Proline Pipe Helix: Structure of the Tus Proline Repeat Determined by ^{1}H NMR‡

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ABSTRACT: The structure of a 22 amino acid peptide, TPPI [Nedved, M. L., Gottlieb, P. A., & Moe, G. R. (1994) *Nucleic Acids Res. 22*, 5024−5030], that is similar to the proline repeat segment of the replication arrest protein, Tus, has been determined by ^{1}H NMR in 50% trifluroethanol. The structure is a novel left-handed helix having 5.56 residues per turn and a regular hydrogen bonding network that is limited to one side of the helix and contains a channel that runs down the helix axis. The latter feature gives the structure an overall pipe-like appearance; hence, the structure has been designated a proline pipe helix. The Tus proline pipe is also amphiphilic with one side consisting of proline and other nonpolar residues while the other side contains mostly basic and other polar residues. Tus and several other proteins that contain a similar proline repeat sequence are DNA binding proteins. It is shown here that the proline pipe helix of TPPI can be accommodated within the major grove of B-form DNA in a manner that positions nearly all of the basic residues near phosphate groups in the DNA backbone. The proline pipe helical motif may be a structural element of many other proteins including integral membrane receptor proteins.

Tus is a 309 amino acid DNA binding protein that has been shown to arrest replication in *Escherichia coli* (Hill, 1992). Tus binds as a monomer with high affinity and specificity to 22−23 base pair DNA sequences known as *Ter* sites (Gottlieb et al., 1992; Hill, 1992). The termination effect of Tus/*Ter* complexes on replication is asymmetric as the movement of replication complexes from only one direction, which is determined by the particular orientation of the *Ter* site, is affected. The mechanism of Tus/*Ter* replication arrest is not known but may involve protein/protein interactions between the complex and subunits of the replicase (Hiasa & Marians, 1994; Skokotus et al., 1994).

The structure of Tus and the segments of Tus that are directly involved in DNA binding have not yet been determined. It has been suggested from an analysis of the amino acid sequence that the N-terminal 223 amino acids of Tus comprise a typical globular protein domain composed primarily of α-helical structure (Nedved et al., 1994). In contrast, the 178 amino acid C-terminal segment is punctuated by several helix-destabilizing glycine and proline residues, suggesting that it has a more complex fold. This general view of the structure based on the Tus sequence was supported by CD[1] studies which showed that the protein contains a mixture of about 40% α-helical and 45% aperiodic conformations (Coskun-Ari et al., 1994). The C-terminal domain of Tus contains a proline-repeat segment (residues 223−243) in which proline occurs every fifth residue, with the exception of Leu228, and is either preceded by or followed by a basic residue. Similar proline repeat segments of 20 to 25 amino acids have been identified in several other nucleic acid binding proteins, polymerases, apolipoproteins, and transmembrane segments of receptor proteins (Nedved et al., 1994; G. R. Moe, P. A. Gottlieb, and J. J. Péne, unpublished observations).

Recently, we described the CD spectrum and DNA binding activity of a 22 amino acid peptide, TPPI, which has an amino acid sequence that is similar to that of the proline-repeat-containing segment of Tus (Nedved et al., 1994). The peptide was found to be disordered in water but exhibited a CD spectrum that is associated with helical hydrogen bonding networks in TFE/water solutions and in the presence of micellar concentrations of the ionic detergent sodium dodecyl sulfate. Computer graphics and energy minimization calculations were used to construct several helical models of TPPI that might account for these observations. One model in particular, which consisted of an amphiphilic, left-handed 5.1_{19} helix, was consistent with the available experimental observations and also converged to a low energy minimum expected for a sterically reasonable conformation (Nedved et al., 1994). In order to determine whether or not the solution conformation is similar to the graphic model, the structure of TPPI in 50% TFE was studied by ^{1}H NMR. We report here the structural determination and characteristic structural features of TPPI in 50% TFE and its possible significance for proteins that contain similar proline repeat sequences.

‡ The Protein Data Bank file name for the 10 structures calculated from the NMR data is 1SUT.

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[1] Abbreviations: CD, circular dichroism; DSS, 2,2-dimethyl-2-silapentane-5-sulfonate; Fmoc, 9-fluorenylmethoxycarbonyl; NMR, nuclear magnetic resonance; NOE, nuclear Overhauser effect; ROE, rotating frame nuclear Overhauser effect; $d_{\alpha N}(i,j)$, $d_{NN}(i,j)$, etc., intramolecular distance between the protons $C^{\alpha}H$ and NH, NH and NH, etc., on residues *i* and *j*; SDS, sodium dodecyl sulfate; TFE, trifluroethanol.

