

STABILITY CONSTANT OF THE 1:1 COMPLEX OF SODIUM WITH GUANOSINE 5'-MONOPHOSPHATE

JUDITH A. WALMSLEY AND THOMAS J. PINNAVAIA

Department of Chemistry, Michigan State University, East Lansing, Michigan 48824

ABSTRACT The stability of the 1:1 complex of sodium ion with the dianion of guanosine 5'-monophosphate has been determined by means of a potentiometric titration employing a specific ion electrode. The stability constant for the reaction $\text{Na}^+ + 5'\text{-GMP}^{2-} \rightleftharpoons \text{Na}(5'\text{-GMP})^-$ was found to be $2.85 \pm 0.36 \text{ M}^{-1}$ at 5°C and an ionic strength of $1.1 \pm 0.1 \text{ M}$. Although 5'-GMP forms ordered self-structures at high concentration in the presence of sodium ions, in dilute solution and at low sodium ion concentrations the Na^+ binding is weak and typical of that for other nucleotides.

INTRODUCTION

In weakly acidic solution ($\text{pH} \sim 5$) guanosine 5'-monophosphate (5'-GMP) spontaneously forms an anisotropic gel (1), but under neutral or slightly basic conditions where the nucleotide is a dianion, soluble aggregates having an ordered structure are formed (2, 3). It has been postulated that self-aggregation occurs through the stacking of planar tetramer units which are formed via interbase hydrogen bonds between donor positions N(1)H and N(2)H and acceptor positions O(6) and N(7) (3–5). The presence of a specific cation is required, and the extent and nature of the self-association appears to be dependent on the size of the cation. Among the alkali metal ions, Na^+ , K^+ , and Rb^+ promote self-association, whereas Li^+ and Cs^+ exhibit little or no tendency to facilitate aggregate formation (6–8). The ordered solution structures of 5'-GMP formed in the presence of Na^+ are believed to be predominantly octamers. The octamers and species composed of more than two tetrameric units give rise to nonequivalent H(8) environments in the ^1H NMR spectrum (3, 6) and to IR frequency shifts for the C=O and C=N stretching vibrations of the nucleotide.

Although various models regarding the kind and number of Na^+ binding sites in aggregated 5'-GMP have been proposed (6, 7), the binding of Na^+ to the monomeric 5'-GMP has not been investigated. In an attempt to understand the role of sodium in the solution chemistry of 5'-GMP, a study of Na^+ binding to the nucleotide was undertaken using a specific ion electrode. The investigation was carried out at a nucleotide concentration where no ordered structure is present in solution as judged by NMR spectroscopy. It was thought that either the stoichiometry

of the Na^+ binding and/or the magnitude of the binding constant might provide insight into the role of the cation in the aggregation of 5'-GMP.

MATERIALS AND METHODS

The 5'-GMP used in the titrations was in the form of the tetramethylammonium (TMA) salt because it has been shown that self-association does not occur in the presence of this cation (9). $(\text{TMA})_2(5'\text{-GMP})$ was prepared by titrating guanylic acid [$\text{H}_2(5'\text{-GMP}) \cdot \text{H}_2\text{O}$; Sigma Chemical Co., St. Louis, MO; 1 g dissolved in 100 ml of deionized distilled water] with 0.1 M TMA hydroxide (Matheson, Coleman and Bell, Gibbstown, NJ) to a pH of 7.9–8.0. The solid was obtained by freeze drying. Solutions for titration were prepared by dissolving solid $(\text{TMA})_2(5'\text{-GMP})$ or dried NaCl in 0.80 M TMA chloride (Aldrich Chemical Co., Inc., Milwaukee, WI). The $5'\text{-GMP}^{2-}$ concentration was determined spectrophotometrically at 252 nm ($\epsilon = 13,700$).

The ionic medium method of Sillen (10) was used. A portion of 0.1 M $(\text{TMA})_2(5'\text{-GMP})$ was pipetted into the cell and equilibrated with stirring at 5°C . The electrode and thermocouple were placed in the solution. One buret was filled with 0.2 M $(\text{TMA})_2(5'\text{-GMP})$ and another buret was filled with standard NaCl solution (1.00 or 3.00 M). The electrode potential was read and a measured portion of NaCl and approximately equal amount of $(\text{TMA})_2(5'\text{-GMP})$ were added. The solution was allowed to equilibrate and the electrode stabilize for 10–15 min before taking a reading. The $5'\text{-GMP}^{2-}$ concentration was held constant at 0.089 M and the TMA^+ concentration was 1.0 M throughout the titration. The titrations were done at $\text{pH } 8.0 \pm 0.1$ and an ionic strength of $1.1 \pm 0.1 \text{ M}$. The electrode was calibrated immediately preceding a titration by adding portions of standard NaCl solutions in 1.0 M TMA chloride to 1.0 M TMA chloride at 5°C . A plot of electrode potential (mV) vs. $\log [\text{Na}^+]$ was linear for sodium ion concentrations above 10^{-2} M . The slope and intercept of the calibration plot were determined using a linear least-squares treatment.

