Acid–base properties of the nucleic-acid model 2′-deoxyguanylyl(5′→3′)-2′-deoxy-5′-guanylate, d(pGpG)$^{3-}$, and of related guanine derivatives†‡

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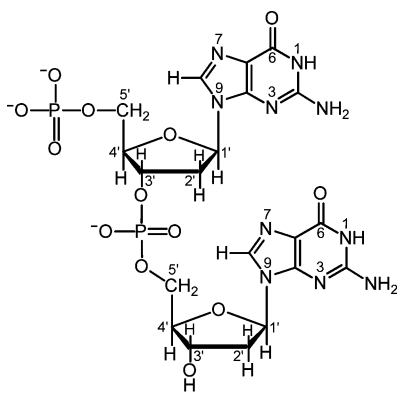
The dinucleotide d(pGpG) is an often employed DNA model to study various kinds of interactions between DNA and metal ions, but its acid–base properties were not yet described in detail. In this study the six deprotonation reactions of H_4[d(pGpG)]$^+$ are quantified. The acidity constants for the release of the first proton from the terminal $P(O)(OH)_2$ group ($pK_a = 0.65$) and for one of the $(N7)H^+$ sites ($pK_a = 2.4$) are estimated. The acidity constants of the remaining four deprotonation reactions were measured by potentiometric pH titrations in aqueous solution (25 °C; $I = 0.1$ M, $NaNO_3$): The pK_a values for the deprotonations of the second $(N7)H^+$, the $P(O)_2(OH)^-$, and the two (N1)H sites are 2.98, 6.56, 9.54 and 10.11, respectively. Based on these results we show how to estimate acidity constants for related systems that have not been studied, *e.g.* pGpG, which is involved in the initiation step of a rotavirus RNA polymerase. The relevance of our results for nucleic acids in general is briefly indicated.

1 Introduction

The anticancer drug Cisplatin, *cis*-$(NH_3)_2PtCl_2$, is well known to interact with DNA.[1] Due to the preferential binding of *cis*-$(NH_3)_2Pt^{2+}$ to the N7 sites of two consecutive guanine residues in DNA, the dinucleotide d(pGpG) has relatively often been studied as model compound.[1,2] There are also studies with a novel antitumor-active dirhodium(II,II) complex[3] and further platinum(II) compounds[4] as well as with closely related oligonucleotides.[5] In addition, the interaction of d(pGpG) with Na^+ and K^+,[6] the antibiotic actinomycin D,[7] as well as the human immunodeficiency virus type 1 integrase[8] have received attention.

Whereas the d(pGpG) unit serves as the primary target in DNA for Cisplatin and related compounds, its ribose relative pGpG does occur in living cells. The cyclic dimer of GMP, c-di-GMP plays a critical role in bacterial cell signaling and is thereby hydrolyzed to pGpG and finally to GMP by a phosphodiesterase.[9] Interestingly, this hydrolysis reaction is strongly Mg^{2+} dependent. The formation of pGpG has also been observed during the replication of the viral RNA by the rotavirus RNA-dependent polymerase:[10] During replication, the dinucleotide pGpG (as well as ppGpG) is formed and serves subsequently as a specific primer for the (−) strand synthesis leading to the conclusion that this dinucleotide is the initiator of replication.[10]

In the course of our attempts to reveal the interrelations between metal ions and nucleic acids,[11,12] we started to use dinucleotides as models to quantify the metal ion-binding properties of single-stranded RNA and DNA.[13] Besides the phosphate diester bridge,[13] the N7 site of guanine residues is especially important for metal ion binding to nucleic acids.[14–16] Thus, we selected d(pGpG)$^{3-}$ (see Fig. 1) as a ligand to be studied because of its wide use as a DNA-model compound[1–3,6–8] as well as in various other investigations;[17] furthermore, its relative pGpG occurs in nature as indicated above. To our surprise we discovered that the acid–base properties in aqueous solution of these dinucleotides have never been described in detail.[18]



d(pGpG)$^{3-}$

Fig. 1 Chemical structure of the trianion of 2′-deoxyguanylyl(5′→3′)-2′-deoxy-5′-guanylic acid, *i.e.*, 2′-deoxyguanylyl(5′→3′)-2′-deoxy-5′-guanylate, abbreviated as d(pGpG)$^{3-}$, and also known[6] as 2′-deoxy[5′-phosphate-guanylyl-(3′-5′)-guanosine]. The two guanosine units are shown in their dominating *anti* conformation.[14] Note, species written in the text without a charge, *e.g.*, d(pGpG), either do not carry one or represent the species in general (*i.e.*, independent of their protonation degree); which of the two possibilities applies is always clear from the context.

