Among the fundamental subunits of nucleic acids, guanosine 5'-monophosphate (5'-GMP) has the unique ability to self-associate spontaneously in either acidic1,2 or neutral3 aqueous solutions, forming ordered helical structures. Although it was suggested in 1962 that a hydrogen-bonded guanine tetramer known as the G-quartet2 (Figure 1a) is the basic building block of such helices, their exact structures have remained unsolved for more than four decades. Here we use nuclear magnetic resonance (NMR) spectroscopic methods to investigate the detailed structures formed by Na₂(5'-GMP) self-assembly in neutral solution. We have found that three types of 5'-GMP aggregates generally coexist in solution, using monomers, dimers, and G-quartets as basic building blocks, respectively. The dimer formation is based on the centrosymmetric structure denoted as GG3² (Figure 1a) in the Jeffrey and Saenger notation.¹² Most interestingly, the G-quartets stack on top of each other forming a right-handed helix where alternating C2'-endo and C3'-endo sugar puckers are found along the helical strand.

Figure 1. (a) Structures of GG3² dimer and G-quartet. (b) H₈ region of a ¹H 2D DOSY NMR spectrum of 1.0 M Na₂(5'-GMP) in D₂O at 278 K.

It has been well-known that the H₈ region of the ¹H NMR spectrum of a concentrated Na₂(5'-GMP) solution exhibits four major signals (H₆, H₇, H₈, and H₉ as shown in Figure 1b). Pinnavaia and colleagues⁷,8,11 interpreted these signals as being due to the presence of C₄ and D₄ stereoisomers of a G-octamer. However, we recently showed that the size of Na₂(5'-GMP) self-aggregates is on the nanometer scale, much larger than that of a G-octamer.¹³

This finding immediately called for a new spectral interpretation and ultimately led to the complete structural determination reported herein. To establish the exact identities of these ¹H NMR signals, we first used diffusion-ordered spectroscopy (DOSY)¹⁴ NMR experiments. As seen in Figure 1b, the H₆ and H₇ signals are associated with the same molecular aggregate that has a much smaller translational diffusion coefficient (D), (8.8 ± 0.5) × 10⁻¹² m²/s at 278 K, than those giving rise to the H₈ and H₉ signals. The D value observed for the H₈ signal, (11.6 ± 0.5) × 10⁻¹² m²/s, suggests that this aggregate is due to the stacking of 5'-GMP dimers (vide infra). The H₈ signal exhibits the largest D, (17.2 ± 0.5) × 10⁻¹² m²/s, and arises from the stacking of monomers.¹³ Because of the presence of four sets of very similar signals, ¹H NMR spectra of Na₂(5'-GMP) are extremely overcrowded in the region containing sugar proton resonances, making it difficult to use a conventional NMR approach for structural determination. To gain structural information about the H₆/H₇ aggregates, we employed a combined DOSY and NOESY approach.¹⁵ Figure 2a shows parts of the 2D ¹H DOSY-NOESY spectrum of 1.0 M Na₂(5'-GMP) in D₂O. Because the signals from 5'-GMP dimers and monomers are diffusively “filtered out” in this spectrum, two sets of ¹H resonances can be clearly identified. Complete spectral assignment for sugar ¹H resonances was established using ¹H DQF-COSY and DOSY-NOESY spectra. Homonuclear (J₁H₁₂) and heteronuclear (JᵣC₅ and JᵣH₈) indirect spin–spin coupling constants were measured from ¹H DQF-COSY, ¹H–¹³C HSQC, and ¹H–¹⁵P COSY spectra, respectively. Sterechemical assignment of the H₅' and H₅'' signals was achieved using J(C₅',H₅') > J(C₅',H₅'').¹⁶ Our density functional theory (DFT) calculations at the B3LYP/6-311++G(d,p) level also confirmed this trend of J-coupling constants; see Supporting Information. All ¹³C resonances were unambiguously assigned from ¹H–¹³C HSQC and HMBC spectra. Because the values of J(H₆, H₇) are too small to be useful, we used a combination of H₁–C₅ HMBC and C₅–H₈ HSQC experiments to establish the connectivity between H₈ signals (H₆ and H₇) and H₆ resonances. Resonances for exchangeable imino protons, N₁H, were assigned using refocused ¹H–¹³C HMBC spectra in which H₈ → C₅ → N₁H connectivity was established. A complete list of ¹H, ¹³C, and ¹⁵P chemical shifts and J-coupling constants are provided in the Supporting Information.

