NUCLEAR MAGNETIC RESONANCE STUDIES OF HYDROGEN BONDED COMPLEXES OF OLIGONUCLEOTIDES IN AQUEOUS SOLUTION. I. pdG-dC AND pdG-dT

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Summary

The self-interaction of two deoxydinucleotides, pdG-dC and pdG-dT, was studied (in aqueous solution) by 100 MHz FT NMR spectroscopy. Concentration studies show that the self-complementary pdG-dC forms hydrogen bonded complexes. An analysis based on the concentration dependence of the chemical shift of the guanine amino protons strongly suggests that hydrogen bonded dimer formation occurs with a K for the dimerization equilibrium of $7.8 \pm 0.7\ M^{-1}$. On the other hand, the non-self-complementary pdG-dT does not give evidence of similar complex formation in the same concentration range which thus illustrates the importance of complementarity in the formation of hydrogen bonded complexes of deoxydinucleotides. Further impetus is therefore provided for the use of dinucleotides in modeling interactions common to nucleic acids.

Nuclear magnetic resonance studies in H_2O solutions (1) provide the opportunity of observing the important resonances of the NH and NH_2 protons of proteins (1) and nucleic acids (2). For example, Crothers et al. (3) have used this approach to observe the exchangeable ring NH protons of the double helical complex of d(A-A-C-A-A) with d(T-T-G-T-T), while, in previous work in D_2O solution, Cross and Crothers (4) observed this complex formation by monitoring the nonexchangeable protons. On the other hand, the association of mononucleotides in aqueous solution primarily results in the vertical stacking of the bases (e.g. see ref. 5), although Raszka and Kaplan (6) have observed downfield shifts of the amino protons of mononucleotides which is evidence for the formation of hydrogen bonded base pairs. In this communication we will show that the

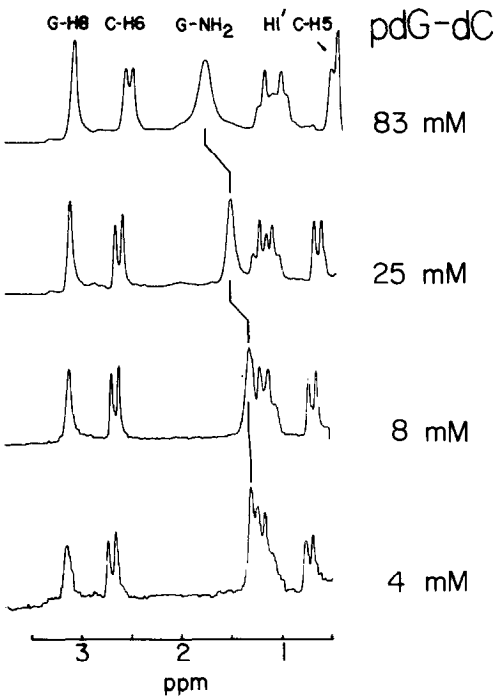

Figure 1. The 100 MHz proton NMR spectra of pdG-dC in H_2O at several concentrations; temperature 2.2±0.3°C, pH 7.15±0.15. Notes: Spectra were accumulated using a 180°-τ-90° pulse sequence (WEFT method) with a supplemental homogeneity spoiling pulse applied to further reduce the H_2O peak, as previously described (10). The sample, purchased from Collaborative Research Corp., was purified by passage through a Chelex-100 column and recovered by lyophilization. The dilutions were performed by adding weighed portions of glass distilled and filtered water. The molarity of the most concentrated sample was determined spectrophotometrically. Lower scale: ppm downfield from the H_2O peak used as reference.

concentration dependence of the chemical shift of the guanine 2-amino group of the deoxydinucleotide pdG-dC provides straightforward evidence of a hydrogen bonded complex between two of these self-complementary deoxydinucleotides. This data thus provides support for the use of the deoxy and ribo-dinucleotides as model compounds for studying drug-nucleic acid interactions (7-9).

The 100 MHz proton nmr spectra of pdG-dC in H_2O at several concentrations are shown in Fig. 1. The guanine 2-amino protons move <u>downfield</u> as the concentration of the nucleotide is increased, while the C-H5 and C-H6 protons move <u>upfield</u> with increasing concentration. The

G-H8 proton chemical shift is essentially constant over this concentration range. The large downfield shift of the amino protons almost certainly results from intermolecular hydrogen bond formation (2-4,6, and references therein). Patel (11) recently reported the 300 MHz proton magnetic resonance spectrum of pdG-dC in H_2O solution, in connection with a study on actinomycin D. However, a concentration dependence study was not performed and in his discussion Patel (11) assumed that the pdG-dC molecules exist as monomers (50 mM, pH 5.8).

