Pergamon

Tetrahedron Letters, Vol. 35, No. 29, pp. 5105-5108, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $7.00+0.00

0040-4039(94)00949-X

Molecular Dynamics and NMR Studies of Single-Stranded PNAs

Shiow-Meei Chen*, Venkatraman Mohan, John S Kiely, Michael C. Griffith and Richard H. Griffey

ISIS Pharmaceuticals
2280 Faraday Avenue
Carlsbad, CA 92008 USA

Abstract:

Proton NMR spectroscopy and molecular dynamics simulations are employed to investigate the conformations of PNA monomers, a dimer and an octamer. The monomers exist as a 70:30 mixture of two amide rotamers interconverting slowly on the NMR time scale at 20 °C. In the major form, the side chain carbonyl group points toward the glycine, which places the methylene protons in proximity to the 2-aminoethyl protons. The minor form places its side chain carbonyl group away from the glycine, and the methylene protons are close in space to the glycine α protons. The PNA CT-dimer has multiple rotamers at 20 °C. In contrast, a NOESY spectrum taken from an octamer indicates only a single conformer in solution at 40 °C.

INTRODUCTION

Peptide Nucleic Acids (PNAs) represent a new class of nucleic acid oligomers which differ dramatically from DNA/RNA or conventional analogs.[1,2] Within the PNA oligomer the standard bases have been retained to insure the fidelity of Watson-Crick pairing, but the sugar phosphate backbone has been replaced with a peptide backbone (see

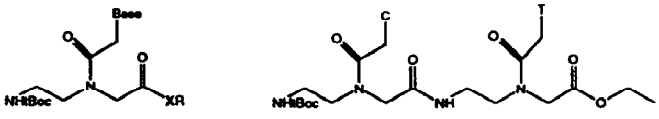

Fig. 1 Single-stranded PNA model compounds used for proton NMR studies.

Figure 1). Mixed pyrimidine/purine PNA sequences form antiparallel duplexes with complementary RNA or DNA. These have greater stability (T_m values) than duplexes between complementary oligonucleotide strands.[3] In addition, homopyrimidine PNAs can bind to DNA in both antiparallel and parallel orientations in a triplex motif.[3] PNAs have potential utility as antisense therapeutic agents and as diagnostics for gene identification and sequencing.[4]

As a mixture of a nucleobase and a modified peptide backbone, PNAs might be expected to exhibit physicochemical properties of both classes of molecules. To date, little structural information has been reported about the conformation of either single-stranded or duplexed PNAs in solution. CD analysis of PNA-RNA duplexes show that the bases stack in an A-form helix, but spectra of the PNA alone have little CD absorbance.[5] This result is unexpected since analysis of the T_m data would indicate that the PNA molecules have some degree of preorganization.[3] Molecular modeling studies have suggested some organization can occur via inter-residue hydrogen bonds.[6,7] Recently, the hydrophobic nature of the PNA backbone has been shown to enhance the stability of

PNA:DNA duplexes.[8] However, the interplay between stacking, hydrogen bonding and conformational mobility about the peptide backbone has yet to be defined, and no experimental information has been available.

We have studied the solution properties of single-stranded PNAs of varying lengths using NMR spectroscopy and molecular dynamics. NMR analyses of PNA monomers and dimer demonstrate that the barriers to rotation about the amide bonds are similar to those observed in peptides. Molecular dynamics and NMR of single-stranded PNA oligomers provide no evidence for preorganization facilitated by hydrogen bonds. Finally, NMR results on a mixed PNA octamer are consistent with a single conformation about both sets of amide bonds, which suggests the conformations of mixed PNAs are dominated by the properties of the nucleobases.

RESULTS AND DISCUSSION

In an earlier study,[6,7] an inter-residue hydrogen bonding scheme has been proposed to contribute to the preorganization of PNA single-stranded oligomers. Such molecular mechanics studies carried out in the gas phase without including explicit solvent molecule are known to overestimate the hydrogen bonding contribution to the total energy.[9] We have employed molecular dynamics simulation[10] to study a PNA CT-dimer in a 30 Å cube of water for possible hydrogen bondings. The time history of the total energy of this dimer shows energetically stable behavior. Data from the first 20 ps of the simulation is attributed to the equilibration process and not included in the final analysis. The time history of the distance between the oxygen of the carbonyl group of the nucleobase side chain and the hydrogen on the secondary nitrogen along the backbone (inter-residue hydrogen bonding distance) of the PNA dimer is shown in Fig. 2. The average distance is over 5 Å, which within this model, unequivocally shows the absence of any possible inter-residue hydrogen bonding.