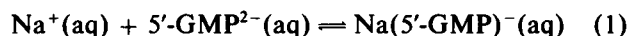
The free $[\text{Na}^+]$ was measured with an Orion model 9611 (Orion Research Inc., Cambridge, MA) sodium ion combination electrode connected to an Analogic millivolt meter (model AN2570 d XIP; Analogic Corp., Wakefield, MA) via a high impedance buffer. The titrations were carried out inside a Faraday cage. The cell was constructed of glass and consisted of a small jacketed beaker. The temperature was maintained at $5 \pm 1^\circ\text{C}$ by circulating H_2O -ethanol through the cell jacket using a Forma Scientific Model 2095 bath. The

Dr. Walmsley's present address is the Department of Chemistry, The University of Toledo, Toledo, Ohio 43606.

temperature of the solution was determined by a glass-encased copper-constantan thermocouple connected to a calibrated Omega Engineering meter (Omega Engineering, Inc., Stamford, CT). The solution was stirred by means of an air-driven magnetic stirrer. The standard solutions were added to the reaction solution from 5-ml burets having scale divisions of 0.01 ml.

RESULTS AND DISCUSSION

The binding of Na^+ to disordered 5'-GMP was found to obey the expressions:



$$K = [\text{Na}(5'\text{-GMP})^-] / [\text{Na}^+][5'\text{-GMP}^{2-}]. \quad (2)$$

Based on the combined data from two independent titrations, the value of K at 5°C was found to be $2.85 \pm 0.36 \text{ M}^{-1}$. The experimental data and theoretical curve are shown in Fig. 1. Several other models were tried in which the ratio of Na^+ to $5'\text{-GMP}^{2-}$ in the complex was assumed to be 2:1, 5:4, and 1: n , where $n = 2-4$. None of these models fit the experimental data.

The 1:1 $\text{Na}(5'\text{-GMP})^-$ complex most likely involves the interaction of Na^+ with a phosphate oxygen, because the magnitude of K is typical of the values found for other nucleotide systems. Smith and Alberty (11) found that the apparent stability constant for $\text{Na}(5'\text{-AMP})^-$ was $2.2 \pm 0.2 \text{ M}^{-1}$ at 25°C and an ionic strength of 0.2 M with TMA bromide as the supporting electrolyte. They also determined K for the binding of one Na^+ to the orthophosphate anion and obtained a value of $3.9 \pm 0.4 \text{ M}^{-1}$ under the same conditions. A Donnan equilibrium study by Strauss and co-workers (12) gave values of K for the binding of Na^+ to DNA in the range 0.7 to 1.6 M^{-1} at 25°C . Although a direct comparison between these stability constants and that for $\text{Na}(5'\text{-GMP})^-$ cannot be made because the determinations were done at differing ionic strengths and temperatures, it can be seen that they are all of similar magnitude.

Since Na^+ binding to the disordered nucleotide is quite weak and typical of ion pairing to phosphate oxygen, it

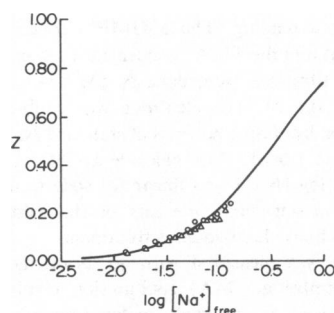


FIGURE 1 Titration curve for the reaction of $5'\text{-GMP}^{2-}$ with Na^+ . \circ , Δ , experimental data; —, theoretical curve for 1:1 complex; $Z = ([\text{Na}^+]_{\text{total}} - [\text{Na}^+]_{\text{free}}) / [5'\text{-GMP}^{2-}]_{\text{total}}$.

appears that the initial role of Na^+ is that of partial neutralization of the negative charge of $5'\text{-GMP}$. It is reasonable to think that this would be a necessary prerequisite for the formation of large $5'\text{-GMP}$ aggregates. However, charge neutralization is probably not the only or the major mechanism by which Na^+ interacts in the formation of large self-aggregates. The critical aggregation reaction is metal ion-size dependent and may involve the binding of Na^+ to donor groups from several $5'\text{-GMP}$ units in the ordered species.