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‡ This study is dedicated to Professor Bernhard Lippert, University of Dortmund, Germany, on the occasion of his 60th birthday, with the very best wishes of the authors for all his future endeavors and with deep appreciation for his friendship, unselfish help and advice over the years to H.S. and R.K.O.S.

Here, we report the six equilibrium constants for the stepwise deprotonation of nearly fully protonated $d(pGpG)^{3-}$, *i.e.* of the $H_4[d(pGpG)]^+$ species. Encompassing the pH range of about 10.5 to 1, our study also includes the acidity constants for the release of the protons from the two (N1)H sites of the guanine residues. The site attribution for the various deprotonation reactions could be achieved in an unequivocal way by comparisons with (mostly) other guanine derivatives. One of the surprising conclusions of this study is that the two nucleobase residues in d(pGpG) react rather independently and thus, do not "feel" much of each other. Regarding nucleic acids this is a remarkable result and different from the observations made with pUpU,[13] where the mutual nucleobase effects are also minor but where the deprotonation of the (N3)H sites of the pyrimidine bases is somewhat shifted towards a lower pH range. Furthermore, the here presented new data, now allows by sophisticated comparisons to estimate the corresponding acidity constants for $H_4(pGpG)^+$ and other dinucleotides, which so far have also not been described.

2 Results

The dinucleoside monophosphates GpG and d(GpG), *i.e.* without a 5′-terminal phosphate group, are known for their tendency to undergo aggregate formation *via* self-association by nucleobase stacking and guanine–guanine hydrogen bonding.[19,20] Such an aggregation is much smaller with the here investigated $d(pGpG)^{3-}$, as a comparison of the self-association properties of $d(GpG)^-$ and $d(pGpG)^{3-}$ has shown.[6] Evidently, the addition of a phosphate group to the 5′-OH of the 2′-deoxyribose residue significantly inhibits this tendency. This agrees with observations made for guanosine and GMP^{2-} where the equilibrium constants defined according to the isodesmic model for an indefinite noncooperative self-association[21] in aqueous solution are $K = 8\ M^{-1}$ (*cf.*[21]) and $1.3\ M^{-1}$ (*cf.*[22]), respectively. Calculations for various guanine derivatives reveal that with the ligand concentrations used in this study, *i.e.*, 0.15 mM (section 4.3), more than 99% of the species are present in their monomeric form.[23] Hence, the following results refer in all instances to the monomeric species.

The dinucleotide $d(pGpG)^{3-}$ (Fig. 1) can accept three protons at its phosphate groups; however, two of these protons are released at a very low pH. For the 5′-terminal $P(O)(OH)_2$ group, a pK_a value can be estimated (see below), though it needs to be emphasized that the pH range of the deprotonation reaction of this proton certainly overlaps with that of the proton released from the phosphate diester bridge, for which in a first approximation a similar pK_a value is expected. Because one proton each can be accepted at the N7 sites of the two guanine residues, the six deprotonation reactions considered here begin with $H_4[d(pGpG)]^+$ and terminate with $d(pGpG–2H)^{5-}$, *i.e.* the species where the two (N1)H sites have also lost their proton. This then leads to the following six deprotonation reactions:

