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THE PREPARATION AND SPECTROSCOPIC CHARACTERIZATION OF A WEAKLY SELF-ASSOCIATING SALT OF GUANYLYL-(3'-5')-GUANOSINE

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The tetramethylammonium salt of guanylyl-(3'-5')-guanosine has been prepared by a cation-exchange technique and it has been found that the tetramethylammonium ion drastically reduces the self-association of GpG in solution. This has allowed the characterization of GpG by FTIR and 1-D and 2-D NMR spectroscopy. A complete, well-resolved ^{1}H NMR spectrum in D_2O has been obtained and all resonances have been assigned. A weak, essentially non-cooperative intermolecular association is observed in solution (15-20 mM) below 40°C. The association occurs via base stacking and base-base hydrogen bonding.

The guanine mono- and dinucleotides have been used frequently as model compounds to study the binding sites of the platinum antitumor agents,(1-3) such as cis-diamminedichloroplatinum(II), and certain antibiotics.(4,5) These agents are believed to bind to adjacent guanine residues or guanine-rich regions in nucleic acids. Although the deoxyribodinucleotide has been used for this purpose, the ribodinucleoside monophosphate, guanylyl-(3'-5')-guanosine has, with a few exception,(6,7) been largely ignored because its strong self-association behavior has rendered it difficult, if not impossible, to study by some experimental techniques. For example, the structurally-informative technique of nuclear magnetic resonance was not previously applicable to GpG solution studies because of severe line-broadening resulting from the association.(8) We have prepared the tetramethylammonium salt of GpG and have found that the TMA ion drastically reduces but does not completely eliminate, the self-association tendency. The import of this work is that the GpG moiety can now be obtained in a form

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Abbreviations: TMA = tetramethylammonium; DSS = 2,2-dimethyl-2-silapentane-5-sulfonate

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which will permit controlled studies of its binding to other biomolecules and of its self-association upon addition of metal ions. We report here the characterization of GpG by FTIR and, for the first time, a complete, well-resolved ^1H NMR spectrum, including assignment of ribose frequencies. This fundamental information is a prerequisite for future studies of the interaction of GpG with other molecules or ions.

It is not surprising that the TMA ion reduces the self-association of GpG in aqueous solution. Tetraalkylammonium ions have been shown to reduce association in poly(G) (9) and to prevent the base stacking,(10) but not base-base hydrogen bonding(11) in guanosine 5'-monophosphate. A weak non-cooperative type of intermolecular self-association is observed for TMAGpG solutions (15-20 mM in D_2O) below 40°C. The association occurs via base stacking and base-base hydrogen bonding.

MATERIALS AND METHODS

NH_4GpG (25 mg, Sigma) was dissolved in 1-2 mL deionized, distilled water (pH adjusted to 8 with TMAOH), and eluted through a Dowex 50W-X8 column which had previously been loaded with TMA ion. The eluate (15-20 mL) was lyophilized; then relyophilized twice from D_2O. Stock solutions (30-37 mM) were prepared by dissolving the solid in 1 mL D_2O. Research solutions were prepared by dilution of stock solutions and the pD adjusted to 8.0-8.1 with DCl. Concentrations were determined by UV spectroscopy; ε at 252 nm = 25.0×10^3 $M^{-1}cm^{-1}$.(12) The purity was checked by TLC on PEI-cellulose precoated plates containing fluorescent indicator using 0.2M LiCl/DMSO (19/1). R_f= 0.37 (13)

The ^1H NMR spectra were run on a Varian 400 VXR (400 MHz) spectrometer. All spectra were run with solvent suppression (gated homonuclear decoupling) except where noted. The TMA ion was used as an internal reference and was set to 0.000 ppm (to convert to the DSS scale, add 3.185 ppm). The 1-D spectra were obtained using a 23° pulse width, 32K data points, a pulse interval of 4.5 sec, and 96 or 192 transients. An FT size of 512x512 was used for the ^1H-COSY experiments. The FTIR spectra were obtained on a Nicolet 60SX spectrometer with 2 cm^{-1} resolution.

