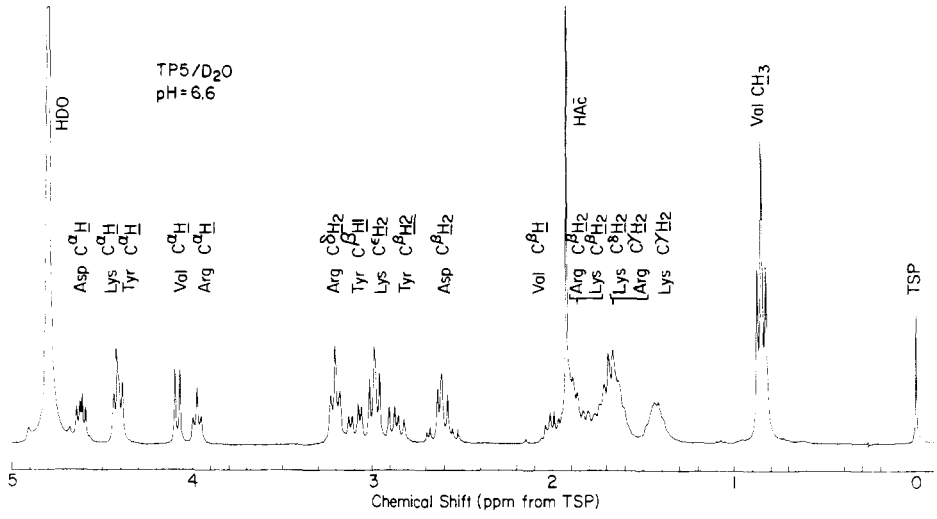

FIGURE 1: The 270-MHz proton Fourier transform NMR spectrum (0–5 ppm) of TP5 (Arg-Lys-Asp-Val-Tyr) in D_2O.

of TP5 by a study of the pH dependence of ^1H chemical shifts, analysis of NH–C$^\alpha$H vicinal coupling constants, and measurement of amide hydrogen exchange rates.

Experimental Procedures

(1) Sample Preparation and Materials. The pentapeptide, TP5, was prepared by solid-phase synthesis. A concentration of ~30 mM in D_2O or H_2O was employed in the proton NMR studies. The sample pH was varied by the addition of appropriate amounts of HCl (DCl) or NaOH (NaOD). The reported pH values in D_2O are the direct pH meter readings standardized with *proteo* buffers and have not been corrected for deuterium isotope effects. This procedure was followed since the isotope effects on the glass electrode approximately cancel those on the ionization constants (Bundi & Wüthrich, 1979a).

(2) NMR Studies. The proton NMR spectra of TP5 were recorded in the Fourier transform (FT) NMR mode at 270 MHz for samples in D_2O and in the correlation NMR mode (Dadok & Sprecher, 1974; Gupta et al., 1974) at 250 MHz for samples in H_2O solution. The assignment of some crucial resonances was accomplished by straightforward spin decoupling experiments in the FT mode and by continuous underwater decoupling (Dadok et al., 1972) in the correlation and FT NMR modes. The chemical shifts of various resonances of TP5 were measured with respect to TSP as the internal reference. The observed chemical shifts were corrected for the titration of this reference (DeMarco, 1977) and were finally expressed with respect to the position of the TSP peak under acidic conditions. All the studies were performed in 5-mm OD standard NMR sample tubes.

Transfer of solvent (H_2O) saturation experiments were performed at ambient probe temperature (30 ± 1 °C) by employing the 250-MHz Carnegie-Mellon University spectrometer operating in the correlation mode as previously described (Glickson, 1975; Glickson et al., 1976, 1977; Krishna et al., 1979, 1980b). The apparent spin–lattice relaxation times of the NH hydrogens of TP5 in H_2O (with 10% D_2O for deuterium lock) were measured at the same temperature on the Brandeis University 270-MHz FT NMR spectrometer employing the 2–1–4 pulse sequence (Redfield et al., 1975; Redfield, 1978). Relaxation times have not been corrected for the small difference in resonance frequencies. The apparent relaxation rates for each of the NH resonances were measured by selective saturation recovery experiments. The presaturation was sufficiently selective so as to minimize the perturbation of neighboring resonances and the water peak. A homospoil pulse applied τ seconds after presaturation was followed by the 2–1–4 observation sequence (typical pulse sequence: 162, 79, 316 μs) after allowing a short interval for recovery from the homospoil pulse. The apparent relaxation rates were estimated by a least-squares fitting of the experimental points (resonance intensity changes vs. τ) on a semilogarithmic scale to a straight line.