$$H_4[d(pGpG)]^+ \rightleftharpoons H_3[d(pGpG)] + H^+ \quad (1a)$$

$$K^H_{H_4[d(pGpG)]} = [H_3[d(pGpG)]][H^+]/[H_4[d(pGpG)]^+] \quad (1b)$$

$$H_3[d(pGpG)] \rightleftharpoons H_2[d(pGpG)]^- + H^+ \quad (2a)$$

$$K^H_{H_3[d(pGpG)]} = [H_2[d(pGpG)]^-][H^+]/[H_3[d(pGpG)]] \quad (2b)$$

$$H_2[d(pGpG)]^- \rightleftharpoons H[d(pGpG)]^{2-} + H^+ \quad (3a)$$

$$K^H_{H_2[d(pGpG)]} = [H[d(pGpG)]^{2-}][H^+]/[H_2[d(pGpG)]^-] \quad (3b)$$

$$H[d(pGpG)]^{2-} \rightleftharpoons d(pGpG)^{3-} + H^+ \quad (4a)$$

$$K^H_{H[d(pGpG)]} = [d(pGpG)^{3-}][H^+]/[H[d(pGpG)]^{2-}] \quad (4b)$$

$$d(pGpG)^{3-} \rightleftharpoons d(pGpG–H)^{4-} + H^+ \quad (5a)$$

$$K^H_{d(pGpG)} = [d(pGpG–H)^{4-}][H^+]/[d(pGpG)^{3-}] \quad (5b)$$

$$d(pGpG–H)^{4-} \rightleftharpoons d(pGpG–2H)^{5-} + H^+ \quad (6a)$$

$$K^H_{d(pGpG–H)} = [d(pGpG–2H)^{5-}][H^+]/[d(pGpG–H)^{4-}] \quad (6b)$$

It should be noted that $d(pGpG–H)^{4-}$ in equilibrium (5a) is to be read as "d(pGpG) *minus* H", meaning that one of the two (N1)H sites has lost a proton, without defining which one. Analogously, in the species $d(pGpG–2H)^{5-}$ both (N1)H sites are deprotonated.

Acidity constants for equilibria (1a) and (2a) could only be estimated (see Table 1 and section 2S in the ESI), whereas the values for the other four equilibria were measured by potentiometric pH titrations. The results are listed in Table 1 together with the acidity constants for several related species.[24–30] Comparisons of the given data allows clear-cut site attributions for the various deprotonation reactions of $H_4[d(pGpG)]^+$ as is for example evident when comparing the acid–base values for the equally charged $d(pGpG)^{3-}$ (entry 5) and GDP^{3-} (entry 11) species.

3 Discussion

The here-determined acid–base properties of $H_4[d(pGpG)]^+$ do not stand alone. Together with the additional data on related compounds, as summarized in Table 1, we can now analyze and quantify in detail the mutual effects of the distinct subunits on each other, *e.g.* of the nucleobase, the 2′-OH group or the phosphate residue. Many comparisons and conclusions are possible, some of which are discussed in the following paragraphs.

At first sight it is somewhat surprising that the 5′-$P(O)_2(OH)^-$ group of $H(pUpU)^{2-}$ (Table 1; entry 6; column 5) is slightly more acidic than the same group of $H[d(pGpG)]^{2-}$ (entry 5; column 5), *i.e.*, $\Delta pK_a = pK^H_{H[d(pGpG)]} - pK^H_{H(pUpU)} = (6.56 \pm 0.03) - (6.44 \pm 0.02) = 0.12 \pm 0.04$. However, from a structural point of view, two differences between $pUpU^{3-}$ and $d(pGpG)^{3-}$ are immediately obvious: Not only the nucleobase moieties, but also the sugar residues differ in the two dinucleotides.

Indeed, it is well known that the replacement of a ribose residue by a 2′-deoxyribose unit in a nucleotide leads to a slight basicity increase.[28,31] This increase occurs irrespective of the type of nucleobase attached to the sugar moiety as the following comparisons of $H(dGMP)^-$ with $H(GMP)^-$ and $d(CMP)^-$ with $H(CMP)^-$ show: $\Delta pK_{a/deoxy} = pK^H_{H(dGMP)} - pK^H_{H(GMP)} = (6.29 \pm 0.01) - (6.25 \pm 0.02) = 0.04 \pm 0.02$ (Table 1; entries 8,9; column 6) and $pK^H_{H(dCMP)} - pK^H_{H(CMP)} = (6.24 \pm 0.01) - (6.19 \pm 0.02) = 0.05 \pm 0.02$ (*cf.* ref. 31).