To further establish that G-quartet formation is responsible for the presence of both sets of ¹H resonances, we obtained 2D ¹H NOESY spectra of 1.0 M Na₂(5'-GMP) in D₂O/H₂O (1:1) at 278 K allowing detection of exchangeable protons. As seen in Figure 2b, the spectral signatures of G-quartet formation, H₆/N₂H₆ and N₁H₆/N₁H NOE cross peaks, are clearly observed. Figure 2b also shows that only the imino proton from the 5'-GMP dimer, N₁H(d), is involved in hydrogen bonding. This observation suggests that the two guanine bases are held together by two N₁H•••O₆=C…C•••N₆ bonds.
chemical exchange cross peaks labeled as "ex". Cross peaks due to chemical exchange are to be the most stable homo base pair, to our knowledge, calculated calculations have long predicted hydrogen bond linkage along the 5'-GMP structures determined from NOE and 3'-J coupling constrains, we decided to perform quantum chemical calculations of 3J(N,N), 3J(i+1), and 3J(i,i+2) (1H,13C). Because these NMR parameters are primarily determined by the molecular geometry, we chose to calculate two isolated 5'-GMP molecules. The computational results provide strong confirmation of the derived 5'-GMP molecular structures (see Supporting Information, Table S4 and Figure S8).

Now how are the two G-quartets, G4(N) and G4(S), stacked on top of each other to form a helix? The interquartet NOE cross peaks highlighted in Figure 2a provide critical clues. The H2(N)—H1(S) cross peak suggests that the head face of G4(S) points to the tail face of G4(N), i.e., head-to-tail stacking. Furthermore, H5(S)—H12(N) and H1(S)—H15(N) cross peaks are consistent with the Zimmerman model in which the two G-quartets are twisted by 30° and stacked in a right-handed fashion with an axial rise of 3.4 Å. Interestingly, Gellert et al. proposed an octamer model for the 3'-GMP helix. But the difference is that the G-quartets in the 3'-GMP octamer are stacked head-to-head (or tail-to-tail) with the same sugar pucker. We have also identified all four hydroxyl 1H resonances from the two sugar conformers. Interestingly, judging from the observed 3J(H) values, three of the four hydroxyl groups, O2-endo, O3-endog, and C3-endog, are involved in strong hydrogen bonding. This observation provides additional hints about the final helical structure. Figure 3a displays a single "strand" of the 5'-GMP quadrupole helix to highlight how individual 5'-GMP molecules are "stitched" together via P-O—H—O hydrogen bonds with C2'-endo and C3'-endo sugar puckers alternating along the helical strand. Moreover, an additional [P(S)—O]1—[H—O···N(i+1)]1+3 hydrogen bond interlocks the helical structure. Figure 3b displays the hydrogen bond linkage along the 5'-GMP helix in a conventional fashion used for polynucleotides. It is striking to notice that the arrangement of adjacent 5'-GMP molecules is such that they are perfectly positioned for phosphodiester bond formation. Such a self-organized structure of 5'-GMP may provide a clue for formation of RNA oligomers under prebiotic conditions. Another notable feature of the 5'-GMP helix is that, within the Gd(S)/Gd(N) octamer, P[S(i)], and P[N(i)], are separated by 6.7 Å, making it possible or even necessary for a Na+ ion to bridge the two negatively charged groups. This mode of Na+ binding to the phosphate groups in 5'-GMP self-assembly was first proposed by Detellier and Laszlo. In comparison, [P[N(i)]1+1] and [P[S(i)]2] are separated by a longer distance, 7.2 Å. Such a P—O—OH—Na+—O—P interaction plays a crucial role in the 5'-GMP helix formation, because replacement of Na+ by K+ or Rb+ would lead to a different yet known ordered structure. Another important structural role that Na+, K+, and Rb+ ions share is to occupy the central channel of the 5'-GMP helix.
Figure 3. (a) A partial structure of the 5′-GMP helix showing key hydrogen bonds. The O⋯O hydrogen bond distances are given. (b) Scheme of the hydrogen bond linkage along the 5′-GMP helix following the convention for polynucleotides. (c) A full turn of the right-handed 5′-GMP quadruple helix in which the central channel is filled with Na⁺ ions (purple balls). (d) The top view of the 5′-GMP helix highlighting the two different types of phosphorus atoms (gold balls). In (c) and (d), hydrogen atoms are omitted for clarity.

Supporting Information Available: Materials and methods, NMR spectra, and computational results (4 tables, and 8 figures). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Bang, I. Biochem. Z. 1910, 26, 293–311.

JA090258Y