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MATERIALS AND METHODS

The peptide TPPI (acetyl-PQNAKLKIKRPVKVQPIAR-RVY-amide) was synthesized and purified as described previously (Nedved et al., 1994). D_2O (99.96 atom % D) and DSS were from Aldrich (Milwaukee, WI). TFE-d_2 (99.5 atom % D) was from Isotec, Inc. (Miamisburg, OH).

NMR Experiments. All NMR experiments were performed with a 7 mM peptide sample in 500 μL of 50% TFE-d_2/50% H_2O. DSS was used as an internal reference set at 0.0 ppm. All experiments were acquired with the transmitter set on the H_2O resonance. Solvent suppression was achieved by irradiation of the H_2O resonance during the delay period. Phase-sensitive (States et al., 1982) COSY (Griesinger et al., 1987), TOCSY (Davis & Bax, 1985), and ROESY (Bax & Davis, 1985) experiments were recorded at 277 K and processed on a Bruker AMX 500 spectrometer. A total of 512 increments were acquired with 48 scans and 32 dummy scans for each increment. In the ω_2 dimension, 1024 complex data points were acquired over a spectral width of 6 kHz. Prior to Fourier transformation, the free induction decay was multiplied by a sine function in both dimensions, and the data in ω_1 were zero-filled to yield a 1024 $\times$ 1024 matrix of real points. The TOCSY experiment used a mixing time of 75 ms, and the ROESY experiments used mixing times of 25, 50, and 100 ms.

Each proton was assigned to a specific resonance in the NMR spectrum using the standard method for sequential assignments (Wüthrich, 1986). A TOCSY experiment was used to assign resonances through scalar interactions corresponding to protons belonging to individual amino acid. The individual amino acid residues and their sequential assignments were then differentiated by $d_{\alpha N}(i,i+1)$ and $d_{NN}(i,i+1)$ interactions observed in the ROESY spectrum at 277 K. In some instances, more than one assignment of a particular medium-range ROE was possible in the ROESY spectra. Most of these ambiguous assignments were resolved by considering unambiguous ROE assignments involving the same residues, determining whether distances calculated from ROE peak volumes were consistent with the closest possible approach of two protons allowed by the covalent structure of the peptide and considering whether an assignment was consistent with the pattern of ROEs established by unambiguous assignments.

Determination of Distance and Dihedral Angle Constraints. The distance constraints were calculated by comparing the ROE buildup rate for each cross peak as a function of mixing time with the ROE buildup rate for the δ and ϵ protons in the tyrosine ring with a known distance of 2.46 Å (Williamson et al., 1985). Distance constraints involving protons for which stereospecific assignments could not be made (e.g., methylene, methyl, and aromatic ring protons) were corrected for the pseudoatom representation as described by Wüthrich et al. (1983). Dihedral angle constraints (θ) were calculated from the $^3J_{NH\alpha}$ coupling constants, which were determined from TOCSY spectra, using the Karplus relationship (Karplus, 1959). A range of $\pm$10% of the calculated distances and $\pm$10° of the calculated dihedral angles were used in the simulated annealing calculations.

Simulated Annealing Calculations. Dynamic simulated annealing calculations (Nigles et al., 1988; Clore et al., 1985) were carried out on a Silicon Graphics Indigo computer using the NMRchitect program of the Insight software suite (Biosym Technologies, San Diego, CA). A starting conformation with a random array of atoms was generated by assigning random values to the coordinates of the atoms comprising the peptide. Close nonbonded contacts were first removed by 100 steps of steepest descents minimization followed by 500 steps of conjugate gradients minimization with all force constants set to very low values. The force constants were then set to values such that the initial potential energy was approximately equal to the kinetic energy at 1000 K. Initial velocities were assigned according to a Maxwell distribution at 1000 K, and all masses were set to uniform values (10 au). The first phase of the calculation consisted of 50 cycles of dynamic simulated annealing, each comprising 1 ps dynamics with a time step of 1 fs at 1000 K. For the first 30 cycles the ROE distance and $^3J_{NH\alpha}$ dihedral force constants were increased rapidly while the covalent and nonbonded force constants were increased slowly over the entire 50 cycles. The second phase of the protocol consisted of five cycles of 2 ps dynamics where the kinetic temperature was cooled down to 300 K. The second phase was followed by 200 cycles of steepest descents minimization and 2000 cycles of conjugate gradients minimization. This protocol was repeated to generate a total of 10 converged structures.