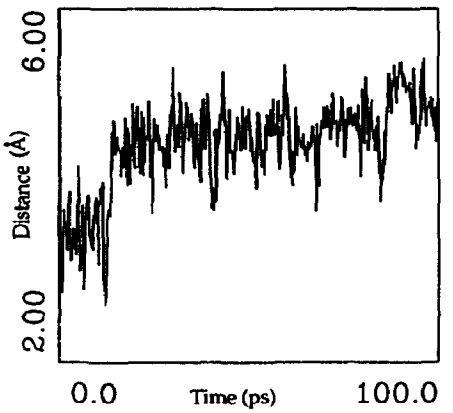

Fig. 2. A diagram showing the time-dependent inter-residue hydrogen bonding distance (for details see text) from a molecular dynamics simulation study of the PNA CT-dimer in water.

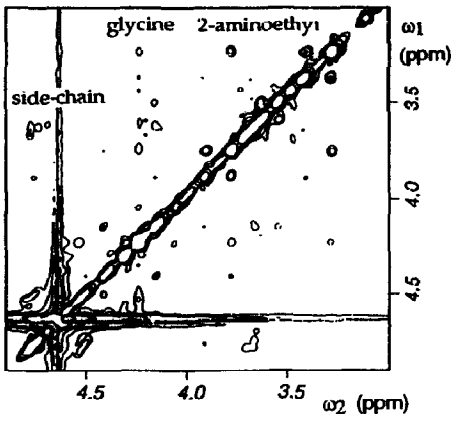

Fig. 3. Region of 500 MHz proton 2D NOESY spectrum of 2 containing crosspeaks between methylene protons arisen from different units of PNA. The spectrum was acquired from a D_2O sample at 310K with 400 ms mixing time. Two rotamers about the tertiary amide bond for each PNA residue exist, a total of four conformational rotamers are observed.

We have examined four PNA monomer esters and amides, a pyrimidine dimer and an octamer using ^1H NMR.[11,12] For monomer esters, amides **1a-d** and the dimer **2**, a 70:30 population of the cis:trans conformers about the tertiary amide bond of the nucleobase side chain is observed by 1 and 2D NMR. The interconversions between amide bond conformational rotamers usually require high activation energies (ΔE_a in the range of 10-25 kcal mol^{-1}).[13] Variable temperature proton 1D NMR studies are performed on the monomers and the dimer. The activation energy for the interconversion and the rate of exchange at 37° C are calculated [13] as 19±2 kcal mol^{-1} and 0.5~2 sec^{-1}, respectively. From NOESY spectra of dimer **2** (Fig. 3), the base protons (8H for purines and 6H for pyrimidines) are

close to the methylene protons on the carbon bearing the nucleobase. These same side chain protons are interchanging between major and minor conformers. In the major form, these methylene protons are close in space to one of the methylene protons of the 2-aminoethyl unit. This requires the major conformer to be in the cis-form. As expected, the minor conformer has these protons near the glycine α-protons. All of the protons are found engaging in slow exchange on the NMR time scale between the cis- and trans-conformer.

Rotamer population distributions of 1c remain unchanged when NMR experiments are undertaken in acetonitrile or in DMSO, and temperature coefficients of the amide protons are estimated [14] to be in the range of -9 to -10 ppb K^{-1}. These two lines of experimental results does not favor intramolecularly hydrogen bonds in PNA. We also examined a PNA octamer to determine if the observations for the smaller PNAs are confirmed. In contrast to the simple model monomers and dimer, the PNA octamer exhibits much simpler NOE cross peak patterns (Fig. 4), which suggests the octamer adopts a single conformation. The preorganized structure of this PNA octamer can be rationalized as analogous to that of ss DNA and ss RNA, where the stablizing elements are likely coming from base stacking.[15]

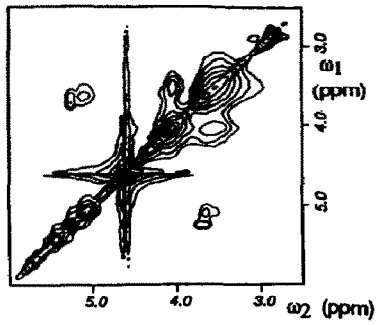

Fig. 4. Region of 500 MHz proton 2D NOESY spectrum of 3 containing methylene protons arisen from side chain (~5 ppm), glycine (~4 ppm) and 2-aminoethyl (3.5~3 ppm). The spectrum was acquired at 313 K with 300 ms mixing time. Strong NOE crosspeaks between methylenes of side chain and 2-aminoethyl indicate that cis-conformation about the tertiary amide bond predominates.

CONCLUSIONS

Molecular dynamics and proton NMR have been used to study the conformational properties of four PNA monomers, a dimer and an octamer. NMR analyses of the PNAs demonstrate the slow interconversion of two rotamers about the tertiary amide bond, with an energy barrier similar to amides in peptides. No intra- or inter-residue hydrogen bonds are observed in the ss PNAs in organic or aqueous solvents. The preorganization observed for a mixed PNA octamer may result from energetically favorable base stacking interactions. The reduced base stacking in homopyrimidine PNAs may allow alternative conformations along the peptide backbone.