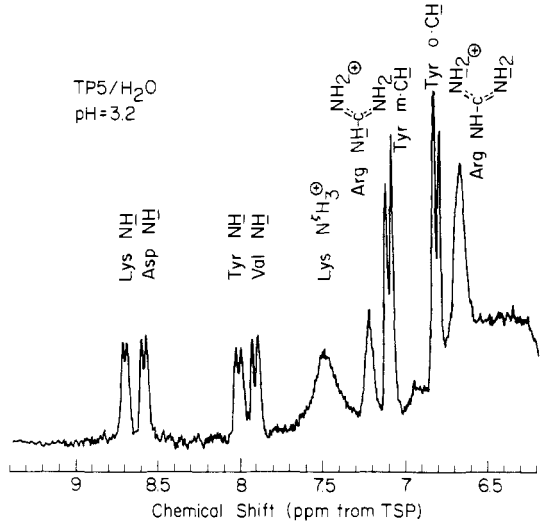

FIGURE 2: The 250-MHz correlation NMR spectrum of TP5 in H_2O showing the low-field proton resonances.

Results and Discussion

(1) Resonance Assignments. A typical 270-MHz proton NMR spectrum (0–5 ppm) originating from the nonexchanging hydrogens of TP5 in D_2O is shown in Figure 1. The corresponding spectrum in H_2O showing the aromatic CH and the exchangeable NH resonances appears in Figure 2. The various resonances have been assigned in a straightforward manner on the basis of (1) multiplet structure, (2) spin-decoupling experiments, and (3) the characteristic pK_a values exhibited by protons adjacent to sites of ionization in the molecule. Because of severe overlap, the multiplet structure

[1] Abbreviations used: NMR, nuclear magnetic resonance; TP5, thymopoietin$_{32-36}$; TSP, sodium 2,2,3,3-tetradeuterio-3-trimethylsilylpropionate.

of individual resonances in the region 1.3–2.0 ppm could be identified only approximately, on the basis of changes in the band shapes in spin-decoupling experiments. Higher operating frequencies and difference double resonance experiments should enable a more precise identification of the resonances within this spectral region (Gibbons et al., 1975).

The doublet at 4.1 ppm must belong to the Val-C$^{\alpha}$H proton since this is the only residue in TP5 with a single β hydrogen. Decoupling of this proton identified the Val-C$^{\beta}$H resonance. The C$^{\alpha}$H resonance of arginine has been identified by its pK_a = 7.41, which is in the range characteristic of N-terminal α-amino ionization in polypeptides (Greenstein & Winitz, 1961). Among the remaining three C$^{\alpha}$H resonances, two were identified on the basis of the multiplet structure exhibited by their β protons. The only amino acids in TP5 for which the α and β protons would be expected to exhibit characteristic ABX type patterns are aspartic acid and tyrosine. Thus, the multiplet structure at 3.1, 2.85, and 2.6 ppm must be associated with the β-proton resonances of these two amino acids. Decoupling of the α protons collapsed the corresponding β resonances to an AB pattern. The aspartic acid and tyrosine resonances were distinguished on the basis of the characteristic pH titrations of the C$^{\beta}H_2$ peaks (the average chemical shift of the two β protons has been monitored). The C$^{\beta}$H multiplet at 2.6 ppm exhibits a pK_a of 3.81 which is in reasonable agreement with the value of 3.9 ± 0.1 reported for the Asp-C$^{\beta}H_2$ resonances in the model tetrapeptide H-Gly-Gly-Asp-Ala-OH (Bundi & Wüthrich, 1979a). The C$^{\beta}H_2$ resonances at 2.85 ppm and at 3.1 ppm titrated with pK_a = 3.7, corresponding to ionization of the C-terminal carboxylate group of the tyrosine residue. Assignment of these peaks to the Tyr-C$^{\beta}H_2$ hydrogens is also supported by the high-field shift of these resonances at pH >9, which is attributed to the phenolic group titration. The assignment of the Asp and Tyr resonances was confirmed further by the magnitude of titration shifts exhibited by their respective C$^{\alpha}$H and C$^{\beta}H_2$ on the basis of their proximity to ionization sites. The remaining C$^{\alpha}$H peak was identified by exclusion as belonging to lysine; at pH 6.6 (Figure 1) its C$^{\alpha}$H resonance overlaps with that of tyrosine. Decoupling the Lys-C$^{\alpha}$H signal identified its C$^{\beta}H_2$ resonance position.