Docking TPPI to DNA. The average structure of TPPI calculated from dynamic simulated annealing was positioned within the major groove of B DNA having the sequence 5′-TGTTGT-3′ using the Insight graphical interface (Biosym Technologies, San Diego, CA). The position of the TPPI helix within the major groove was chosen to optimize possible contacts between the charged groups of basic residues and phosphate groups in the DNA backbone. The few steric overlaps between the extended side chains of the peptide and the DNA were removed through manual manipulation and energy minimization using Discover (Biosym Technologies, San Diego, CA).

RESULTS AND DISCUSSION

The complete resonance assignments and $^3J_{NH\alpha}$ coupling constants for TPPI in 50% TFE at 277 K are given in Table 1. In addition to the expected intraresidue and sequential $d_{\alpha N}(i,i+1)$ ROEs, numerous medium-range ROEs having a periodic spacing of five to six residues propagated throughout the entire peptide were observed (Figures 1 and 2). Periodic connectivities such as these are characteristic of helical structures, although the periodicity of five to six residues observed here is quite unusual (Wüthrich, 1986). The extensive set of $d_{\beta N}$ and $d_{\beta\alpha}$ ROEs and the lack of $d_{\alpha N}$ ROEs having the same periodicity indicate the TPPI helical structure is left-handed based on the differences in the orientation of side chains relative to the NH groups in right-handed versus left-handed helices.

The distance constraints calculated from the ROE peak volumes and the dihedral angle constraints calculated from the $^3J_{NH\alpha}$ coupling constants were used in simulated annealing structure calculations. The backbone heavy atoms of the ten converged structures are shown in Figure 3. As expected from the type and periodicity of the observed ROE connectivities, all of the calculated structures are left-handed helices having, on average, 5.56 residues per turn. The structures calculated here are quite similar to the graphical model constructed earlier by Nedved et al. (1994). The