Similarly, from a comparison of the acid–base properties of $H(UMP)^-$ and $H(GMP)^-$, it follows that the guanine residue causes an increase in basicity at the 5′-terminal phosphate group:

Table 1 Negative logarithms of the acidity constants for the deprotonation of the P(O)(OH)$_2$, (N7)H$^+$, and (N1)H sites in H$_4$[d(pGpG)]$^+$ [entry 5; eqn (1–6)], together with some related data[a,b]

			pK_a of the sites			
Number	Acids[c]	P(O)(OH)$_2$	(N7)H$^+$	P(O)$_2^-$(OH)	(N1)H	Ref.
1	H(Guo)$^+$		2.11 ± 0.04		9.22 ± 0.01	23
2	H(dGuo)$^+$		2.30 ± 0.04		9.24 ± 0.03	24
3[d]	H$_2$(GpG)$^+$		1.49 ± 0.03/2.51 ± 0.03		9.34 ± 0.07/10.38 ± 0.10	25,26
4[e]	H$_2$[d(GpG)]$^+$		1.69 ± 0.10/2.71 ± 0.10		9.37 ± 0.03/10.39 ± 0.07	25
5	H$_4$[d(pGpG)]$^+$	0.65 ± 0.3[f]	2.4 ± 0.2[f]/2.98 ± 0.13	6.56 ± 0.03	9.54 ± 0.08/10.11 ± 0.14	—
6	H$_2$(pUpU)$^-$	1.0 ± 0.3		6.44 ± 0.02	8.99 ± 0.03/9.63 ± 0.08[g]	13
7	H$_2$(UMP)	0.7 ± 0.3		6.15 ± 0.01	9.45 ± 0.02[g]	27
8	H$_3$(GMP)$^+$	0.3 ± 0.2	2.48 ± 0.04	6.25 ± 0.02	9.49 ± 0.02	23
9	H$_3$(dGMP)$^+$	0.35 ± 0.2[h]	2.69 ± 0.03	6.29 ± 0.01	9.56 ± 0.02	28
10[i]	H$_2$(3′dGMP)$^\pm$		2.29 ± 0.04	6.14 ± 0.01	9.45 ± 0.02	—
11	H$_3$(GDP)$^\pm$	0.77 ± 0.20	2.67 ± 0.02	6.38 ± 0.01	9.56 ± 0.03	29
12[j]	H$_4$(pGpG)$^+$	0.6 ± 0.3	2.2 ± 0.2/2.80 ± 0.16	6.52 ± 0.01	9.50 ± 0.09/10.10 ± 0.18	—

[a] All constants were determined by potentiometric pH titrations in aqueous solution (25 °C; I = 0.1 M, $NaNO_3$) except for a few, which were estimated (see below). The errors given are three times the standard error of the mean value or the sum of the probable systematic errors, whichever is larger. The error limits of differences between constants as they appear in the text were calculated according to the error propagation after Gauss. [b] So-called practical, mixed or Brønsted acidity constants[30] are listed (section 4.2). [c] Definitions: dGMP^{2-} (= 5′dGMP^{2-}), 2′-deoxyguanosine-5′-monophosphate; d(GpG)$^-$, 2′-deoxyguanylyl(3′→5′)-2′-deoxyguanosine; 3′dGMP^{2-}, 2′-deoxyguanosine-3′-monophosphate; dGuo, 2′-deoxyguanosine; GDP^{3-}, guanosine-5′-diphosphate; GMP^{2-}, guanosine-5′-monophosphate; GpG$^-$, guanylyl(3′→5′)guanosine, Guo, guanosine; pGpG^{3-}, guanylyl(5′→3′)-5′-guanylate; pUpU^{3-}, uridylyl-(5′→3′)-5′-uridylate; UMP^{2-}, uridine-5′-monophosphate. [d] The values for the (N7)H$^+$ sites are from ref. 26. [e] The values for the (N7)H$^+$ sites are estimates; see ref. 25. [f] Estimated values; see text in sections 2 and 3. [g] These protons are released from the (N3)H sites of the uracil residues. [h] This constant was estimated by correcting the value of H$_3$(GMP)$^+$ for the effect of the 2′-deoxyribose residue which amounts to 0.05 ± 0.02 log units (see text in section 3). [i] B. Song, J. Zhao, H. Sigel, results to be published. [j] For details see section 3S in the ESI.

i.e., $\Delta pK_{a/NMP} = pK^H_{H(GMP)} - pK^H_{H(UMP)} = (6.25 \pm 0.02) - (6.15 \pm 0.01) = 0.10 \pm 0.02$ (Table 1; entries 7,8; column 5).