The two triplets at 3.2 ppm and at 3 ppm in Figure 1, with an intensity of two protons, have been assigned respectively to Arg-C$^{\delta}H_2$ and Lys-C$^{\epsilon}H_2$. This assignment is consistent with the chemical shift data on solvated peptides (McDonald & Phillips, 1969) and is further confirmed by the high-field shift of Lys-C$^{\epsilon}H_2$ for pH >9 because of ionization of the lysine side-chain amino group. The guanidino group is normally expected to titrate above pH 11.6. The remaining resonances in the band from 1.3 to 2 ppm have been identified by successive decoupling of the assigned resonances.

The low-field region in the NMR spectrum of TP5 in H_2O is shown in Figure 2. We have originally assigned the various backbone NH resonances by straightforward underwater decoupling of the corresponding CH protons in the correlation NMR mode (Dadok et al., 1972). We have recently confirmed these assignments by "underwater decoupling in the FT NMR mode". Typical results of these experiments are shown in Figure 3. In this procedure, the dynamic range problem associated with the strong H_2O signal has been minimized by presaturation of the water resonance (Redfield, 1978; Bleich & Glasel, 1975; Hoult, 1976; Krishna, 1976). At the end of presaturation, the decoupler frequency is set on the C$^{\alpha}$H that is to be decoupled, and a nonselective observation pulse is applied in the usual manner. The two doublets at 6.8 ppm

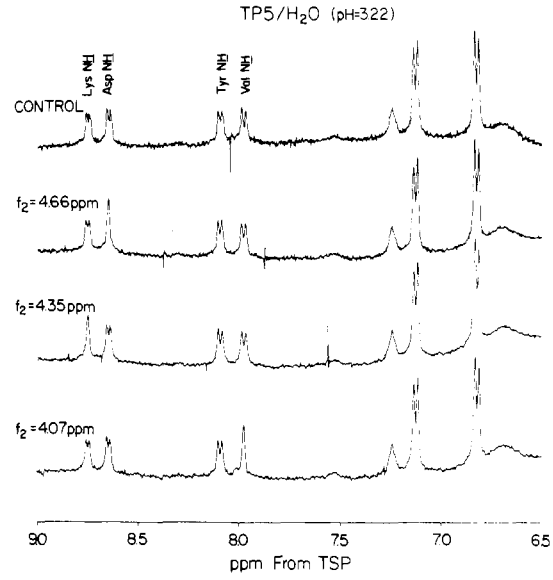

FIGURE 3: Typical spectra of TP5–H_2O (36 mM, pH 3.22) showing underwater decoupling in the conventional FT NMR mode. In these experiments, the dynamic range problem associated with the water resonance is minimized by first presaturating the water with radio-frequency (rf) irradiation before the nonselective observation pulse is applied. In practice, the presaturation is easily accomplished by multiple point irradiation of the water peak. In this procedure, a list of frequencies spanning the water resonance is defined, and the decoupler is made to jump from one frequency to another in a repetitive manner, spending ~10 ms at each, until the residual water signal is minimal. The top trace is the control spectrum and shows the various peptide NH resonances. The remaining traces show the underwater decoupling spectra obtained by shifting the irradiation frequency to the various C$^{\alpha}$H resonance positions (indicated on the traces) after presaturation of water and maintaining the decoupler at these frequencies during data acquisition. The strength of irradiation was the same for presaturation and spin decoupling ($\gamma H_2/2\pi \simeq 35$ Hz). These experiments were performed on a WH-400 NMR spectrometer equipped with an Aspect-2000 computer. The spectra shown were obtained (5-mm tube, 20° flip angle, 200 scans, quadrature detection) with a 10-mm ^{13}C probe adapted for ^{1}H observation (using decoupler coils). The quality of the spectra, though reasonable under these conditions, can be further improved by minimizing the residual water signal accumulation through a proper rf phase synchronization of observing and decoupler frequencies along with phase shifting (Redfield, 1978; Hoult, 1976; Krishna, 1976). Since most peptide NH's show exchange minimum in the pH range 2–3.5, adjustment of sample pH to this range assures that the diminution in the NH proton intensity due to a transfer of saturation from water is minimal.

and at 7.1 ppm in Figure 2 arise from the aromatic proton resonances. The broad resonance at 7.5 ppm has been assigned, tentatively, to the $-N^{\zeta}H_3^+$ protons of lysine. The resonance from the α-amino group is expected to be too broad because of fast exchange with the solvent. The peak of approximately single-proton intensity at 7.2 ppm has been assigned to the guanidino-N$^{\epsilon}$H– of arginine. The remaining resonance at 6.65 ppm has been assigned to the guanidino-C$^{\zeta}(NH_2)_2$ protons of arginine, on the basis of similar assignment for these protons in angiotensin (Glickson et al., 1973).

