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THE EFFECT OF METAL ION COMPLEX FORMATION ON ACIDIC DEPURINATION OF 2'-DEOXYADENOSINE AND 2'-DEOXYGUANOSINE

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Abstract - The substitution inert N7-(dien)Pt(II) complex of 2'-deoxyguanosine has been shown to undergo acidic depurination 200 times less readily than the uncomplexed nucleoside, whereas the corresponding N1- and N7-complexes of 2'-deoxyadenosine are depurinated almost as rapidly as the nucleoside itself. These observations have been compared to the influences that several substitution labile metal ions exerted on the rate of depurination. Accordingly, the effects of (dien)Pd(II), Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) ions on the acidic hydrolysis of 2'-deoxyadenosine and 2'-deoxyguanosine have been accounted for by competitive attachment of protons and metal ions to the N1 and N7 sites. The applicability of metal ions in chemical DNA sequencing is briefly discussed.

Chemical DNA sequence determination according to the method of Maxam and Gilbert[1] is based on specific base modifications. Alternatively, the reactivity of base residues may be influenced by complexing with metal ions. Since the complex formation depends on both the chemical nature of the interacting species and pH of the reaction mixture,[2-5] metal ions offer a potential system with which to remove selectively a certain type of nucleic acid base. For example, it has been reported recently[6] that reaction of DNA with $K_2[PdCl_4]$ at pH 2.0 followed by a piperidine workup results in a specific cleavage of adenine residue. The increased selectivity of the

depurination reaction was attributed to enhanced hydrolysis of 2'-deoxyadenosine due to complexing with $PdCl_3^-$. This observation prompted us to study the influence of metal ions on the competitive depurination of 2'-deoxyadenosine and 2'-deoxyguanosine in more detail.

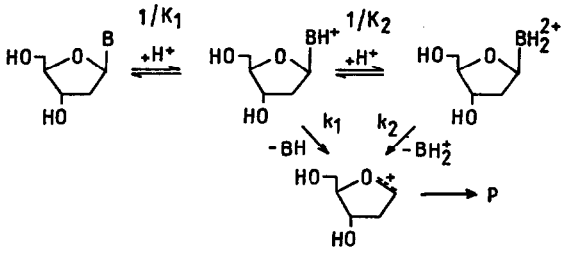
The complex formation has been shown to protect N-glycosidic bond against acidic hydrolysis. For example, increasing concentrations of Ag(I) and 3d transition metal ions retard the hydrolysis of several acyclic nucleoside analogues , viz. 1-(1-ethoxyethyl)benzimidazole,[7] and 9-(1-ethoxyethyl)-purine[8] and -adenine,[9] at a constant hydronium ion concentration. In each case the rate-retardation has been accounted for by competitive attachment of protons and metal ions to the starting materials. Formation of metal ion complexes decreases the extent of protonation, and since the complexed substrates are cleaved less readily than the protonated ones, deceleration takes place. Similarly, N7 coordinated $(NH_3)_5Ru(III)$ complex of 2'-deoxyguanosine is hydrolyzed almost 2000 times less readily than the N7 protonated species.[10]

In the present paper the influences of several substitution labile metal ions, viz. Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and (dien)Pd(II) ions, on the competitive hydrolysis of 2'-deoxyadenosine and 2'-deoxyguanosine have been studied. A monofunctional palladium aquo ion, $[(dien)Pd(H_2O)]^{2+}$, was employed instead of $[PdCl_4]^{2-}$ to reduce the number of competitive equilibria. For comparative purposes, hydronium ion-catalyzed depurinations of N1 and N7 coordinated $(dien)Pt^{2+}$ complexes of 2'-deoxyadenosine and N7 coordinated complex of 2'-deoxyguanosine were also studied. The ligand exchange reactions of these platinum complexes are so slow that the Pt-N bonds are not cleaved concurrent with hydrolysis of the N-glycosidic bond, and hence direct information on the relative reactivities of N1- and N7-bonded species may be obtained.

RESULTS AND DISCUSSION

The acidic hydrolysis of 2'-deoxyguanosine[11] and 2'-deoxyadenosine[12] has been shown to involve a rapid initial protonation of the base moiety followed by a rate-limiting heterolysis of the resulting mono- or dication to give free purine base and a resonance stabilized glycosyl carbonium ion (Scheme 1). Reaction proceeds predominantly through the monocation at $pH > pK_1$ and through the dication at higher acidities. A first-order dependence on oxonium ion activity is observed over a wide acidity range, even at $pH = pK_1$, since

$\underline{k}_1/\underline{K}_1$ and $\underline{k}_2/\underline{K}_2$ are approximately equal.[11,12] With adenine nucleosides the first protonation takes place at N1[13,14] and the second at N7,[15] with guanine nucleosides at N7[16] and N3,[17] respectively.