Using calorimetry (13) and ultraviolet hypochromicity (1), Chantot and Guschlbauer found evidence for the presence of unstacked tetramers in dilute aqueous solutions of $3'\text{-GMP}$. Although an unstacked tetramer has not been observed for $5'\text{-GMP}$, it is a possible structure in dilute solution. Since each $5'\text{-GMP}$ molecule in such a tetramer would be expected to be in rapid exchange with monomer, averaging of the NMR resonances would result. From our measurements, species such as $\text{Na}_n(5'\text{-GMP})_n^-$, where $n > 1$, are indistinguishable from $\text{Na}(5'\text{-GMP})^-$. Therefore we cannot confirm the existence or absence of an unstacked tetrameric species in the solutions.

Finally, we note that in the titration of 0.089 M $5'\text{-GMP}$, deviations from 1:1 complex formation were observed at Na^+ concentrations above 0.15 M. Although we have not determined the nature of the species being formed, analysis indicates an increase in the number of Na^+ bound per $5'\text{-GMP}$ over that for the 1:1 complex.

This research was supported in part by the National Institutes of Health, grant number GM-24516.

Received for publication 15 December 1981.

REFERENCES

1. Chantot, J. F., and W. Guschlbauer. 1972. Mechanism of gel formation by guanine nucleosides. *Jerus. Symp. Quantum Chem. Biochem.* 4:205-214.
2. Miles, H. T., and J. Frazier. 1972. Formation of a new 5'-guanylic acid helix in neutral solution. *Biochem. Biophys. Res. Commun.* 49:199-204.
3. Pinnavaia, T. J., H. T. Miles, and E. D. Becker. 1975. Self-assembled 5'-guanosine monophosphate. Nuclear magnetic resonance evidence for a regular, ordered structure and slow chemical exchange. *J. Am. Chem. Soc.* 97:7198-7200.
4. Gellert, M., M. N. Lipsett, and D. R. Davies. 1962. Helix formation by guanylic acid. *Proc. Natl. Acad. Sci. U.S.A.* 48:2013-2018.
5. Zimmerman, S. B. 1976. X-ray study by fiber diffraction methods of a self-aggregate of guanosine-5'-phosphate with the same helical parameters as poly(rG). *J. Mol. Biol.* 106:663-672.
6. Pinnavaia, T. J., C. L. Marshall, C. M. Mettler, C. L. Fisk, H. T. Miles, and E. D. Becker. 1978. Alkali metal ion specificity in the solution ordering of a nucleotide, 5'-guanosine monophosphate. *J. Am. Chem. Soc.* 100:3625-3627.
7. Borzo, M., C. Detellier, P. Laszlo, and A. Paris. 1980. ^1H , ^{23}Na , and ^{31}P NMR studies of the self-assembly of the 5'-guanosine monophosphate dianion in neutral aqueous solution in the presence of sodium cations. *J. Am. Chem. Soc.* 102:1124-1134.

8. Detellier, C., and P. Laszlo. 1980. Role of alkali metal and ammonium cations in the self-assembly of the 5'-guanosine monophosphate dianion. *J. Am. Chem. Soc.* 102:1135-1141.
9. Bouhoutsos-Brown, E. 1980. Structure directing alkali and alkaline earth metal ions in the solution ordering of guanosine monophosphates. Ph.D. Dissertation. Michigan State University, East Lansing, Michigan.
10. Sillen, L. G. 1971. Polynuclear complexes in solution. In *Coordination Chemistry*. A. E. Martell, editor. Van Nostrand Reinhold, New York. 491-541.
11. Smith, R. M., and R. A. Alberty. 1956. The apparent stability constants of ionic complexes of various adenosine phosphates with monovalent cations. *J. Phys. Chem.* 60:180-184.
12. Strauss, U. P., C. Helfgott, and H. Pink. 1967. Interactions of polyelectrolytes with simple electrolytes. II. Donnan equilibria obtained with DNA in solutions of 1-1 electrolytes. *J. Phys. Chem.* 71:2550-2556.
13. Chantot, J. F. 1972. Nucleoside conformations. IX. A calorimetric study of gel formation by guanylic acids. *Arch. Biochem. Biophys.* 153:347-356.