(*2*) *pH Titration Data.* The changes in the chemical shifts of well-resolved peaks in the NMR spectrum of TP5, as a function of pH, are shown in Figure 4 and 5. The pK_a values estimated by least-squares fitting to the standard equations corresponding to one- or two-proton titrations (Dwek, 1973) are also shown, and most of these are associated in a straightforward manner with the inductive effect of neighboring ionization sites. The pK_a values of 7.41 and 3.33–3.70 for the N-terminal α-amino and C-terminal α-carboxylate groups, respectively, are in the range expected for polypeptides (Greenstein & Winitz, 1961). The pK_a (=7.41) of the N-

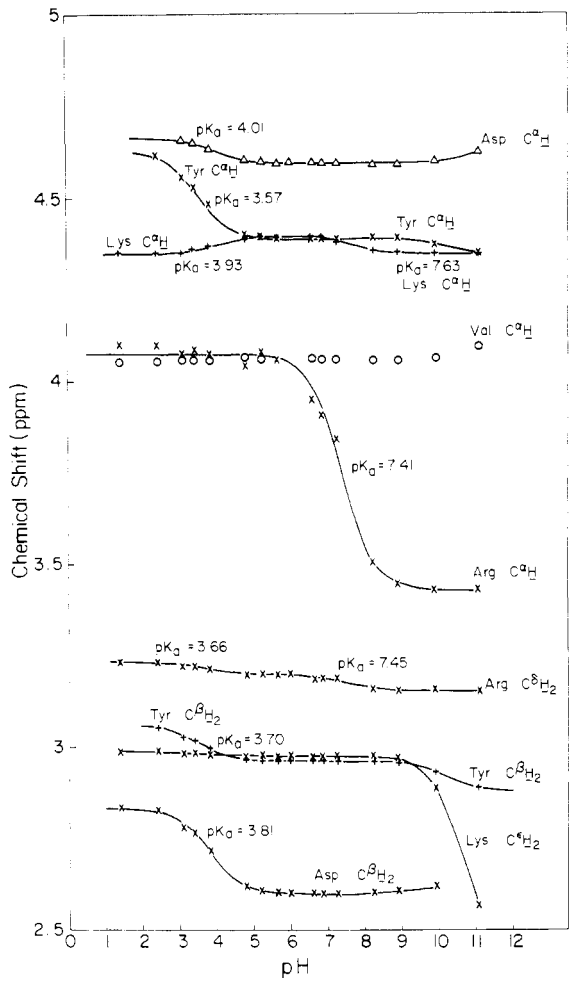

FIGURE 4: Titration shifts of the proton resonances of TP5 in D_2O.

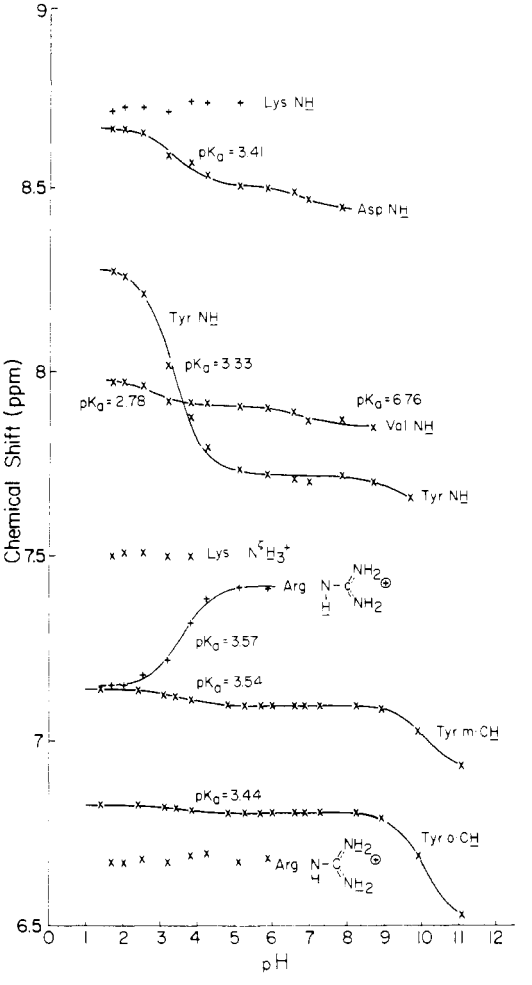

FIGURE 5: Titration shifts of the low-field proton resonances of TP5 in H_2O.