Scheme 1

Acidic depurination of (dien)Pt(II) complexes. Binding of (dien)Pt(II) ion to N7 of 2'-deoxyguanosine retards acidic cleavage of N-glycosidic bond by more than two orders of magnitude, the first order rate constants obtained with uncomplexed nucleoside and its N7-(dien)Pt(II) complex being 2.8 10^{-3} s^{-1} and 2 10^{-5} s^{-1}, respectively, in 0.10 mol dm^{-3} aqueous perchloric acid at 323.2 K. The large reactivity difference is expected, since (dien)Pt(II) at N7 blocks the preferential site of protonation. As with $(NH_3)_5Ru(III)$,[10] N7 platinated guanine appears to be a poor leaving-group compared to the N7 protonated species. In contrast, binding of (dien)Pt(II) ion to N7 of 2'-deoxyadenosine leaves the most basic nitrogen atom (N1) free, although undoubtedly reduces its basicity. As seen from Fig. 1, the N7 complexed 2'-deoxyadenosine reacts at pH > 2 almost as rapidly as the uncomplexed substrate. At higher acidities a moderate rate-retardation is observed, since the hydrolysis rate of the N7-(dien)Pt(II) complex levels off to a constant value under conditions where the N1 site may be expected to become fully protonated. The formation of diprotonated species is retarded by N7 coordination, and hence a deceleration is observed at high acidities. The dependence of the observed rate constant, $\underline{k}$(obs.), on $[H^+]$ may be expressed by eqn. (1), where the partial constants, $\underline{k}_3$ and $\underline{K}_3$, are those indicated in Scheme 2.

$$\underline{k}(\text{obs.}) = \underline{k}_3[H^+]/(\underline{K}_3 + [H^+]) \qquad (1)$$

Least-squares fitting[18] gave the values: $\underline{k}_3$ = 1.40 10^{-3} s^{-1} and $\underline{K}_3$ = 4.1 10^{-2} mol dm^{-3}. The value obtained for $\underline{K}_3$ is in a fairly good agreement with earlier ^{1}H NMR studies,[19] according to which N7 coordinated (dien)Pt(II) complex of 9-methyladenine undergoes protonation in the pH range 1-3. On the basis of the data reported earlier,[12,15] the first-order rate constants, $\underline{k}_1$ and $\underline{k}_2$, for the cleavage of N1 monocation and N1,N7 dication of 2'-deoxyadenosine (Scheme 1) may be estimated to be 6 10^{-6} s^{-1} and 0.6 s^{-1},

respectively, under the experimental conditions. The value obtained for k_3 expectedly falls between these limits.

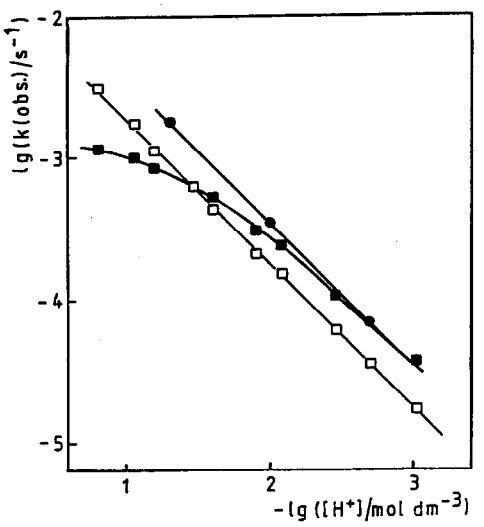
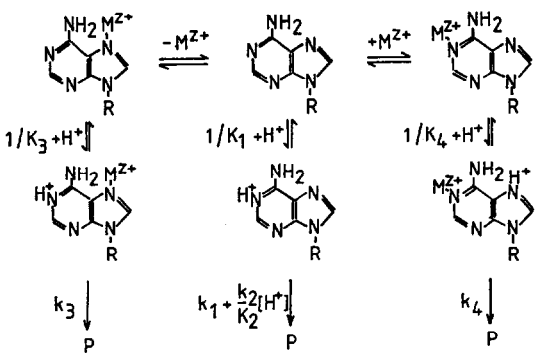


Fig.1: First-order rate constants for the depurination of 2'-deoxyadenosine (●) and its N1- (□) and N7-(dien)Pt^{2+} (■) complexes at 323.2 K. I = 0.10 mol dm^{-3} ($NaClO_4$).