RESULTS AND DISCUSSION

^1H NMR spectra of 15 mM TMAGpG in D_2O at representative temperatures are shown in Figure 1. The presence of some self-association is revealed in the spectrum at 9°C by the line broadening and modest upfield shifts in the resonance frequencies. Although the H1' doublets are not resolved, the H8 and H1' resonances of the two guanine residues are distinct. Even as low as 2°C (not shown) this is so, although the H8 lines are beginning to overlap. The degree of intermolecular association is greatly reduced at 23°C, with both H1' doublets resolved as well as the H2' and H3' resonances of the 3'-terminus residue (-pG). From the poorer resolution of the 5'-terminus (Gp-) resonances, it appears that this residue is more strongly involved in intermolecular interactions at this temperature. At 59°C, the resolution of

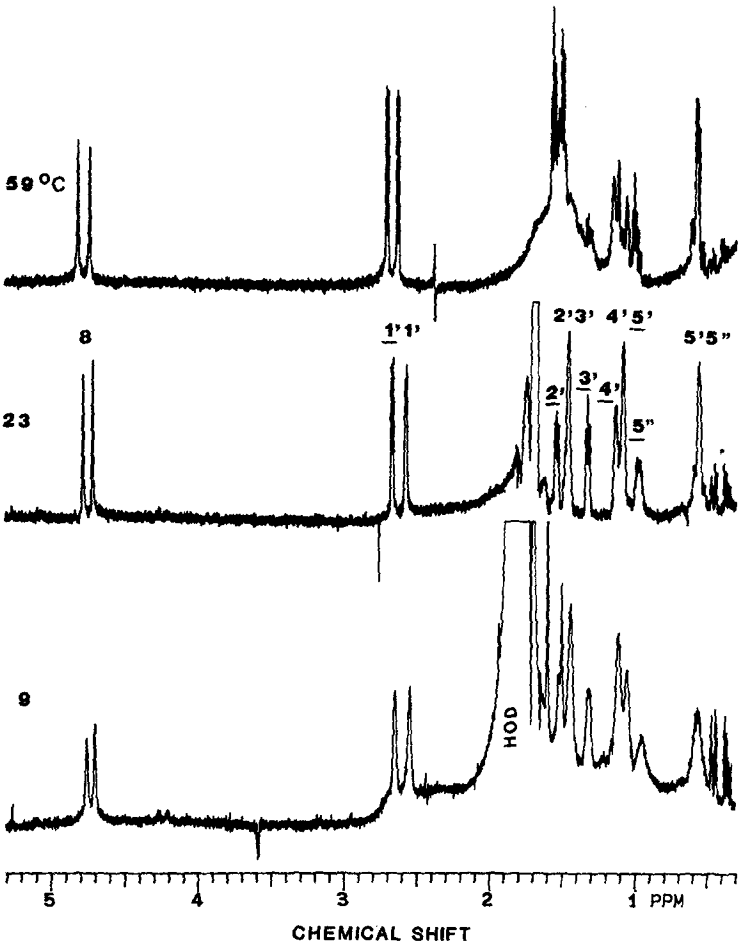

Figure 1. 400 MHz ^1H NMR spectra of 15 mM TMAGpG in D_2O; pD 8.1. Referenced to TMA^+ at 0.000 ppm. Underlined assignments refer to -pG residue and are based on Fig. 3. H2' and H3' of Gp- overlap at 23°C. Spectra at 23 and 59°C run with solvent suppression (gated decoupling). * = impurity.

the multiplets and the constant or nearly constant δ values indicate that GpG exists as the monomer at this temperature.

A plot of the H8 chemical shift vs. temperature (Fig. 2) reveals that the thermal transition between associated and monomeric species occurs over a fairly broad temperature range, centered at 20-25°C. Although there is a small amount of sigmoidal character to the thermal melting curve, the transition is basically non-cooperative in nature, indicative of associated species which are not highly ordered. The rather low temperature at which the aggregates dissociate means that they are not very stable. In contrast to the TMA salt of GpG, a T_m of 50°C (cooperative) has been reported for GpG in the presence of a mixture of Na^+ and NH_4^+ (0.16M) at pH 7.(14)

The downfield shift of the proton resonances with increasing temperature is indicative of destacking of the bases. At low temperatures where the

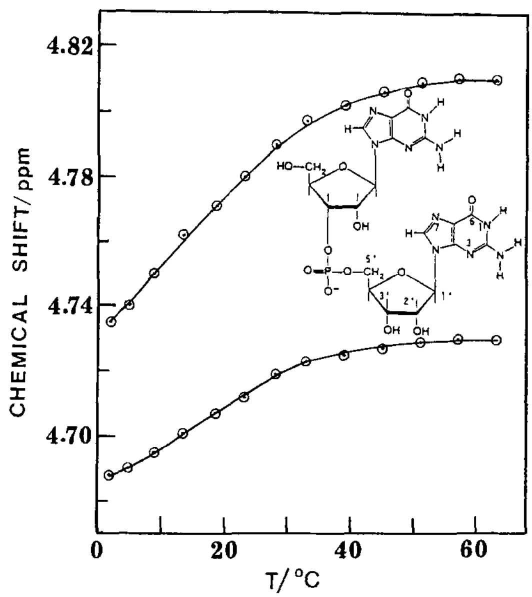

Figure 2. ^1H NMR of 15 mM TMAGpG in D_2O at pD 8.1 as a function of temperature; H8 resonances.