terminal α-amino group is in good agreement with that reported for bradykinin (Paiva & Juliano, 1977). The ionization constant of 3.81 for the side-chain carboxylate group of aspartic acid is in good agreement, within experimental error, with the value of 3.9 reported for this residue in the model tetrapeptide H-Gly-Gly-Asp-Ala-OH (Bundi & Wüthrich, 1979a). The ortho and meta protons of tyrosine exhibit the titrations of both the C-terminal carboxylate group and the phenolic group. The guanidino-N$^{\epsilon}$H- of arginine exhibits a low-field shift titration with a pK_a = 3.57, even though there are no adjacent groups that titrate in this range. The only nearest sites of ionization are the guanidino group, which is expected to titrate around 12.4 (Greenstein & Winitz, 1961), and the α-amino group, which is found to titrate at 7.41. Thus, the titration of arginine-N$^{\epsilon}$H- is probably a manifestation of a specific conformation of the pentapeptide. One possible mechanism that could explain the low-field shift is the existence of an intramolecular hydrogen bond between the guanidino-N$^{\epsilon}$H and one of the carboxylate groups. A similar mechanism has been invoked by Bundi & Wüthrich (1979b) to explain the unexpected low-field titrations of amide protons in some linear tetrapeptides. The alternate mechanism consisting of a conformational change as the pH is changed, viz., deshielding experienced by the guanidino proton as it moves away from a shielding group (e.g., the tyrosine aromatic ring), should also be considered. The small but significant high-field shift with a pK_a of 3.66 experienced by Arg-C$^{\delta}H_2$ is probably related to the perturbation experienced by the guanidino proton. Similarly, the low-field shift experienced by Lys^2-C$^{\alpha}$H with a pK_a = 3.93 may be associated with the titration of the

Table I: Vicinal NH-C$^{\alpha}$H Coupling Constant Data and Possible Sets of Torsional Angles for the TP5 Fragment[a]

residue	J_{cor}[b] (Hz)	torsional angles, ϕ (deg)[c]
Lys^2-NH	7.6 ± 0.2	34–49, 71–86, −78 to −86, −154 to −162
Asp^3-NH	7.7 ± 0.2	34–50, 70–86, −79 to −86, −154 to −161
Val^4-NH	9.0 ± 0.2	44–76, −89 to −98, −142 to −151
Tyr^5-NH	8.6 ± 0.4	40–80, −86 to −95, −145 to −154

[a] Values of torsional angles were derived by assuming a single conformation and by using the Karplus curve given in Figure 5 of the review article by Bystrov (1976). [b] $J_{cor} = 1.09J_{obsd}$, where J_{obsd} is the observed doublet separation and 1.09 refers to the correction for substituent effects (Bystrov, 1976). The pH range of 1.5–3.5 was used to measure the coupling constants since the amide proton resonances exhibited broadening for pH >3.5. The NH doublet separations have been corrected to take into account partial overlap of lines in the manner indicated by Bystrov (1976). [c] The additional dispersion arising from the error limits on J values has not been included in the values shown for torsional angles.

β-carboxylate group of aspartic acid. For the remaining protons we could not estimate pK_a values due to insufficient data points.

(3) Vicinal NH–C$^{\alpha}$H Coupling Constants. The various NH–C$^{\alpha}$H coupling constants measured for the TP5 fragment are listed in Table I. Also shown in the table are the various possible backbone torsional angles (ϕ) that are consistent with the observed coupling constants if a single preferred conformation is assumed for TP5. The magnitudes of the coupling constants and especially those for Lys^2 and Asp^3 suggest that

Table II: Amide Hydrogen Exchange Rates (K_{ex}) of TP5 in H_2O at 30 °C

pH	rates (s^{-1})[a]			
	Lys²-NH	Asp³-NH	Val⁴-NH	Tyr⁵-NH
1.84	0	*b*	0	*b*
3.15	0.6	*b*	0	0
4.1	2.8	*b*	0	0
5.02	27.6	*b*	0	0
5.6	78.7[c]			
6.05	112.1[c]	5.0	0.4	0
6.97		21.6	2.4	0
7.88		98.00[c]	12.6[c]	
8.71			69.6[c]	

[a] A zero rate implies that the amide proton exchange rate is negligible compared to its spin–lattice relaxation rate. [b] Rate could not be estimated since the corresponding $C^{\alpha}H$ is decoupled in the transfer of solvent saturation experiment and η could not be measured. [c] Estimated from the line width as $K_{ex} \simeq \pi(\Delta_{ex} - \Delta_{nex})$ where Δ_{ex} and Δ_{nex} are the full widths (in hertz) at half-height of the amide hydrogen in the presence and absence (i.e., negligible) of chemical exchange, respectively.

they may also reflect conformationally averaged values. The constraints imposed by the observed coupling constants on the various possible models for the solution conformation of TP5 will be considered later.