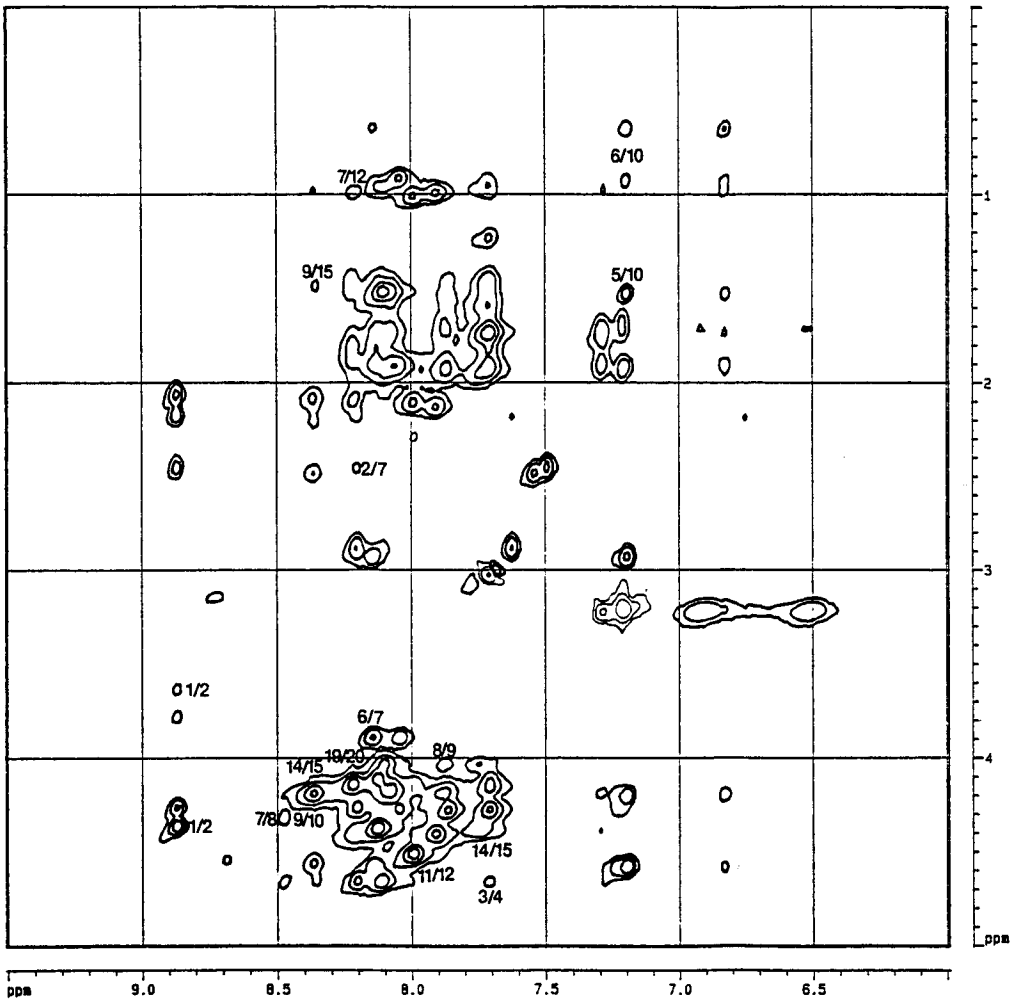

FIGURE 1: Portion of a 500 MHz ROESY spectrum of TPPI in 50% TFE at 277 K showing the amide to CαH connectivities. This portion of the spectrum was important in making the resonance assignments and in identifying the pattern of unusual medium-range connectivities (*i*,*i*+5 and *i*+6) that are characteristic of the structure adopted by the peptide.

Table 1: Assignments and $^3J_{NH\alpha}$ Coupling Constants for TPPI at 277 K, pH 4

residue	NH	$C_\alpha H$	$C_\beta H$	$C_\gamma H$	$C_\delta H$	$C_\epsilon H$	NH	$^3J_{NH\alpha}$
Pro1		4.42	2.00/2.44	2.40	3.68/3.82			
Gln2	8.92	4.30	2.12/2.22	2.49			6.76/7.50	8.41
Asn3	8.27	4.70	2.92				6.84/7.62	7.16
Ala4	7.78	4.15	1.30					7.83
Lys5	7.79	4.16	1.80/1.95	1.60	1.50	3.08	7.77	7.38
Leu6	8.10	3.92	1.95	0.95	0.67			10.15
Lys7	8.19	4.40	1.80/1.95	1.60	1.50	3.08	7.77	10.49
Ile8	7.78	4.06	1.95	1.60	1.00			7.23
Lys9	7.90	4.35	1.80/1.95	1.60	1.50	3.08	7.77	7.79
Arg10	8.19	4.73	1.83/1.92	3.27			6.87/7.20	7.97
Pro11		4.55	2.10/2.40	2.35	3.68/3.87			
Val12	8.05	4.23	2.17	1.03				6.35
Lys13	7.80	4.09	1.80/1.95	1.60	1.50	3.08	7.77	7.23
Val14	7.95	4.23	2.17	1.03				7.11
Gln15	8.42	4.62	2.12/2.22	2.51				7.83
Pro16		4.32	2.03/2.44	2.40	3.68/3.83			
Ile17	7.76	4.35	1.95	1.60	1.00			6.61
Ala18	7.81	4.09	1.29					7.23
Arg19	7.92	4.26	1.72/1.95	3.27			6.87/7.20	7.11
Arg20	8.12	4.31	1.77/1.92	3.27			6.87/7.34	7.57
Val21	8.05	4.20	2.14	1.05				8.32
Tyr22	8.20	4.65	3.20/2.98		6.95	6.62		9.15

helices are also amphiphilic with Pro1, Leu6, Pro11, Ile12, Pro16, Ile17, and Ala18 forming a continuous hydrophobic surface on one side. This may provide an explanation for why the same structure is also stabilized by SDS micelles (Nedved et al., 1994). Also, the amphiphilic nature of the helix suggests that the structure requires tertiary interactions with the hydrophobic core of a protein or a lipid−water interface of a membrane for stability in water alone.

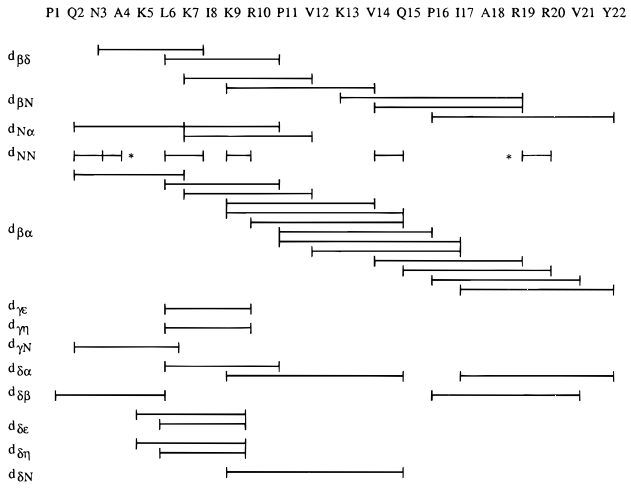

FIGURE 2: Summary of the medium-range ROEs observed for TPPI in 50% TFE at 277 K. The asterisks indicate ROEs that are too close to the diagonal to be observed.