The cytosine 4-amino protons are difficult to monitor during the titration. These protons have been shown to be nonequivalent due to restricted rotation of the amino group (6,12) as shown in Figure 2. In addition, the exchange of the cytosine 4-amino protons with the solvent can also help to account for the low intensity of the cytosine amino resonances in Fig. 1, even though this exchange may not be fast enough to substantially broaden the resonances. This transfer of saturation effect will be discussed in detail in a separate paper on proton fourier transform spectroscopy in aqueous solutions (13).

The chemical shift of the guanine 2-amino protons of the deoxydinucleotide pdG-dT (Fig. 3) changes less than 5 Hz over the concentra-

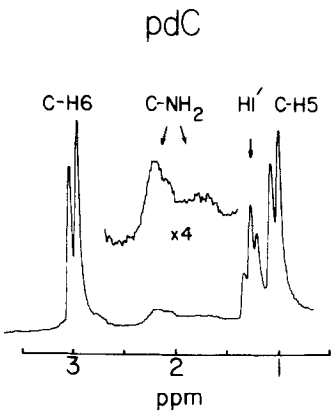

Figure 2. The 100 MHz proton NMR spectrum of pdC in H_2O; concentration 87 mM, temperature 0.0°C, pH 7.00. Lower scale: ppm downfield from H_2O used as reference.

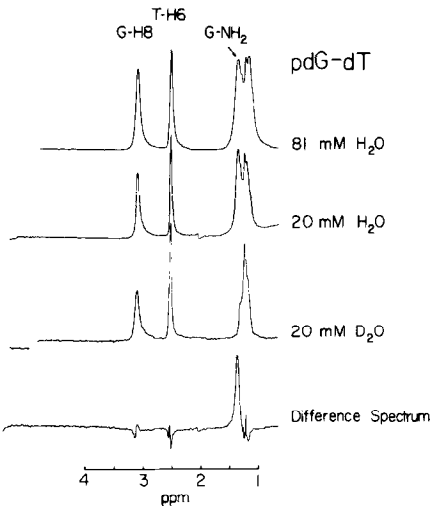

Figure 3. The 100 MHz proton NMR spectra of pdG-dT in H_2O and D_2O; temperature 1.7±0.3°C; pH (meter reading) 7.0 The notes of Figure 1 apply here also.

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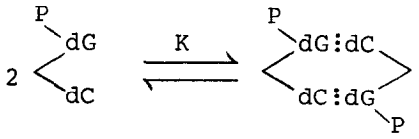
tion range of 5 mM to 80 mM. Thus the guanine 2-amino protons of pdG-dT are not involved in intermolecular hydrogen bond formation in this concentration range. A comparison of the spectra for the self-complementary dinucleotide pdG-dC with the spectra for the non-complementary dinucleotide pdG-dT illustrates the effect of complementarity (e.g. see ref. 9). On the basis of previous experiments on ribodinucleoside monophosphates (e.g. Bangerter and Chan (14) or Ts'o et al. (15)), we did not expect to observe significant shifts of the non-exchanging base protons as a function of increasing dinucleotide concentration in this concentration range. The chemical shifts of the non-exchanging protons of pdG-dT changed less than 4 Hz over the concentration range of 5 mM to 80 mM. If the chemical shifts of the non-exchanging protons of the pdG-dC dinucleotide are likewise not influenced by intermolecular vertical base stacking in this concentration range, then the observed concentration dependence of the chemical shifts of the C-H5 and C-H6 protons (Fig. 1) must result from formation of the hydrogen bonded complex. Although the observed chemical shift changes are in accord with the

formation of a double helical complex containing Watson-Crick base pairs, further experiments are required before a detailed analysis is warranted.

