CHEMISTRY LETTERS, pp. 547-550, 1975. Published by the Chemical Society of Japan

OLIGONUCLEOTIDE STUDIES. XI.[1] DETERMINATION OF BASIC IONIZATION CONSTANTS OF GUANYLYL-3',5'-GUANOSINE

Naotake OGASAWARA and Yasuo INOUE

Department of Biophysics and Biochemistry, Faculty of Science,
The University of Tokyo, Hongo, Tokyo 113

From circular dichroic data, by utilyzing the method developed by Ang for determining the overlapping pK values, basic ionization constants have been evaluated for guanylyl-3',5'-guanosine. This work establishes the first instance of the determination of the apparent ionization constants for the two protonation steps involved in homodinucleotides. A change of the circular dichroism with pH is convincing evidence for the existence of intermediate stacked monoprotonated species.

Although the pK values of bases, nucleosides, and mononucleotides have been determined either potentiometrically or spectrophotometrically, no successive basic or acidic ionization constants for oligonucleotides have been reported in the literature. In a study of the conformational changes of homodinucleotides in acidic media, it became necessary to know the pK values for these compounds. A study of the particular dinucleoside monophosphate named in the title is warranted by the intrinsic behavior previously observed for oligo G[2-4] and poly G[5,6] in their partly or fully protonated form. Furthermore, the recent theoretical findings[7] that the stacking interactions are more favorable for half-protonated pairs than for neutral pairs in decreasing order of relative stability: guanine-guanine ($\overset{+}{\text{G}}$-G) > thymine-thymine ($\overset{+}{\text{T}}$-T) > cytosine-cytosine ($\overset{+}{\text{C}}$-C) > adenine-adenine ($\overset{+}{\text{A}}$-A) prompt us to report our results on a study of the protonation of GpG. The evidence for the two stage protonation of GpG thus forms the subject of matter of this communication.

Pure GpG was prepared as described previously.[8,9] CD spectra were obtained at 25° by using a Jasco J-20 or J-10 spectropolarimeter with a 3-mm silica cell. Measurements of pH were made with a Radiometer 26 pH meter at 25°. Solution of GpG ($A_{252} \simeq 2.5$; ionic strength had been adjusted by NaCl to 0.5), after heated at

60 to 70° for 30 min, was titrated by introducing HCl gas through a capillary tube. The values of basic ionization constants were obtained from experimental plots of $\Delta\varepsilon$ against P and then Q, using equations (2) and (3), by a least-squares computer treatment of data.

Protonation of GpG. Protonation of the guanine residue is known to occur on the N7 atom,[10] and the basic pK was estimated as 2.17 at 25° and ionic strength, 0.1.[11] Thus, the ionization equilibria in GpG can be summarized by the scheme shown in Fig. 1 where $\overset{+}{\text{G}}\text{pG}$ and $\text{Gp}\overset{+}{\text{G}}$ are a pair of sequence isomers of the monoprotonated GpG and $\overset{+}{\text{G}}\text{p}\overset{+}{\text{G}}$ is the diprotonated form. The portion inside the braces in Fig. 1 exists