The 10 structures show an RMS distribution of 2.45 Å for all atoms (0.83 Å for the backbone atoms) with respect to each other and an RMS deviation of 1.65 Å (0.56 Å for the backbone atoms) from the average structure. The center of the peptide has low deviations with a narrow spread while the greatest deviations occur at the ends of the peptide (Figure 3). This is consistent with many small peptides which have been shown to have frayed ends (Dyson & Wright, 1991). The average violation of the distance constraints determined by ROE interactions is 0.19 Å for the 24 sequential constraints, 1.00 Å for the 62 medium-range constraints, and 0.82 Å for all 86 constraints.

Several features of the calculated helical structures are distinctly different from previously observed right-handed α and 3_{10} helices and the left-handed polyglycine II and poly(L-proline) II helices. First, the helical structures are a repeating composite of several secondary structures rather than a single, repeating conformation. This is readily apparent from considering the ϕ,ψ values for the average structure shown in Figure 4. Designating proline to be residue i of the five residue repeat with $\phi \approx -52°$ and $\psi \approx -155°$, residue $i+1$ falls in the region of a parallel β-sheet ($\phi \approx -90°$, $\psi \approx -94°$), residues $i+2$ and $i+3$ have ϕ,ψ values in the region of a left-handed ω-helix ($\phi \approx 65°$, $\psi \approx 84°$), and residue $i+4$ falls in a sterically unfavorable region ($\phi \approx 75°$, $\psi \approx -120°$) as a consequence of preceding proline within a helical conformation.

Interestingly, Lys5 and Arg20, which do not precede proline residues but occupy $i+4$ positions in the repeat, adopt the same sterically strained conformations as those of residues that do precede proline. This suggests that the characteristic, position-dependent conformations of each residue in the TPPI helical structures can be maintained without proline at every fifth position, as occurs in Tus and several other proteins having similar repeats, as long as there

