Calcium Ions Do Accelerate the DNA Binding of New Antitumor-Active Platinum Aminophosphonate Complexes

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The presence of Ca^{2+} has a marked effect on the reaction kinetics of platinum phosphonate complexes with d(GpG). Compounds studied are [*cis*-Pt(NH_3)$_2$(bmpaa)] and [Pt(*R*,*S*-dach)(ntmp)]. The formation of GG-*N7*,*N7* chelates and the release of the phosphonate ligand as observed by NMR is significantly accelerated by the presence of Ca^{2+}, which is ascribed to its interaction with the phosphato groups in the ligands.

Introduction

The therapeutic activity of the well-known antitumor drug cisplatin [*cis*-diamminedichloroplatinum(II)] is thought to originate from its interaction with DNA, resulting in inhibition of DNA synthesis.[1] The main reaction product has proven to be the G*N7*,G*N7*-intrastrand crosslink.[2] Numerous analogs of cisplatin have been synthesized and tested in order to improve the therapeutic properties. A rational approach to the development of cytostatics involves the use of carrier functions that cause a specific accumulation of the potential antitumor agents in the target organs or in the target cells. Platinum complexes containing phosphonic acid ligands have been designed for the treatment of bone tumors,[3] since bisphosphonates show a high affinity for bone and other calcified tissues.[4] Four interesting examples of such compounds, i.e. [*cis*-Pt(NH_3)$_2$(bmpaa)] (**a**), [Pt(*R*,*S*-dach)(apmd)] (**b**), [*cis*-Pt(NH_3)$_2$(ntmp)] (**c**), and [Pt(*R*,*S*-dach)(ntmp)] (**d**)[5] are shown in Figure 1, of which [*cis*-Pt(NH_3)$_2$(bmpaa)] (**a**) is expected to enter clinical trials.[6]

Previous work has shown that [*cis*-Pt(NH_3)$_2$(ntmp)] (**c**) and [Pt(*R*,*S*-dach)(ntmp)] (**d**) are capable of forming GG-*N7,N7* chelates (in analogy with cisplatin), concomittant with the release of the phosphonate ligand.[7] Because of the slow reaction kinetics of these platinum complexes, observed at physiological pH, an activation mechanism was supposed to be present in vivo. The principal objective of the work reported here has been to investigate this activation mechanism and, in particular, the influence of the presence of Ca^{2+} on the reaction kinetics.

Results and Discussion

Reaction of [*cis*-Pt(NH_3)$_2$(bmpaa)](**a**) or [Pt(*R*,*S*-dach)(apmd)] (**b**) with d(GpG) [for the structure of d(GpG), see Figure 2] in water, results in the formation of G*N7*,G*N7* chelates, as deduced from the NMR data, in analogy with the Pt phosphonate compounds **c** and **d** (resulting chelates are [*cis*-Pt(NH_3)$_2${d(GpG)-N7(1),N7(2)}] in case of compound **a** and [Pt(*R*,*S*-dach){d(GpG)-N7(1),N7(2)}] in case of compound **b**).[7] In addition free phosphonate ligand is formed. Since the primary amine is a strongly coordinating ligand and usually acts as a non-leaving group it is rather surprising that in the same molecule, i.e. [Pt(*R*,*S*-dach)(apmd)] (**b**), RNH_2 can act as non-leaving group (dach), but also as leaving group (apmd).

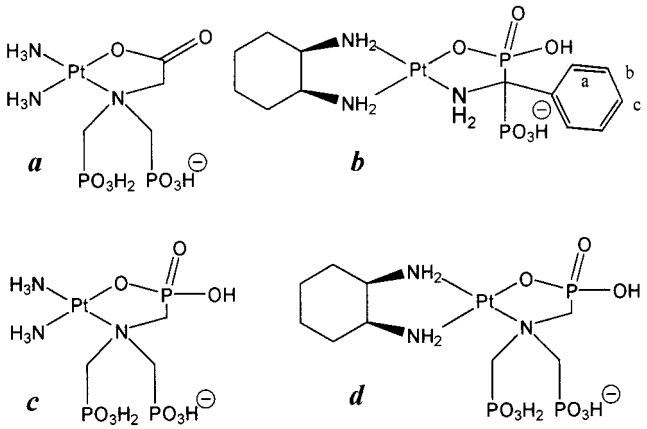

Figure 1. Structures of the platinum phosphonate complexes[*cis*-Pt(NH_3)$_2$(bmpaa)] (**a**), [Pt(*R*,*S*-dach)(apmd)] (**b**), [*cis*-Pt(NH_3)$_2$(ntmp)] (**c**) and [Pt(*R*,*S* dach)(ntmp)] (**d**)

At physiological pH compound **a** is not very reactive towards d(GpG) (see Table 1). The same lack of reactivity towards d(GpG) is observed for compound **d** at physiological pH (see Table 1; see Figure 3). Therefore, an activation mechanism has been proposed to occur in vivo.[7] Initially, it was investigated whether S-containing nucleophiles (cysteine, methionine, gluthatione) could act as activators, because of their known affinity[8] for platinum compounds. However, although initially the reaction of **d** with G bases proceeds faster in the presence of cysteine, methionine or GSH (as determined by UV-Vis spectroscopy), the resulting