By using the two increments described above for 2′-OH and nucleobase substitution, one can estimate the acidity constant for H[d(pGpG)]$^{2-}$: $pK^H_{H[d(pGpG)]estimate} = pK^H_{H(pUpU)} + \Delta pK_{a/deoxy} + \Delta pK_{a/NMP} = (6.44 \pm 0.02) + (0.04 \pm 0.02) + (0.10 \pm 0.02) = 6.58 \pm 0.03$. This estimated acidity constant is in excellent agreement with the measured one, $pK^H_{H[d(pGpG)]} = 6.56 \pm 0.03$ (Table 1; entry 5; column 5), thereby proving the internal consistency of the constants listed in Table 1.

Application of comparisons as described above also allows calculation of pK_a values for other (2′-deoxy)dinucleotides based on the acidity constants available for the corresponding nucleoside 5′-monophosphates.[18,23,27,28] For example, the differences (see Table 1) $pK^H_{H[d(pGpG)]} - pK^H_{H(dGMP)} = (6.56 \pm 0.03) - (6.29 \pm 0.01) = 0.27 \pm 0.03$ and $pK^H_{H(pUpU)} - pK^H_{H(UMP)} = (6.44 \pm 0.02) - (6.15 \pm 0.01) = 0.29 \pm 0.02$, which are on average $\Delta pK_{a/DN,NMP} = 0.28 \pm 0.03$, may be used to estimate acidity constants for H(pApA)$^{2-}$, H(pCpC)$^{2-}$, H[d(pCpC)]$^{2-}$ and H[d(pTpT)]$^{2-}$. The results are $pK^H_{H(pApA)} = 6.49 \pm 0.03$, $pK^H_{H(pCpC)} = 6.47 \pm 0.04$, $pK^H_{H[d(pCpC)]} = 6.52 \pm 0.03$, and $pK^H_{H[d(pTpT)]} = 6.64 \pm 0.03$ (25 °C; I = 0.1 M, $NaNO_3$).§

As the nature of the nucleobase residue has an effect on the acidity of the 5′-terminal phosphate group, one can expect that changes at the phosphate–sugar moiety will also have an effect on the acid–base properties of the nucleobase. Comparison of the acid–base properties of the various guanine derivatives containing either a ribose or a 2′-deoxy unit (Table 1; entries 1,2; 8,9; column 4), reveals that the N7 site of the deoxy compounds are by $\Delta pK_a = 0.2$ more basic. On the other hand, when considering the deprotonation of the (N1)H site in the same pairs of compounds (column 6), the effect of the 2′-OH group is very minor; on average the 2′-deoxy compounds are only by 0.04 ± 0.015 (1σ) log units more basic. Interestingly, the acidifying effect of the 2′-OH group on the first and second deprotonation step of the 5′-terminal phosphate group is with ΔpK_a values of about 0.04 also very small but of the same order as observed at the (N1)H site (Table 1; entries 5,12 and 8,9; columns 3 and 5). Overall, these results indicate that the electron-withdrawing influence of the 2′-OH is strongest experienced at N7 of the nearby imidazole moiety, which is attached to C1′, and it has only very little influence on the more distant (N1)H and 5′-terminal phosphate positions.