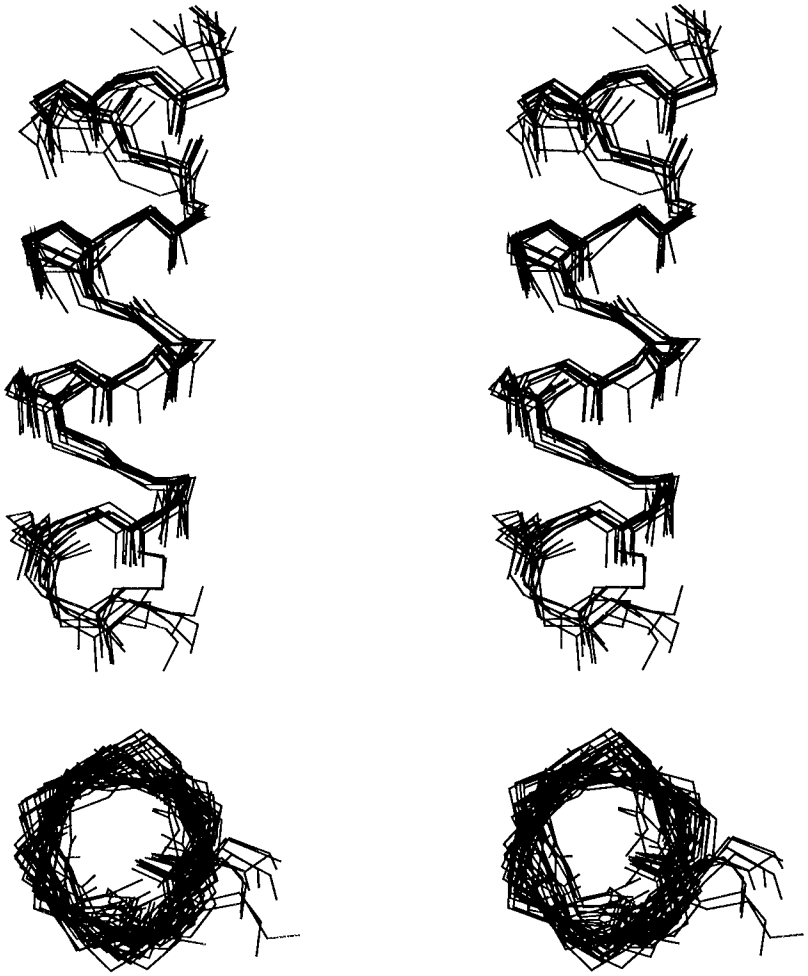

FIGURE 3: Stereoview of the backbone heavy atoms for the 10 converged structures calculated using constraints obtained from the NMR experiments in dynamic simulated annealing structure calculations. Side view with the amino terminus at the top (upper pair). Top view down the helix axis showing the channel (lower pair).

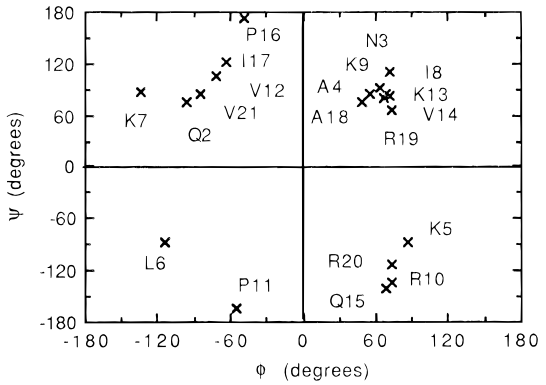

FIGURE 4: Plot of the dihedral ϕ,ψ angles for the average of 10 structures calculated from the NMR data for TPPI in 50% TFE at 277 K.

are a sufficient number of repeats to form the nucleus of the helix. However, additional examples will have to be characterized in order to confirm whether or not this is generally true. As a consequence of the position-dependent variation in backbone conformation throughout each turn, the pitch of the helix is also variable. The pitch is largest for the portion of each turn from the residue preceding proline to the residue that follows proline and decreases nearly to the point of being flat in the intervening segment.

The hydrogen bonding network is a second unusual feature of the TPPI helical structures. Unlike regular α and 3_{10} helices in which backbone NH and C═O groups of each residue participate in forming a continuous network of hydrogen bonds running down the entire length of the helix, the backbone hydrogen bonding network of the calculated TPPI structures are limited to a portion of each turn. Typically, hydrogen bonds occur between the backbone C═O groups of residues at positions $i+1$, $i+2$, and $i+3$ (where residue i is proline or Leu6) with NH groups of residues at positions $i+2$, $i+3$, and $i+4$ of the following turn. The network is essentially that of a 5.56_{19} helix. The C═O group of residue $i+4$ tends to point toward the helix axis while the orientation of the amide group connecting i with $i+1$ is variable though always closer to perpendicular to the helix axis than parallel with it. The hydrogen bonding network observed in the calculated structures accounts for the helical CD spectrum of TPPI in 50% TFE and in micellar SDS (Nedved et al., 1994).

A third distinguishing feature of the TPPI helix is the channel running along the helix axis. The structure resembles a pipe; hence, we refer to it as a proline pipe helix. The presence of the channel is significant because other possible helical structures such as π or ω helices, which would also have axial channels, have never been observed. It has been suggested that such helices would be unstable due to the loss of van der Waals contacts of the peptide backbone (Schultz & Schirmer, 1979). The existence of the channel in the proline pipe helix is a consequence of the proline repeat which forces the peptide backbone to fold back on itself in a series of turns yet sterically precludes the formation of the more compact α helix. The channel is large enough to accommodate water molecules which may provide additional stabilizing hydrogen bond and van der Waals contacts.

Tus is a DNA binding protein, and it has been shown that TPPI binds to DNA, although it binds nonspecifically and with considerably lower affinity than Tus itself (Nedved et al., 1994). Also, several other proteins that have been found to contain similar proline repeat segments are DNA binding proteins (Nedved et al., 1994; G. R. Moe, P. A. Gottlieb, and J. J. Péne, unpublished observations). Several crystallographic studies have shown that important contacts in protein/DNA complexes are frequently mediated by α helices located within the major groove of DNA. Therefore, it was of interest to determine whether the dimensions of the proline pipe could be accommodated in a similar fashion within the major groove of DNA.