(*4*) *Amide Hydrogen Exchange Rates.* Differential exchange rates of NH hydrogens of peptides and proteins serve as indexes of solvent exposure [for example, see reviews by Schellman & Schellman (1964), Hvidt & Nielsen (1966), Willumsen (1971), Otteson (1971), Englander et al. (1972), and Harrington et al. (1966)] and thus could provide secondary and tertiary structural details of these molecules. The amide hydrogen exchange rates of TP5 have been measured by a combination of transfer of solvent saturation and saturation recovery experiments. A preliminary account of these experiments and typical experimental spectra have been presented elsewhere (Krishna et al., 1980b). Within experimental error, saturation recovery for each amide hydrogen has been found to be a single exponential (Krishna et al., 1980a). This result and the observed pH dependence of the apparent relaxation rates are consistent with the assumption of a high-motility limit for the TP5 fragment (Hvidt, 1964; Krishna et al., 1980a,b). In this limit the fractional intensity change in the amide hydrogen magnetization when the solvent is saturated under steady state is (Krishna et al., 1980a)

$$\eta = \frac{M_z - M_0}{M_0} = -\frac{\langle K \rangle}{\langle R \rangle + \langle K \rangle} \qquad (1)$$

The recovery of the amide hydrogen magnetization in a selective saturation recovery experiment is given by eq 2. In

$$M_z(t) = M_0(1 - e^{-(\langle R \rangle + \langle K \rangle)t}) \qquad (2)$$

eq 1 and 2, η is the fractional intensity change, M_z and M_0 are the longitudinal amide hydrogen magnetization and its thermal equilibrium value, respectively, and $\langle R \rangle$ and $\langle K \rangle$ are respectively the conformationally averaged values of the spin–lattice relaxation rate and the exchange rate. They are defined by

$$\langle R \rangle = \sum_i P_i R_i$$

$$\langle K \rangle = \sum_i P_i K_{iS} = K_{ex} \qquad (3)$$

where P_i is the fractional population of the ith conformer, and R_i and K_{iS} are the spin–lattice relaxation rate and chemical exchange rate, respectively, of the amide proton in the ith conformation. The summation in eq 3 runs over all the conformations. The exchange rates K_{ex} ($=\langle K \rangle$) of the individual amide hydrogens determined from the experiments by using eq 1 and 2 are given in Table II.

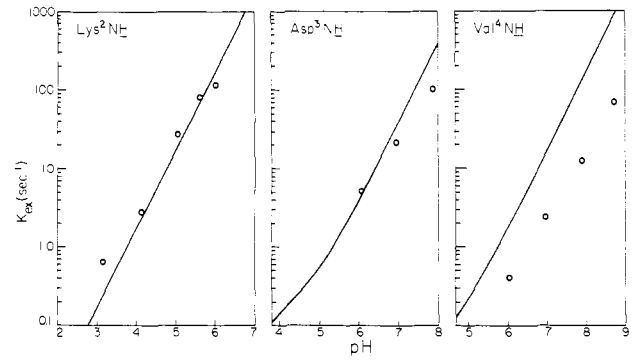

FIGURE 6: Comparison of observed amide hydrogen exchange rates (circles) given in Table II of this paper with predicted rates (solid lines) for solvated peptides at 30 °C. The predicted rates were calculated with the computed rate constants for NA-Ala-Ala-N'MA from the rate data (at 25 °C) in Table I and the relative inductive effects of side chains given in Table IV of Molday et al. (1972). An activation energy of 17 kcal/mol (Englander et al., 1979) was assumed in correcting the data from 25 to 30 °C. The theoretical plot for Lys²-NH was calculated by using $K_{ex} = k_H[H_3O^+] + k_{OH}[OH^-]$, where k_H and k_{OH} are respectively the acid- and base-catalyzed rate constants computed from the model compound data. $[H_3O^+]$ and $[OH^-]$ are respectively the hydronium and the hydroxyl ion concentrations. For Asp³-NH and Val⁴-NH the predicted rates were calculated by using eq 1 of Molday et al. (1972) to take into account the titration of the β-carboxylate group of aspartic acid.