Another interesting observation is that the release of the two (N1)H protons from d(pGpG)$^{3-}$ (entry 5; column 6) occurs with a ΔpK_a difference of 0.57 ± 0.16, and this is within the error limits identical with the statistically expected value of 0.6 for a symmetrical diprotonic acid.[13] Exactly the same observation is made for pUpU^{3-} where $\Delta pK_a = 0.64 \pm 0.08$ (entry 6). These results indicate that the two nucleobase residues present in each of the two nucleotides react rather independently and do not "feel" much of each other. This is different for the two dinucleoside monophosphates GpG$^-$ and d(GpG)$^-$ where the acidity differences are about 1.0 pK unit (Table 1; entries 3,4). This is an indication that in the two latter cases some intramolecular stacking occurs between the guanine residues whereas, once a 5′-phosphate group is present, this appears no longer to be the case. It follows that d(pGpG) occurs in dilute solution in an open form. This conclusion is in perfect agreement with the mentioned observation (section 2; first paragraph) that d(GpG)$^{3-}$ stacks much better than d(pGpG)$^{3-}$.[6]

With the above considerations in mind, and seeing in Table 1 for H$_2$(GpG)$^+$ that ΔpK_a for the two (N1)H and the two (N7)H$^+$ sites is identical, one may also estimate the pK_a value for the release

§ The above values follow from $pK^H_{H(pApA)} = pK^H_{H(AMP)} + \Delta pK_{a/DN,NMP} = (6.21 \pm 0.01)$ [*cf.*[23]] + $(0.28 \pm 0.03) = 6.49 \pm 0.03$, $pK^H_{H(pCpC)} = pK^H_{H(CMP)} + \Delta pK_{a/DN,NMP} = (6.19 \pm 0.02)$ [*cf.*[27]] + $(0.28 \pm 0.03) = 6.47 \pm 0.04$, $pK^H_{H[d(pCpC)]} = pK^H_{H(dCMP)} + \Delta pK_{a/DN,NMP} = (6.24 \pm 0.01)$ [*cf.*[31]] + $(0.28 \pm 0.03) = 6.52 \pm 0.03$, and $pK^H_{H[d(pTpT)]} = pK^H_{H(dTMP)} + \Delta pK_{a/DN,NMP} = (6.36 \pm 0.01)$ [*cf.*[28]] + $(0.28 \pm 0.03) = 6.64 \pm 0.03$.

of the first proton from the two (N7)H$^+$ sites in H_3[d(pGpG)] by deducting the (N1)H difference (0.57 ± 0.16; Table 1; entry 5; column 6) from the pK_a value of the second (N7)H$^+$ site (2.98 ± 0.13) to give $pK^H_{H_3[d(pGpG)]} = 2.4 \pm 0.2$; this value is listed in entry 5 of Table 1 (column 4) (see also section 3S in the ESI). It should be noted in this context that $pK^H_{H[d(pGpG)]} = 2.98 \pm 0.13$ for the deprotonation of the second (N7)H$^+$ site was experimentally determined only at pH ≥ 3.6 (see section 4.3) which means that in this pH range any contribution of the first (N7)H$^+$ site is negligibly small.

Application of the discussed systematic variations allows an estimation of the various acidity constants for the ribose-containing dinucleotide H_4(pGpG)$^+$, which has widely been studied.[9,32] Because to the best of our knowledge no such values exist in the literature, the estimations are listed in entry 12 of Table 1 (for details see section 3S in the ESI).

At this point is seems appropriate to ask which of the two (N7)H$^+$ or (N1)H sites, respectively, is deprotonated first, the 5′ or the 3′ guanine residue? Interestingly, addition of the 3′-P(O)$_2$(OH)$^-$ group to dGuo has no effect on the basicity of N7; both pK_a values are within their error limits identical (Table 1; entries 2,10; column 4), whereas addition of the same group to the 5′-position enhances the basicity of N7 remarkably, *i.e.*, $\Delta pK_a = pK^H_{H_2(5'dGMP)} - pK^H_{H(dGuo)} = (2.69 \pm 0.03) - (2.30 \pm 0.04) = 0.39 \pm 0.05$. Presumably there are two reasons for this behavior: (i) the negative charge of the P(O)$_2$(OH)$^-$ group is closer to (N7)H$^+$ in H_2(5′-dGMP)$^\pm$ than in H_2(3′-dGMP)$^\pm$. (ii) The proton at N7 is sterically in a position to form a hydrogen bond (possibly also involving a water molecule) with the 5′-P(O)$_2$(OH)$^-$ group. Both effects will inhibit the release of the proton from the (N7)H$^+$ site in H_2(5′GMP)$^\pm$. It may be added that the formation of similar hydrogen bonds between NH sites and phosph(on)ate groups is known.[33] Clearly, formation of the indicated hydrogen bond is not possible in H_2(3′-GMP)$^\pm$ for steric reasons.