Computer graphic docking experiments were conducted with a B-form DNA model of the central six base pairs of the conserved *Ter* site DNA sequence (5′-TGTTGT-3′) and the average structure of TPPI obtained from simulated annealing calculations. As shown in Figure 5, the TPPI proline pipe structure fits well within the major groove of the DNA duplex without any unfavorable overlaps between the amino acid side chains and the DNA base pairs. Unlike an α helix, the proline pipe helix spans the entire width of the major groove. Moreover, there is a striking correspondence between the position and spacing on the helix surface of Lys5, Arg10, Gln15, and Arg20 on one side and Lys7 and Lys13 on the other side with the position and spacing of the DNA phosphate groups at the edges of the

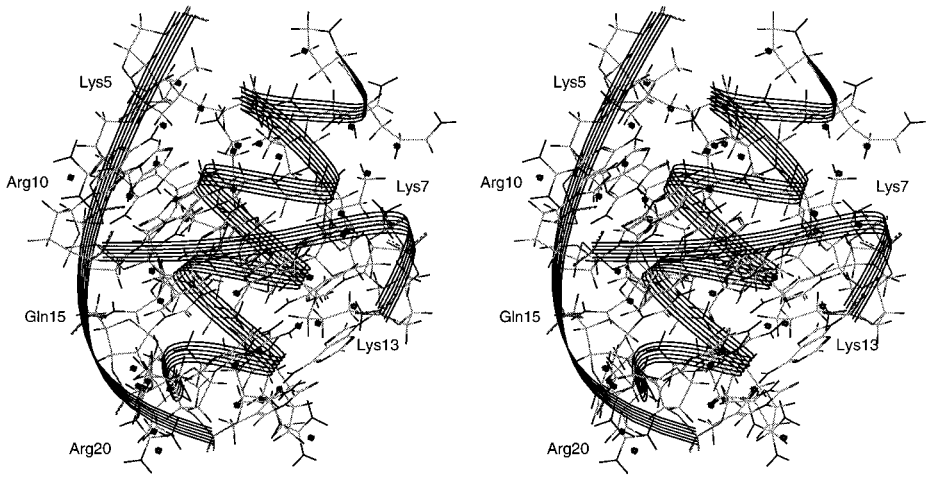

FIGURE 5: Stereoview of a graphic model of the calculated average structure of TPPI docked within the major groove of a B-form DNA duplex having the sequence 5′-TGTTGT-3′. Both the amino terminus of the TPPI proline pipe helix and the 5′-end of the top DNA strand are at the top of the figure. Basic and polar side chains that are near DNA phosphodiester groups are annotated.

major groove. The peptide occupies a binding site of approximately four base pairs, and there are five basic residues in close proximity to phosphate groups in the DNA backbone. These results are in good agreement with previous DNA binding studies which showed that the TPPI binding site size was 4.3 ± 0.5 base pairs and the estimated number of cations release upon binding was 4.7 ± 0.3 (Nedved et al., 1994).

The docking experiments indicate that the proline repeat of Tus represents, at most, a fraction of the protein/DNA interactions in the Tus/*Ter* complex. *Ter* sites are 22−23 base pairs in length with an absolutely conserved subsite of 11 base pairs. The dimensions of the TPPI proline pipe helix and B-DNA, however, only allow continuous interactions with about four base pairs. A proline pipe helix longer than about 20 residues would extend out and away from the DNA as the major groove of B-DNA continues about a helical path. Interestingly, all of the proline pipe repeats that have been identified in DNA binding proteins have been limited to about 20 residues in length (Nedved et al., 1994; G. R. Moe, P. A. Gottlieb, J. J. and Péne, unpublished observations). In contrast, the proline pipe repeats identified in lipid binding proteins and transmembrane segments of receptor proteins are longer.

Given the extraordinary characteristics of the proline pipe helix, it is of concern whether the structure of TPPI in 50% TFE is a relevant or even possible structural element of the native Tus protein. For example, aqueous TFE solutions are known to promote α-helical structure in peptides even when the peptide segments are not helical in the native protein (Waterhous & Johnson, 1994). However, as described above, the proline pipe helix of TPPI is distinctly amphiphilic and, further, is stabilized by binding to SDS micelles. The dominance of hydrophobic interactions in protein folding and the fact that most α helices in native proteins are amphiphilic suggest that the amphiphilic character of the Tus proline pipe sequence is unlikely to be coincidental. Similarly, the striking correspondence between the dimensions of the TPPI proline pipe helix and the distribution of basic amino acids on the surface with the dimensions of the major groove of B DNA and the position of phosphate groups along the backbone suggests a functional role for the helical structure observed.