REFERENCES AND NOTES

1. Nielsen, P. E.; Egholm, M.; Berg, T. H.; Buchardt, O. *Science* **1991**, *254*, 1497-1500.
2. Egholm, M.; Buchardt, O.; Neilsen, P. E.; Berg, R. H. *J. Am. Chem. Soc.* **1992**, *114*, 1895-1897.
3. Egholm, M.; Buchardt, O.; Christensen, L.; Behrens, C.; Frier, S. M.; Driver, D. A.; Berg, R. H.; Kim, S. K.; Norden, B.; Nielsen, P. E. *Nature* **1993**, *365*, 566-568.
4. Nielsen, P. E.; Egholm, M.; Berg, R. H.; Buchardt, O. *Anti-Cancer Drug Design* **1993**, *8*, 53-63.
5. Kim, S. K; Nielsen, P. E.; Egholm, M.; Buchardt, O.; Berg, R. H.; Norden, B. *J. Am. Chem. Soc.* **1993**, *115*, 6477-6481
6. Almarsson, O.; Bruice, T. C.; Kerr, J.; Zuckermann, R. N. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 7518-7522.
7. Almarsson, O.; Bruice, T. C. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 9542-9546.
8. Mohan, V.; Griffey, R. H. *Chem. Phys. Lett.* submitted.
9. McCammon, J.; Harvey, S. *Dynamics of Proteins and Nucleic Acids* **1987**, Cambridge Press, Cambridge.
10. *Molecular Dynamics:* PNA dimer (CT) has been model-built in canonical B-DNA conformation. The protocol of molecular dynamics study started with placing the nucleotide in a 30.0 Å cubical box. Following by solvating with pre-equilibrated water molecules and by removing bad contacts to obtain a density of approximately 1 g cm^{-3}, and eventually a total of 855 waters were left in the central box. All atom CFF91

forcefield [16] has been employed and a three-point flexible interactions were computed via Coulomb's law using a dielectric constant of 1.0. The cutoff used for the intermolecular interactions was 15.0 Å. Periodic boundary conditions were applied in all directions. The simulation was first initiated by 5000 steps of steepest descent minimization and then carried out with a time step size of 1 fs for a total of 100 ps at constant volume and constant temperature. Initial velocities were assigned from a Maxwell-Boltzmann distribution at 100 K. The heating period was divided into three steps. In each step, the temperature of the system is raised slowly by 100 K over a period of 5 ps. This heating process is repeated until the final temperature of 300 K is obtained.

11. Each PNA amide monomers **1a-d** (3.0 mg) was dissolved in 0.5 ml of D_2O containing d_4-methanol (15-50%). The dimer **2** was dissolved in 100% D_2O and in 95% H_2O containing 5% D_2O to give a solution of 9 mg ml^{-1}. The octamer **3** was dissolved in 50 mM ammonium acetate, pH 6.7 resulted in a concentration of 175 A^{260} ml^{-1}. In order to minimize the competitions from the solvent for the possible intrinsic hydrogen bonds in the ss PNA, **1c** was also dissolved in d_3-acetonitrile and in d_6-DMSO. Proton 1D and 2D NMR experiments were conducted in a Bruker AMX-500 spectrometer. All spectra were acquired and plotted in the phase-sensitive mode. The transmitter was placed on the solvent resonance, and the TPPI method [17] was used to achieve quadrature detection in the ω_1 dimension. Standard pulse sequences and phase cyclings were used for obtaining 2QF-COSY [18], NOESY [19]and ROESY [20] spectra. All NMR data were processed using an Iris-Crimson computer with Felix software.[16]
12. The PNAs were prepared by the method of Egholm.[2] For the monomers 3-aminopropane was employed as the incoming amine. The monomer gave satisfactory CHN analyses as hydrates. The dimer and octamer were single peaks by HPLC analysis after preparative HPLC purification and lyophilization. These gave correct molecular weights by electrospray MS.
13. Günther, H. *NMR spectroscopy* **1980**, Wiley, New York, pp 234-280.
14. Deslauriers, R.; Smith, I.C.P. *Biological Magnetic Resonance*, **1980**, *2*, 286-288.
15. Saenger, W. *Principles of Nucleic Acid Structure* **1983**, Springer-Verlag, New York, pp 298-320.
16. Biosym Technologies, Inc., San Diego, CA.
17. Marion, D.; Wüthrich, K. *Biochem. Biophys. Res. Commun.* **1983**, *113*, 967-974.
18. Rance, M.; Sorensen, O. W.; Bodenhausen, G.; Wagner, G.; Ernst, R. R.; Wüthrich, K. *Biochem. Biophys. Res. Commun.* **1983**, *117*, 479-485.
19. Macura, S.; Ernst, R. R. *Mol. Phys.* **1980**, *41*, 95-117.
20. Bothner-By, A. A.; Stephens, R. L.; Lee, J. *J. Am. Chem. Soc.* **1984**, *106*, 811-813.

(*Received in USA* 4 *April* 1994; *revised* 5 *May* 1994; *accepted* 13 *May* 1994)