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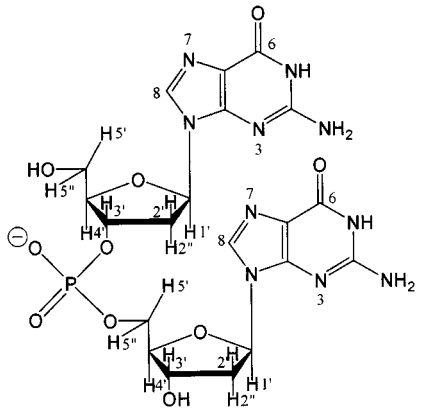

Figure 2. A schematic representation of the dinucleotide anion 2-deoxyguanylyl(3→5′)-2-deoxyguanosine, d(GpG)

product proved to be [Pt(*R*,*S*-dach)(nucl-*S*)(G-*N7*)] (as observed by NMR) and this was found not to be very reactive towards a second G base. Since coordination to a second G base is thought to be necessary for antitumor activity, S-containing nucleophiles are unlikely candidates for an activation mechanism in vivo because they inactivate, rather than activate, platinum phosphonate complexes.

The bmpaa ligand is not released from [*cis*-$Pt(NH_3)_2$(bmpaa)] in the presence of $CaCl_2$, for weeks at 310 K, if no G bases are present. So the above release of bmpaa is not just caused by the $CaCl_2$. Instead the attack of the G base on Pt is significantly enhanced by the presence of Ca^{2+}. The slightly enhanced reactivity in the presence of NaCl could be due to the presence of Na^+ which may also reduce the negative charge on the phosphonate groups, although less pronounced than for Ca^{2+}.

To study the possible effect of (de)protonation, the same experiment has been repeated at slightly acidic pH (pH 6.5) (see Table 1). With these complexes the presence of Ca^{2+} also enhances the reaction, as in the case of pH 7.0. An additional effect, however, must be present under these conditions, since overall the reaction is faster at pH 6.5 than at pH 7. A possible explanation could be that the H^+ ions can also slightly reduce the negative charge on the phosphonate ligand (a similar effect as Na^+, vide supra). Another explanation comes from the known pK_a values of [*cis*-$Pt(NH_3)_2(OH_2)_2$] (pK_{a1} = 5.02, pK_{a2} = 6.93).[9] This implies that at pH 6.5 for the aquated form of cisplatin the predominant species is [*cis*-$Pt(NH_3)_2(OH_2)(OH)]^+$ whereas at pH 7.0 almost all Pt is present as [*cis*-$Pt(NH_3)_2(OH)_2$]

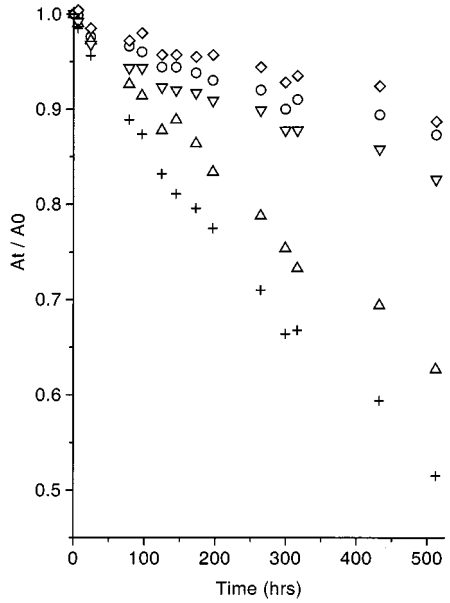

Figure 3. Influence of Ca^{2+} on the reaction of d(GpG) with platinum phosphonate complexes at pH 7 as determined from UV data. ◇ = [Pt(*R*,*S*-dach)(ntmp)]; ○ = [*cis*-$Pt(NH_3)_2$(bmpaa)]; + = [Pt(*R*,*S*-dach)(ntmp)] + Ca^{2+}; ▽ = [*cis*-$Pt(NH_3)_2$(bmpaa)] + Na^+; △ = [*cis*-$Pt(NH_3)_2$(bmpaa)] + Ca^{2+}. A_t/A_o is defined as: $[A_t(\lambda_{max}) - A_t(\lambda_{min})]/[A_o(\lambda_{max}) - A_o(\lambda_{min})]$

which is relatively inert, relative to the aqua species. Although for the Pt phosphonate complexes a direct attack of the G base on the carboxylate is involved (considered to be step 1), it cannot be ruled out that, in case of the subsequent step, the *N*-coordinated phosphonate ligand is hydrolyzed before the second G base will coordinate. This coordination of the second G would occur slower at pH 7.0, due to the presence of coordinated OH, whereas at pH 6.5 relatively more aqua ligand would be present.