In conclusion, the 22 amino acid peptide TPPI has been found to adopt a novel helical structure in 50% TFE. The unusual features of the proline pipe helix include, variable pitch in the helical turns, position-dependent ϕ,ψ dihedral angles within each turn, a limited hydrogen bonding network, and a channel along the helix axis. These characteristics are evidently the effect of steric constraints imposed by the proline repeat amino acid sequence. Based on the identification of many other amino acid sequences in a variety of proteins that contain similar repeats, it is possible that the proline pipe helix may be a relatively common protein secondary structure.

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REFERENCES

Bax, A., & Davis, D. G. (1985) Practical aspects of two-dimensional transverse NOE spectroscopy, *J. Magn. Reson. 63*, 355−360.

Coskun-Ari, F. F., Skokotas, A., Moe, G. R., & Hill, T. M. (1994) Biophysical characteristics of Tus, the replication arrest protein of *Escherichia coli*, *J. Biol. Chem. 269*, 11379−11385.

Clore, G. M., Gronenborn, A. M., Brunger, A. T., & Karplus, M. (1985) Conformation of a heptadecapeptide comprising the DNA binding helix F of the cyclic AMP receptor protein of *Escherichia coli*. Combined use of ^{1}H nuclear magnetic resonance and restrained molecular dynamics, *J. Mol. Biol. 185*, 435−455.

Davis, D. G., & Bax, A. (1985) Assignment of complex ^{1}H NMR spectra via two-dimensional homonuclear Hartmann−Hahn spectroscopy, *J. Am. Chem. Soc. 107*, 2820−2821.

Dyson, H. J., & Wright, P. E. (1991) Defining solution conformations of small linear peptides, *Annu. Rev. Biophys. Biophys. Chem. 20*, 519−538.

Gottlieb, P. A., Wu, S., Zhang, X., Tecklenburg, M., Kuempel, P., & Hill, T. M. (1992) Equilibrium, kinetic and footprinting studies of the Tus-Ter protein-DNA interaction, *J. Biol. Chem. 267*, 7434−7443.

Griesinger, G., Sorensen, O. W., & Ernst, R. R. (1987) Practical aspects of the E.COSY technique. Measurement of scalar spin−spin coupling constants in peptides, *J. Magn. Reson. 75*, 474−492.

Hiasa, H., & Marians, K. J. (1994) Tus prevents overreplication of oriC plasmid DNA, *J. Biol. Chem. 269*, 26959−26968.

Hill, T. (1992) Arrest of bacterial DNA replication, *Annu. Rev. Microbiol. 46*, 603−633.

Karplus, M. (1959) Contact electron-spin coupling of nuclear magnetic moments, *J. Phys. Chem. 30*, 11−15.

Nedved, M. L., Gottlieb, P. A., & Moe, G. R. (1994) CD and DNA binding studies of a proline repeat-containing segment of the replication arrest protein Tus, *Nucleic Acids Res. 22*, 5024−5030.

Nilges, M., Clore, G. M., & Gronenborn, A. M. (1988) Determination of three-dimensional structures of proteins from interproton distance data by dynamical simulated annealing from a random array of atoms, *FEBS Lett. 239*, 129−135.

Schultz, G. E., & Schirmer, R. H. (1979) *Principles of Protein Structure*, pp 67−71, Springer-Verlag, New York.

Skokotas, A., Wrobleski, M., & Hill, T. M. (1994) Isolation and characterization of mutants of Tus, the replication arrest protein of *Escherichia coli*, *J. Biol. Chem. 269*, 20446−20455.

States, D. J., Haberkorn, R. A., & Ruben, D. J. (1982) A two-dimensional nuclear Overhauser experiment with pure absorption phase in four quadrants, *J. Magn. Reson. 48*, 286−292.

Waterhous, D. V., & Johnson, W. C., Jr. (1994) Importance of Environment in Determining Secondary Structure in Proteins, *Biochemistry 33*, 2121−2128.

Williamson, M. P., Havel, T. F., & Wüthrich, K. (1985) Solution conformation of proteinase inhibitor IIA from bull seminal plasma by ^{1}H nuclear magnetic resonance and distance geometry, *J. Mol. Biol. 182*, 295−315.

Wüthrich, K. (1986) *NMR of Proteins and Nucleic Acids*, Wiley, New York.

Wüthrich, K., Billeter, M., & Braun, W. (1983) Pseudo-structures for the 20 common amino acids for use in studies of protein conformations by measurements of intramolecular proton−proton distance constraints with nuclear magnetic resonance, *J. Mol. Biol. 160*, 949−961.

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