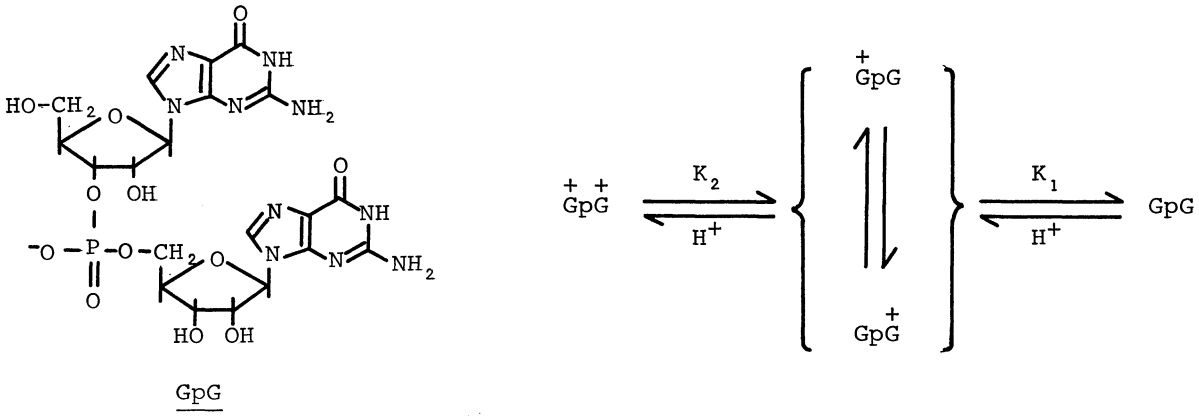

Fig. 1. Structure of GpG and scheme for the ionization of GpG.

in dynamic equilibrium. The protonation behavior of GpG in aqueous solution has been studied by CD spectral measurements. A particular feature of the change of CD spectrum with pH shown by GpG is the increase in intensity of two positive bands near 260 and 290 nm until pH decreases to about 2.0, followed by a decrease with a further increase in hydrogen-ion activity. A plot of $\Delta\varepsilon$ at 292 nm against pH is bell-shaped (Fig. 2), reflecting the formation and disappearance of the monoprotonated species. The application of Ang's method[12] for the determination of overlapping ionization constants of diprotic acids to the observed CD data enables us to determine pK_1 and pK_2 values of GpG.

If $\Delta\varepsilon_1$, $\Delta\varepsilon_2$, $\Delta\varepsilon_3$, and $\Delta\varepsilon$ are the CD of the species $\overset{++}{\text{G}}\text{pG}$, $\overset{+}{\text{G}}\text{pG}$ plus $\text{Gp}\overset{+}{\text{G}}$, GpG, and the mixture of these species at any fixed wavelength, the following relationship is obtained:

$$\{a_H\}^2(\Delta\varepsilon - \Delta\varepsilon_1) + a_H K_2(\Delta\varepsilon - \Delta\varepsilon_2) + K_1K_2(\Delta\varepsilon - \Delta\varepsilon_3) = 0 \qquad (1)$$

Let pH_1 and pH_2 be found on either side of the maximum for some specified value of $\Delta\varepsilon$ in Fig. 2. Then if $\{a_H\}_1$ and $\{a_H\}_2$ are the corresponding hydrogen-ion activities, two simultaneous equations of the form of equation (1) can be set up, one for

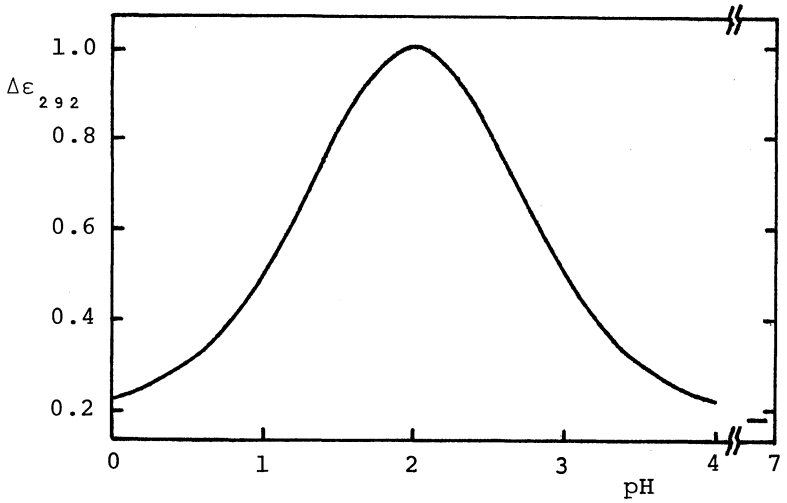

Fig. 2. CD-pH titration curve of GpG at 292 nm. (Temp., 25°; Ionic strength, 0.5 M)

each hydrogen-ion activity. Solving these equations, gives

$$\Delta\varepsilon = \Delta\varepsilon_2 - K_1 \cdot \frac{(\Delta\varepsilon - \Delta\varepsilon_3)[\{a_H\}_1 + \{a_H\}_2]}{\{a_H\}_1\{a_H\}_2} \quad (2)$$

and

$$\Delta\varepsilon = \Delta\varepsilon_1 + K_2 \cdot \frac{\Delta\varepsilon_2 - \Delta\varepsilon}{\{a_H\}_1 + \{a_H\}_2} \quad (3)$$