The enhanced reactivity of platinum phosphonate complexes in slightly acidic media and in the presence of Ca^{2+} may explain their antitumor properties and selectivity for bone tumors. Since in tumor tissue the pH is known to be (approximately) 0.5 pH units lower than in normal tissues,[10] the reactivity of Pt phosphonates may be enhanced in tumor tissues. In addition, the phenomenon called "bone buffering" may also contribute to the enhanced reactivity of phosphonate compounds in tumors. Bone buffering occurs during prolonged acidosis or other pathological conditions.[11] Calcium phosphate (present as hydroxyapatite in

Table 1. $t_{1/2}$ values (hours) for the several reactions of d(GpG) with [*cis*-$Pt(NH_3)_2$(bmpaa)] and [Pt(*R*,*S*-dach)(ntmp)] (10^{-5} M) as determined from UV data (standard deviations in parentheses)

Compound	pH	$t_{1/2}$	Compound	pH	$t_{1/2}$
[*cis*-$Pt(NH_3)_2$(bmpaa)]	6.5	501 (20)	[Pt(*R*,*S*-dach)(ntmp)]	6.5	479 (20)
+$CaCl_2$	6.5	189 (22)	+ $CaCl_2$	6.5	295 (18)
[*cis*-$Pt(NH_3)_2$(bmpaa)]	7.0	2391 (24)	[Pt(*R*,*S*-dach)(ntmp)]	7.0	2871 (21)
+$CaCl_2$	7.0	685 (7)	+ $CaCl_2$	7.0	521 (10)
+NaCl	7.0	1641(17)			

the inorganic part of the bone) is relatively insoluble; however at lower pH the calcium phosphate can go into solution and the levels of Ca^{2+} and phosphate rise. The increased phosphate concentration is thus able to buffer the H^+ ions, whereas the increased Ca^{2+} level can enhance the reactivity of the platinum phosphonate complexes.

Attack of the G base on Pt does result in release of bmpaa, which is significantly enhanced by the presence of Ca^{2+} The interaction of the calcium ions with the negatively charged phosphonate groups is held responsible for this. This interaction results in less negative charge around the platinum and thus the attack of the incoming G-base is less hindered by electronic repulsion. These observations suggest a possible activation mechanism in vivo induced by calcium and slightly acidic conditions and would nicely explain the antitumor properties of these platinum phosphonate complexes.

Experimental Section

Syntheses: The compounds were synthesized as described elsewhere,[7] and characterized by elemental analysis and IR spectra.

NMR Spectra: The NMR spectra were recorded on a Bruker WM300 spectrometer at 297 K in D_2O (99.95%, Merck). – ^{1}H-NMR data (δ in ppm) were scaled in solution to TMA (tetramethylammonium nitrate, δ = 3.18 downfield TMS), and referenced to TMS and ^{31}P-NMR data were referenced to H_3PO_4 (85%). [*cis*-Pt(NH_3)$_2$(ntmp)] and [Pt(*R*,*S*-dach)(ntmp)] were characterized with ^{1}H- and ^{31}P-NMR spectroscopy as described before.[12] Chemical shift data for the other Pt compounds are: [*cis*-Pt(NH_3)$_2$(bmpaa)]: $\delta H_{CH\text{-}P}$ = 3.56, 3.33; $\delta H_{CH\text{-}COO}$ = 4.30; $\delta^{31}P$ = 10.20. [For comparison: free bmpaa ligand: $\delta H_{CH\text{-}P}$ = 3.60; $\delta H_{CH\text{-}COO}$ = 4.13; $d^{31}P$ = 7.88]. [Pt(*R*,*S*-dach)(apmd)]: δH_a = 7.99, δH_b = 7.43 and δH_c = 7.36; $\delta^{31}P$ = 12.86. [For comparison: free apmd ligand: δH_a = 7.68, δH_b = 7.35 and δH_c = 7.27; $\delta^{31}P$ = 10.55].

Reactions: One equivalent of d(GpG) was allowed to react with one equivalent of complex **a** or complex **d** at 310 K in the dark (10^{-5} M) and the reaction was followed by UV-VIS spectroscopy ((DMS-200 UV-Visible Spectrophotometer, Varian). The pH was kept constant by addition of small amounts (μL) of NaOH (0.1 N) to the reaction mixture. Reactions in the presence of likely activators, such as S-containing nucleophiles, $CaCl_2$ or NaCl, were performed as described above, using the following concentrations: cysteine, methionine, gluthatione, and (*S*)-methylgluthatione ($5\cdot10^{-5}$ M and $5\cdot10^{-6}$ M); $CaCl_2$ ($5\cdot10^{-4}$ M); NaCl (10^{-3} M). After completion of the reaction (no changes in the UV-VIS spectrum can be observed any more) the reaction products were lyophilized and analyzed by NMR. The excess of Ca^{2+} was removed by using oxalic acid and the precipitate was removed by filtration.

Acknowledgments

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[5] Abbreviations used: bmpaa = bis(methylphosphate)aminoacetate; dach = diaminocyclohexane; ntmp nitrilotrismethylenephosphonic acid; apmd = 1-amino-1-phenyl-methane-1,1-diphosphonic acid.

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