However, when $pK^H_{H_2(5'dGMP)} = 2.69$ is compared with the *average* pK_a value [= 2.69 = (1/2)(2.4 + 2.98)] of the two (N7)H$^+$ sites in H_3[d(pGpG)], one notes with surprise that the values are identical. However, one should point out that the charge effect and hydrogen bond formation can also operate in this case as described above. Consequently, these comparisons indicate that the two N7 sites in H[d(pGpG)]$^{2-}$ have the same basicity and this conclusion is in accordance with the mentioned statistical difference of 0.6 pK units between the two pK_a values of the (N7)H$^+$ sites (Table 1; entry 5; column 4). Furthermore, because the same difference of 0.6 pK units also applies to the pK_a values for the two (N1)H sites of d(pGpG)$^{3-}$ (column 6), one has to conclude that the acid–base properties of the two guanine residues in d(pGpG)$^{3-}$ are identical because they behave as is expected for symmetrical diprotonic acids. This is an important conclusion regarding nucleic acids.

Finally, we want to discuss the effect of a phosphate group on the deprotonation reaction of a (N1)H unit. Addition of a PO_3^{2-} group to the 3′ or 5′ site of dGuo to give 3′-dGMP^{2-} or 5′-dGMP^{2-} enhances the basicity of the (N1)$^-$ sites by $\Delta pK_a = 0.21 \pm 0.04$ or 0.32 ± 0.04, respectively.¶ The effect of a further phosphate group, and thus a negative charge, can be determined by comparing the pK_a values of 3′-dGMP^{2-} (9.45 ± 0.02) and 5′-dGMP^{2-} (9.56 ± 0.02) with the first one of the d(pGpG)$^{3-}$ species (9.54 ± 0.08). As these values are rather similar, this indicates that the difference in charge between the two mononucleotides and the dinucleotide as well as the presence of two guanine residues in the latter, has little influence on the release of the first proton from the d(pGpG)$^{3-}$ species. This observation differs from the one made[13] with pUpU^{3-} where the two neighboring uracil residues show an increased acidity relative to that of an isolated uracil group (see also entries 6 and 7 in Table 1, column 6). Consequently, predictions about the mutual influence of neighboring nucleobases in nucleic acids are difficult to achieve at this stage and clearly, more data for comparisons are needed to reach this goal.

¶ The above differences result from $pK^H_{3'dGMP} - pK^H_{dGuo} = (9.45 \pm 0.02) - (9.24 \pm 0.03) = 0.21 \pm 0.04$ (see Table 1; entries 2,10; column 6) or $pK^H_{5'dGMP} - pK^H_{dGuo} = (9.56 \pm 0.02) - (9.24 \pm 0.03) = 0.32 \pm 0.04$ (see entries 2,9).