The function, $(\Delta\varepsilon - \Delta\varepsilon_3)[\{a_H\}_1 + \{a_H\}_2]/\{a_H\}_1\{a_H\}_2 = P$, can be calculated directly, because $\Delta\varepsilon_3$ is obtained directly from the CD measurement at a neutral pH. Thus, a plot of $\Delta\varepsilon$ against P is linear with slope $-K_1$ and intercept $\Delta\varepsilon_2$. The latter is used in equation (3) to find the function, $(\Delta\varepsilon_2 - \Delta\varepsilon)/[\{a_H\}_1 + \{a_H\}_2] = Q$. A plot of $\Delta\varepsilon$ against Q now gives K_2 and $\Delta\varepsilon_1$. These have been carried out by utilyzing points in the middle portions of the steep sections of the $\Delta\varepsilon$ vs. pH plot in Fig. 2. Since pK_2 for GpG is less than 2, it will be impossible to work in solutions of pH low enough to justify the approximation $\Delta\varepsilon_1 = \Delta\varepsilon$, because there is a possibility of appreciable protonation on the phosphodiester anionic site in strongly acidic solutions (pK value of the primary phosphate group is near 0.). GpG is found to follow equations (2) and (3), and a least-squares computer analysis of the readings taken at 292 nm above pH 0.98 gave $pK_1 = 2.52$ and $pK_2 = 1.46$ from the gradients of the relations, and the values of 1.49 and 0.17 for $\Delta\varepsilon_2$ and $\Delta\varepsilon_1$ from the intercepts (Fig. 3). Thus the close similarity in the $\Delta\varepsilon_1$ value of $\overset{+}{G}pG\overset{+}{}$ estimated from the method of Ang and that obtained from the direct measurement in 0.5 M HCl appears to be coincidental. At intermediate pH values, the monoprotonated species appears to be important, and the CD spectrum of the monoprotonated GpG, i.e., $\overset{+}{G}pG$ plus $Gp\overset{+}{G}$, has been calculated with the aid of the presently obtained values of pK_1