Figure 6 shows a comparison of the experimental data points for the exchange rates of TP5 with the theoretical curves predicted on the basis of solvated model peptides (Molday et al., 1972). Examination of Figure 6 reveals that the three amide protons, Lys²-NH, Asp³-NH, and Val⁴-NH, do not exhibit any significant acid catalysis in the pH range (2–9) employed in our studies. This observation is consistent with the known behavior of peptides—they exhibit minimum exchange rates normally between pH 2 and 3 (Hvidt & Nielsen, 1966; Molday et al., 1972), and, consequently, only base catalysis is observed for peptides above pH 2.5. The rate constant for Lys²-NH is largest of all, due mainly to inductive effects resulting from the positively charged N-terminal α-ammonium group (Scheinblatt, 1965, 1966; Molday et al., 1972). The experimental points for Lys²-NH are in good agreement with the theoretical curve for which a base-catalyzed rate constant of $\langle k_{OH}^{Lys} \rangle = 1.20 \times 10^{10}$ M^{-1} s^{-1} is predicted from the model compound data (Molday et al., 1972). Such rates are characteristic of diffusion-controlled reactions (Molday & Kallen, 1972). The low-field resonance position and the relatively large base-catalyzed rate constant are characteristic of the NH resonance of the penultimate residue (Glickson et al., 1976).

The experimental rates for Asp³-NH are essentially in good agreement with rates estimated from model compound data of Molday et al. (1972). The latter rates are expected to correspond to rates of solvated groups in polypeptides within about a factor of 2 (Molday et al., 1972). For Val⁴-NH, however, the experimental points are all systematically smaller than the predicted rates by more than a factor of 5. This appears to be a significant difference. For the Tyr⁵-NH, the chemical exchange rates are too small to be measured by the transfer of solvent saturation technique in the pH range employed. The inductive effect of the proximal negatively charged α-carboxyl group is expected to decrease the base-catalyzed exchange rate of adjacent peptide group (Molday et al., 1972).

The magnitudes of the chemical exchange rates (Table II) of the various amide hydrogens in TP5 are compatible with the assumption that TP5 exists in a highly motile dynamic equilibrium among several conformations and that some of these conformations are highly solvated. If solvated structures were absent in the conformational equilibrium, the exchange rates would have been expected to be very small with lifetimes on the order of minutes, hours, or even longer. Since the rates for Lys^2-NH and Asp^3-NH are in good agreement with the predicted rates for solvated peptides, these two amide protons remain essentially accessible to the solvent among the various conformations. The substantially diminished exchange rates for Val^4-NH, on the other hand, imply that this amide proton is not readily accessible to the solvent. This inaccessibility may be due to either (1) steric blockage of Val^4-NH from the solvent within the folded region of the peptide or (2) an internal hydrogen bond involving Val^4-NH. We believe that mechanism 1 is very unlikely to be realized in a peptide as small as TP5. Further, the chemical shift of this proton also suggests that it is not shielded from solvent. Hence we favor mechanism 2 as the likely reason for the diminished exchange rate of Val^4-NH.

Several possible conformational models of TP5 may be considered in order to explain the NMR data. Among the folded structures the type I and type II β turns involving the first four residues of TP5 (Venkatachalam, 1968; Lewis et al., 1973a) could explain the diminished exchange rate for Val^4-NH, since these models would predict an internal hydrogen bond between Arg^1-C=O and Val^4-NH. However, a type I β turn is defined by the torsional angles $\phi_{i+1} \simeq -60$, $\psi_{i+1} \simeq -30$, $\phi_{i+2} \simeq -90$, and $\psi_{i+2} \simeq 0$, whereas a type II β turn is defined by $\phi_{i+1} \simeq -60$, $\psi_{i+1} \simeq 120$, $\phi_{i+2} \simeq 80$, and $\psi_{i+2} \simeq 0$ (Lewis et al., 1973a). Thus, both these models predict a NH–C^{α}H coupling constant in the range 3–4 Hz for the Lys^2 residue, whereas the measured coupling constant is 7.6 Hz. It is therefore clear that the type I and II β turns, though successful in explaining the exchange data, do not make a dominant contribution to the conformational equilibrium.[2] Similarly, it may be concluded that the type III turn with angles $\phi_{i+1} \simeq -60$, $\psi_{i+1} \simeq -30$, $\phi_{i+2} \simeq -60$, and $\psi_{i+2} \simeq -30$ (Lewis et al., 1973a) is also not dominant.