resonances are broadened, both inter- and intramolecular stacking are likely to be occurring. At 59°C the chemical shifts indicate that a portion of the GpG monomers remain in a stacked conformation. A stacking constant of 0.17 at 60°C for the equilibrium unstacked $\rightleftharpoons$ stacked has been calculated from the thermodynamic data of Ogasawara and Inoue.(15)

The proton assignments indicated in Fig. 1 were determined by a ^1H COSY experiment at 23°C. (Fig. 3). They are consistent with the chemical shifts of guanine residues reported for heterodinucleoside monophosphates.(16) The assignment of the two sets of ribose resonances to the Gp- and -pG residues was based on the assumption that the highest field ribose resonance was that of the H5',5" of the Gp- residue, as assigned in the guanosine monophosphates,(17,18) and heterodinucleotides.(16)

A ^1H COSY experiment at 57°C permitted the assignment of the H2' at 1.557 ppm (-pG) and 1.485 ppm (Gp-). At this temperature the overlap of the H2' and H3' resonances of the Gp- residue was removed and the multiplet located at 1.52 ppm, between the two H2' multiplets, was assigned to H3'(Gp-) on the basis of its chemical shift and observation of the splitting due to coupling with the phosphorus. The positions of the H3' resonance downfield from that of the H2' resonance in the 5'-terminus has not been observed in guanine-containing ribodinucleotides previously, but has been found in ApA at 72°C.(19) This is a result of the conformation about the C3'-O3' and P-O3' bonds of the phosphodiester. Above the melting temperature of the associated species, GpG behaves like a "normal" dipurine nucleoside monophosphate,

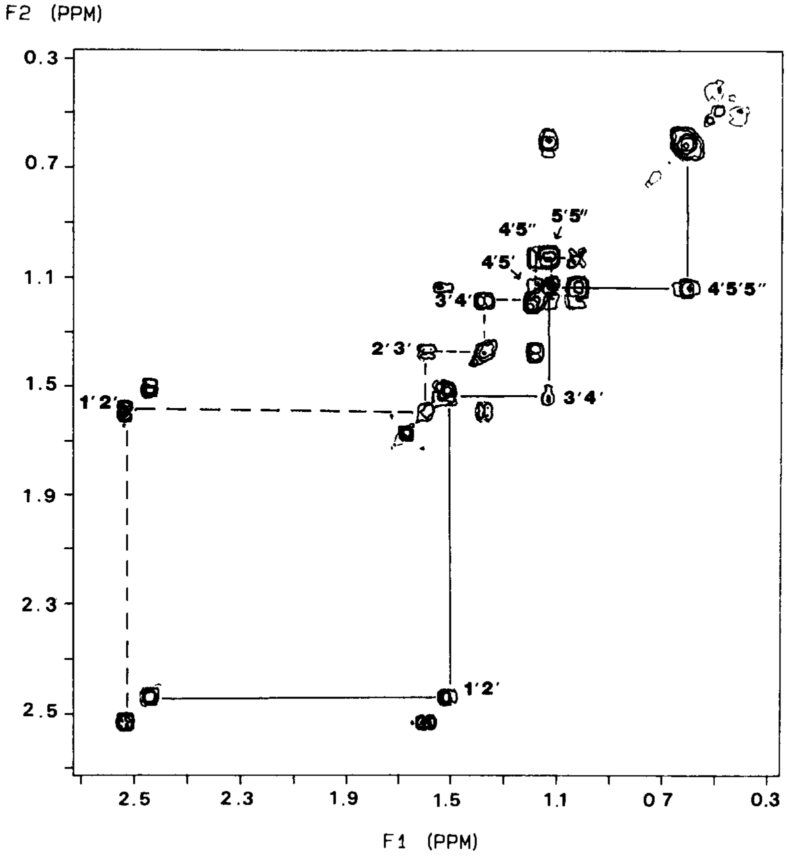

Figure 3. ^{1}H COSY spectrum of 15 mM TMAGpG in D_2O at pD 8.1 and 23°C. ---- connectivities in -pG; —— connectivities in Gp-. See Fig. 1 for 1-D spectrum. Referenced to TMA$^+$; gated decoupling on HOD line.

such as ApA. The proton NMR at 60-70°C of both compounds exhibit reversal of the chemical shift positions of H2' and H3', both are monomers existing in part as a stacked conformer, and both have very similar H1'-H2' coupling constants(19) (4.8 Hz for Gp- and 5.2 Hz for -pG at 59°C).