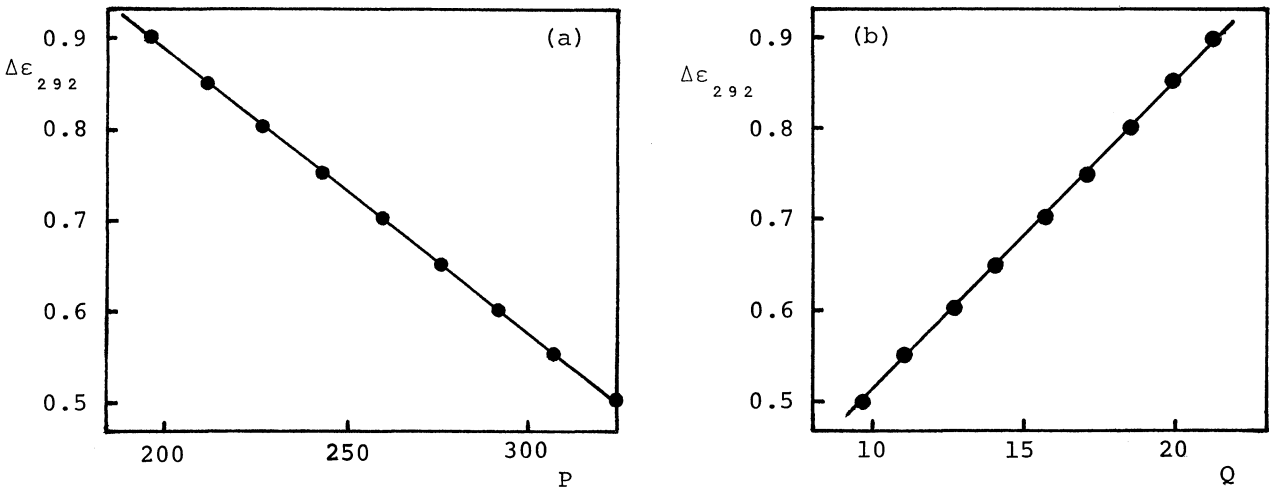

Fig. 3. Plot of $\Delta\varepsilon_{292}$ against (a) function P and (b) function Q for GpG. The solid lines are: $\Delta\varepsilon_{292} = 1.48_7 - 3.04\times10^{-3}P$ and $\Delta\varepsilon_{292} = 0.16_5 + 3.44\times10^{-2}Q$.

and pK_2. At 292nm $\Delta\varepsilon_2$ is more akin to $\Delta\varepsilon_{292}$ of a model compound, $m^{7}\overset{+}{G}pG$, having chromophore structure similar to that of $\overset{+}{G}pG$ than to $\Delta\varepsilon_{292}$ of $Gpm^{7}\overset{+}{G}$, suggesting that the formation of $\overset{+}{G}pG$ is preferred to that of $Gp\overset{+}{G}$ [$\Delta\varepsilon_{292}$ = 2.38 l/cm·mole for $m^{7}\overset{+}{G}pG$ and $\Delta\varepsilon_{292}$ = -0.05 l/cm·mole for $Gpm^{7}\overset{+}{G}$ after correction for the effect due to substitution of a methyl group][13].

Entry into oligonucleotide system by a preferential protonation promises to provide a variety of further interesting models in the chemistry of oligonucleotides Further experiments along these lines are under way.

Acknowledgments. We thank Professor K. Imahori for allowing us access to the Jasco J-20 spectropolarimeter in his laboratory. We also thank Sankyo Co., Ltd., Tokyo, for the provision of purified ribonuclease T_1.

References and Notes

1) Part X, Y. Watanabe and Y. Inoue, FEBS Lett., 35, 344 (1973).
2) Abbreviations used are: CD, circular dichroism; GpG, guanylyl-3',5'-guanosine; $\overset{+}{G}pG$ and $Gp\overset{+}{G}$, half-protonated GpG at a 5'-terminal base and a 3'-terminal base, respectively; oligo G and poly G, oligo- and polyguanylic acids, respectively; $m^{7}\overset{+}{G}pG$, N^7-methylguanylyl-3',5'-guanosine at pH 4.5.
3) S. K. Podder, Biochemistry, 9, 2415 (1971).
4) J-F. Chantot, T. Haertle, and W. Guschlbauer, Biochimie, 56, 501 (1974).
5) F. Pochon and A. M. Michelson, Proc. Nat. Acad. Sci. U. S., 53, 1425 (1965).
6) T. L. V. Ulbricht, R. J. Swan, and A. M. Michelson, Chem. Commun., 63 (1966).
7) F. Jordan and H. D. Sostman, J. Amer. Chem. Soc., 95, 6544 (1973).
8) K. Satoh and Y. Inoue, Biochem. J., 114, 271 (1969).
9) H. Ikenaga and Y. Inoue, Biochemistry, 13, 577 (1974).
10) J. M. Broomhead, Acta Cryst., 4, 92 (1951).
11) L. G. Bunville and S. J. Schwalbe, Biochemistry, 5, 3521 (1966).
12) K-P. Ang, J. Phys. Chem., 62, 1109 (1958).
13) Intimate details will be presented in a forthcoming paper: N. Ogasawara, Y. Watanabe, and Y. Inoue, to be submitted to J. Amer. Chem. Soc.

(Received March 20, 1975)