We may also attempt to rationalize the above argument by making an estimate of the fractional population of the hydrogen-bonded conformer. If we assume a two-state model for the purpose of discussion, then a diminution by a factor of 6 for the Val^4-NH exchange rate would place a lower limit of 83% for the population of the hydrogen-bonded conformer. In order to match the observed coupling constant of 7.6 Hz for the Lys^2-NH, an assumption of the type I, II, or III β turns for the hydrogen-bonded structure would imply that the solvated conformation must have a coupling constant of $\sim$25 Hz, which is unrealistic. Of course, this line of reasoning may not be valid, if there is more than one conformation significantly populated in which Val^4-NH is hydrogen bonded.

One structure that appears to be consistent with the experimental data is a 1 $\leftarrow$ 3 bend (γ turn) for the Asp^3 residue. The γ turn or C_7 conformation consists of a seven-membered ring system (Avignon et al., 1969; Nemethy & Printz, 1972; Matthews, 1972; Avignon et al., 1973; Lewis et al., 1973b; Madison et al., 1974; Meraldi et al., 1975; Hruby et al., 1978; Pease & Watson, 1978). It is defined (Lewis et al., 1973b) by the torsion angles $\phi_{i+1} \simeq \pm 80$ and $\psi_{i+1} \simeq \pm 80$ (where the upper and lower signs correspond respectively to the equatorial and axial conformations) and is characterized by a hydrogen bond between the C=O of the ith residue and the NH of the $i + 2$ residue.[3] The existence of such a structure for Asp^3 is also indicated by preliminary theoretical calculations (J. L. DeCoen, personal communication). In addition to successfully predicting a hydrogen bond for Val^4-NH, this model is also consistent with the observed vicinal coupling constant data for the Asp^3 residue.[4] Hence a γ turn (C_7 structure) for the Asp^3 residue is reasonable as a dominant conformation of TP5 in aqueous solution.[4] Another conformation that is compatible with the NMR data is a five-membered ring system, C_5 (Lewis et al., 1973a; Avignon et al., 1969), for the Val^4 residue. This structure is defined by the torsional angles $\phi_{i+1} \simeq -150$ and $\psi_{i+1} \simeq 150$ (Lewis et al., 1973a) and is characterized by a hydrogen bond between NH and C=O of the $i + 1$ residue. Such a C_5 structure for the Val^4 residue is compatible with both the diminished exchange rate and the vicinal coupling constant (9 Hz) for the Val^4-NH. The possibility of such a structure for the valine residue of TP5 is also consistent with the preliminary theoretical calculations (J. L. DeCoen, personal communication).

Conclusions

The main features of TP5 in aqueous solution are as follows. (1) The NMR data are compatible with the assumption of a highly motile dynamic equilibrium among several conformations. (2) Lys^2-NH and Asp^3-NH remain essentially in a solvated environment within the conformational equilibrium. (3) Val^4-NH exhibits a diminished exchange rate perhaps as a result of an intramolecular hydrogen bond. (4) The guanidino-N^{ϵ}H of Arg^1 titrates with a pK_a of 3.6. This may be due to a folded conformation of TP5 involving association of this proton with one of the carboxylate groups of TP5. It has been shown that the alanine analogue of TP5 (Ala-Lys-Asp-Val-Tyr) and the tetrapeptide Lys-Asp-Val-Tyr are biologically inactive (Goldstein et al., 1979). Thus, the location of Arg at the N terminal appears to be consistent with the structural and conformational requirements essential to eliciting biological activity from TP5. The positive charge on the guanidino group might provide the necessary electrostatic interaction for receptor binding. Whether the biological activity is also facilitated to some extent by the conformation involving the guanidino-N^{ϵ}H could only be answered after studying the appropriate analogues of TP5. (5) The chemical exchange rates and the NH–C^{α}H vicinal coupling constant data are consistent with a γ turn for the Asp^3 residue and/or a C_5 structure for the Val^4 residue being dominant conformers of TP5 in aqueous solution. Further investigations are in progress to determine whether only one or both of these structures are significantly populated.

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[2] In drawing this conclusion, we have assumed that the angles given by Lewis et al. (1973a) for the minima corresponding to the β turns are accurate to within $\pm 10°$. If the angles for the β turns differ significantly from these values (e.g., if $\phi_{i+1} \simeq -80$), then these turns also will be compatible with the NMR data on TP5.

[3] A second hydrogen bond, as originally included by Nemethy & Printz (1972) in their definition of γ turn, is not observed in TP5. Such a hydrogen bond would involve Lys^2-NH which we find is solvated.

[4] While it is generally agreed that C_7 conformations may be preferred in nonpolar solvents, the status of these conformations in aqueous solvents is not yet certain (Avignon et al., 1973; Burgess & Scheraga, 1973).

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