The concentration dependence of the chemical shift of the guanine 2-amino protons may be used to determine the equilibrium constant of hydrogen bond formation. Assuming that the hydrogen bond formation results from the formation of a dimer (i.e. a double helix)



Eq. (1)

the appropriate expression is

$$\delta_{obs} - \delta_M = (\delta_D - \delta_M)\left\{\frac{4K[N]_o + 1 - \sqrt{(8K[N]_o + 1)}}{4K[N]_o}\right\}$$

Eq. (2)

where δ_{obs} is the observed chemical shift, δ_M is the chemical shift of the protons in the monomer state; δ_D is the chemical shift of the protons in the dimer, and $[N]_o$ is the stoichiometric concentration of the nucleotide. The values obtained from a nonlinear least squares regression analysis are $K = 7.8 \pm 0.7\ M^{-1}$, $\delta_M = 1.22 \pm 0.01$ ppm and $\delta_D = 2.71 \pm 0.06$ ppm. The excellent agreement between the observed and calculated chemical shifts (for the parameters given above) is illustrated in Fig. 4. This agreement supports the interpretation of the data in terms of the formation of a double helix (Eq.(1)). The magnitude of K reported here ($7.8 \pm 0.7\ M^{-1}$) is reasonable when compared to the estimate value of K for the base pairing of GMP + CMP ($0.6\ M^{-1}$, ref. 6) and the value of K ($100\ M^{-1}$) measured by Crothers et al. (3) for a mixture of complementary pentanucleotides. As expected in this comparison, the stability of the helix increases with the length of the helix.

We also note that we have not been able to observe the resonance of the hydrogen bonded guanine NH proton. This is not surprising in view of the work of Crothers et al. (3) in which the ring NH protons were ob-

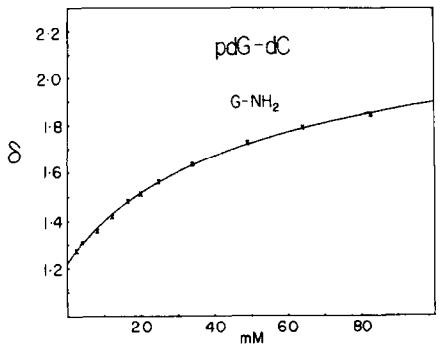


Figure 4. The chemical shift of the G-NH_2 protons of pdG-dC as a function of concentration at 2.2±0.3°C and pH 7.15±0.15. The water peak was used as reference. The solid curve is the least squares fit to the experimental points (x).

served to broaden into the baseline far below the T_m of the pentanucleotid double helix. In addition, transfer of saturation effects may also play a role in our experiments (13). However, the guanine 2-amino protons seem to provide a reliable measure of helix formation. Finally, the present techniques should provide a means of obtaining valuable information on a variety of oligonucleotide complexes (e.g. codon-anticodon recognition), as well as drug-nucleic acid interactions using oligonucleotides as models for DNA and RNA.

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References

1. Glickson, J.D., McDonald, C.C., and Phillips, W.D. (1969) Biochem. Biophys. Res. Comm. 35, 492-498.
2. Kearns, D.R., Patel, D., and Shulman, R.G. (1971) Nature 229, 338-339.
3. Crothers, D.M., Hilbers, D.W., and Shulman, R.G. (1973) Proc. Nat. Acad. Sci. U.S. 70, 2899-2901.
4. Cross, A.D., and Crothers, D.M. (1971) Biochemistry 10, 4015-4023.
5. Ts'o, P.O.P., Schweizer, M.P., and Hollis, D.P. (1969) Annals New York Acad Sci. 158, 256-297.
6. Raszka, M., and Kaplan, N.O. (1972) Proc. Nat. Acad. Sci. U.S. 69, 2025-2029.
7. Krugh, T.R. (1972) Proc. Nat. Acad. Sci. U.S. 69, 1911-1914.
8. Krugh, T.R., and Neely, J.W. (1973) Biochemistry 12, 4418-4425.

9. Krugh, T.R., Wittlin, F.N., and Cramer, S.P. (1975) Biopolymers, in press.
10. Mooberry, E.S., and Krugh, T.R. (1975) J. Magnetic Resonance, in press.
11. Patel, D.J. (1974) Biochemistry 13, 2388-2395.
12. Shoup, R.R., Becker, E.D., and Miles, H.T. (1971) Biochem. Bio. Phys. Res. Comm. 43, 1350-1353.
13. Krugh, T.R. and Schaefer, W., in preparation.
14. Bangerter, B.W., and Chan, S.I. (1969) J. Am. Chem. Soc. 91, 3910-3921.
15. Ts'o, P.O.P., Kondo, N.S., Schweizer, M.P., and Hollis, D.P. (1969) Biochemistry 8, 997-1029.