4 Experimental

4.1 Materials

The synthesis of 2′-deoxyguanylyl(5′→3′)-2′-deoxy-5′-guanylate, *i.e.*, of the trisodium salt of d(pGpG)$^{3-}$, was achieved by the *in-solution* phosphoramidite methodology.[34] Thus, 5′-*O*-dimethoxytrityl-*N*^{2}-isobutyryl-2′-deoxyguanosine-3′-*O*-(2-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite was reacted with 3′-*O*-acetyl-*N*^{2}-isobutyryl-2′-deoxyguanosine in the presence of 1*H*-tetrazole in CH_3CN solution to give after I_2–pyridine–H_2O oxidation of the intermediate phosphite, the fully protected dinucleoside monophosphate. After selective removal of the dimethoxytrityl group with 3% dichloroacetic acid in CH_2Cl_2, the dinucleoside monophosphate was 5′-*O*-phosphorylated with bis-*O*,*O*-(2-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite reagent followed by oxidation of the phosphite intermediate with I_2–pyridine–H_2O.[35] The obtained fully protected 5′-*O*-phosphorylated dinucleotide was purified by silica gel chromatography and deprotected by a 20 h treatment with 30% aqueous ammonia at 55 °C. The crude product was purified by ion-exchange chromatography on DEAE Sephadex A-25 (elution with a linear gradient of triethylammonium bicarbonate from 0.05 to 0.5 M). The purified dinucleotide was then transformed into its trisodium salt by passing through Dowex 50 W × 8 (Na$^+$ form) and lyophylized to give a white solid in 34% yield (based on the protected nucleoside). The compound gave with analytical reversed phase HPLC a single peak. The structure of d(pGpG) was confirmed by using spectroscopic methods: proton-decoupled ^{31}P NMR (Bruker Avance, 200 MHz; D_2O) showed two singlets at $\delta = -0.40$ ppm (internucleotide phosphorus) and 4.35 ppm (terminal phosphate); FAB MS (Finnigan MAT 95; negative ions) gave *m*/*z* 675.0 (calculated MW 676.42 for free acid). The observed ^{31}P NMR chemical shifts are fully consistent with the data reported for d(pGpG) salts;[36] other synthetic routes[37] have also been applied.[3]

All the other materials and reagents were the same as used previously and the NaOH stock solutions were also prepared as described.[13] The aqueous stock solutions of d(pGpG) were freshly prepared daily and the pH of the solutions was adjusted close to 8.0 with sodium hydroxide. The exact concentration of the ligand solutions was determined in each experiment by evaluation of the corresponding titration pair, that is, the differences in NaOH consumption between solutions with and without ligand (see below).

4.2 Potentiometric pH titrations

The pH titrations were carried out with a Metrohm E536 potentiograph connected to a Metrohm E665 dosimat and a Metrohm 6.0253.100 Aquatrode-plus combined double-junction macro glass electrode. This equipment was calibrated with buffer solutions (pH 4, 7, 9; all based on the NBS scale, now U.S. National Institute of Science and Technology, NIST) obtained from Metrohm AG, Herisau, Switzerland. The acidity constants determined at $I = 0.1$ M ($NaNO_3$) and 25 °C are so-called practical, mixed or Brønsted constants[13,30] which may be converted into the corresponding concentration constants by subtracting 0.02 from the measured and listed pK_a values.[30] The ionic product of water (K_W) and the conversion term mentioned do not enter into the calculations because the differences in NaOH consumption between solutions with and without ligand (see below) are evaluated.

4.3 Determination of the acidity constants

The acidity constants $K^{H}_{H_2[d(pGpG)]}$, $K^{H}_{H[d(pGpG)]}$, $K^{H}_{d(pGpG)}$, and $K^{H}_{d(pGpG-H)}$ of $H_2[d(pGpG)]^-$ [eqn (3–6)] were determined by titrating aqueous solutions (30 mL) of HNO_3 (0.5 mM) (25 °C; $I = 0.1$ M, $NaNO_3$) under N_2 with NaOH (up to 3.5 mL, 0.02 M) in the presence and absence of $d(pGpG)^{3-}$ (0.15 mM). The experimental data were evaluated with a curve-fitting procedure using a Newton–Gauss non-linear least-squares program by employing every 0.1 pH unit the difference in NaOH consumption between the two mentioned titrations, *i.e.*, with and without ligand. The acidity constants of $H_2[d(pGpG)]^-$ were calculated in the pH range 3.6 to 10.2, corresponding to 81% neutralization (initial) for the equilibrium $H_2[d(pGpG)]^-/H[d(pGpG)]^{2-}$ and about 50% (final) for $d(pGpG–H)^{4-}/d(pGpG–2H)^{5-}$. These neutralization degrees explain why the error limits of the first and the last acidity constant are relatively large. The final results for $K^{H}_{H_2[d(pGpG)]}$, $K^{H}_{H[d(pGpG)]}$, $K^{H}_{d(pGpG)}$ and $K^{H}_{d(pGpG-H)}$ are the averages of the values from four independent pairs of titrations.

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