The proton-decoupled ^{31}P NMR spectrum (Jeol FX-90Q, 36.2 MHz) of 9 mM TMAGpG in D_2O at pD 8.0 and 27°C consists of a single line at -0.52 ppm (upfield from external H_3PO_4, $\nu_{1/2}$=1.2 Hz) This is in the chemical shift range normally observed for ribodinucleoside monophosphates.(20)

The infrared spectra of D_2O solutions as a function of temperature (Fig. 4) were obtained on a Nicolet 60SX FT spectrometer The observed IR thermal behavior is very much like that reported for poly(G) (9), which has a four-stranded double-helical structure in the solid state.(21) The band at 1675 cm^{-1} (20°C and below) arises primarily from the carbonyl stretching vibration. At low temperatures, this absorption displays a hyperchromic effect and a shift to higher frequency. The intensity effect is consistent with planar base-base hydrogen bond formation(22) and the frequency change is

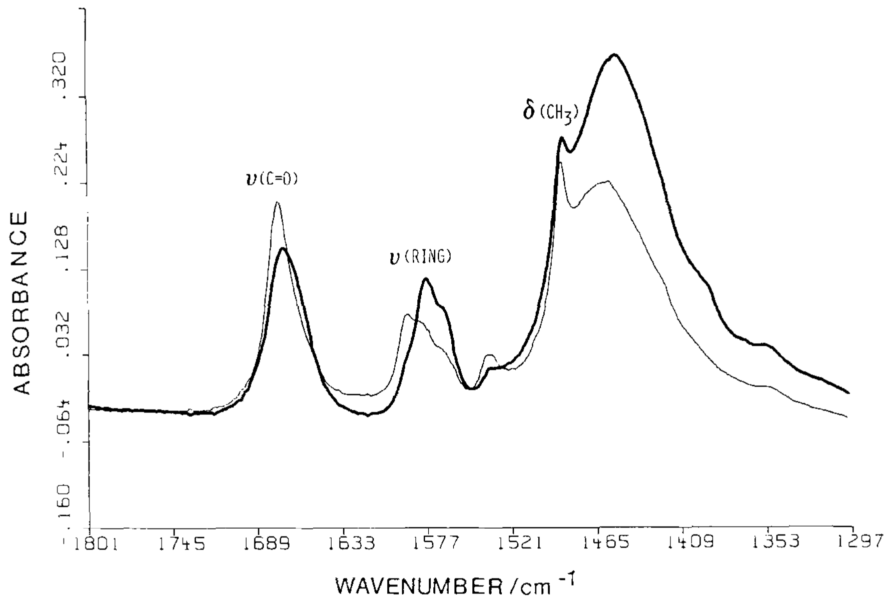

Figure 4. FTIR spectrum of 15 mM TMAGpG in D_2O; pD 8.0. Light line at 22°C; heavy line at 60°C. The band at 1455 cm^{-1} is an HOD bend.

that typically observed when base-water H-bonds are replaced by base-base H-bonds.(23,24) The low temperature hypochromism of the band centered near 1580 cm^{-1}, which arises from guanine ring vibrations, is due to base stacking.(22) It can be concluded that intermolecular association is occurring via both H-bonding and base stacking.

Although the melting profiles for the associated GpG are of the same shape for both IR and NMR data, the IR data are shifted to higher temperature, with the maximum change centered at 35-45°C. It is possible that the two kinds of measurements are reflecting different changes in the solute species. However, the design of the low and high temperature IR cells(25) and the location of the thermocouple are such that the observed difference could arise from inaccuracy in measuring the solution temperature. The imprecision would result in the measured solution temperature being too high above and too low below room temperature, consistent with the higher dissociation temperature observed by IR.

Several types of structures can be proposed for the associated species. A tetramer of GpG molecules in which four bases (one from each of the four GpG's) H-bond to form a planar cyclic tetramer of guanines (N7 and O6 acceptors, N2-H and N1-H donors) and which stack upon an identical structure formed by the other four bases, has been proposed.(14,26) This would constitute the basic unit of a four-stranded helix, and several such species could stack upon each other. However, there are several other guanine H-bonding schemes which would give dimers of GpG.(27)

The main objective of this research has been to prepare a salt of GpG whose intermolecular self-association is sufficiently small to allow its characterization by NMR spectroscopy and to permit studies of its binding to other molecules. The mechanism by which the TMA ion reduces self-association is not known, but it may interfere with the intermolecular stacking of guanine bases.

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