It is slightly surprising that blocking of the most basic nitrogen atom (N1) in 2'-deoxyadenosine with (dien)Pt(II) retards the depurination only by a factor of 2 (Fig. 1). However, the following facts make the small rate-retardation understandable. Firstly, the basicity difference between N1 and N7 sites is relatively small. Kim and Martin[20] have reported the pK_a value of the N7 monocation (N1 unprotonated) to be 1.1. Secondly, N1-(dien)Pt^{2+} does not reduce the basicity of N7 as much as N7-Pt(dien)$^{2+}$ reduces the basicity of N1. ^1H NMR spectroscopic measurements have indicated that N1 coordinated (dien)Pt(II) complex of 9-methyladenine is detectably protonated already at pH 1.[19] Thirdly, N7 protonated adenine ring is from 10 to 50 times better leaving-group than the N1 protonated.[14] If the acidity constant, K_4, is assumed to be of the order of 0.1-1 mol dm^{-3}, the rate constant, k_4, for the



Scheme 2

cleavage of the N1Pt,N7H$^+$ species fall on the range from 3 10^{-3} to 2 10^{-2} s^{-1} (Scheme 2), *i. e.* larger than k_3 but still considerably smaller than k_2.

In conclusion, it is seen that coordination of a metal ion to N7 of guanine residue retards acidic depurination much more than coordination to N1 or N7 sites of 2'-deoxyadenosine.

Effect of (dien)Pd(II) ion on acidic depurination. In contrast to (dien)Pt$(H_2O)^{2+}$, the ligand exchange reactions of (dien)Pd$(H_2O)^{2+}$ are under the experimental conditions so fast[21] that complexing with purine deoxyribonucleosides takes place in a rapid pre-equilibrium stage of the depurination reaction. (dien)Pd$(H_2O)^{2+}$ ion was observed to retard the hydrolysis of 2'-deoxyguanosine by a factor of 65 at both pH 2.0 and 3.0, the rate-retardation being independent of the concentration of metal ion at $0.01 < [(dien)Pd^{2+}]/mol\ dm^{-3} < 0.03$ mol dm^{-3}. The equilibrium data of Kim and Martin[20,22] show that 2'-deoxyguanosine is under these conditions a mixture of N1H,N7(dien)Pd^{2+} and N1(dien)Pd^{2+},N7(dien)Pd^{2+} complexes, the former complex prevailing at pH 2.0 and the latter at pH 3.0. The mole fractions of all other species are more than 10^4 times smaller. Since the rate-retardations are almost equal at pH 2.0 and 3.0, both of these species appear to undergo depurination much less readily than uncomplexed 2'-deoxyguanosine, in consistence with the behavior of the substitution inert N7-(dien)Pt(II) complex.

The influence of (dien)Pd$(H_2O)^{2+}$ ion on the hydrolysis of 2'-deoxyadenosine is presented in Fig. 2. At pH 2.0 the observed rate constant, k(obs.), passes through a broad minimum with increasing concentration of (dien)Pd$(H_2O)^{2+}$, whereas at pH 3.0 a continuous acceleration is observed. The data of Kim and Martin,[20,22] which refer to ionic strength of 0.5 mol dm^{-3} (KNO_3) at 307 K, indicate that (dien)Pd(II) ion binds to both N1 and N7 sites of adenine ring, the N1,N7 dicoordinated complex predominating under the concentrations employed. The distribution curves of different species at pH 2.0 are presented in Fig 3. Comparison of these curves with the rate profile suggests that the small rate-retardation observed at low concentrations of (dien)Pd$(H_2O)^{2+}$ results from formation of mono- and dicoordinated species, at least one of which is less reactive than uncomplexed 2'-deoxyadenosine. The results obtained with the substitution inert (dien)Pt(II) complexes indicate that this may well be the case. However, this deceleration is overcompensated at high (dien)Pd$(H_2O)^{2+}$ concentrations by an acceleration, the origin of which remains obscure. It is noteworthy that this acceleration takes place in the concentration range, where the mole fraction of the N1,N7 dicoordinated species already

exceeds 0.9. Evidently, participation of a third (dien)Pd(II) ion must be assumed to explain the rate-acceleration. At pH 3.0 the rate of this "palladium promoted" reaction is so high compared to that of acidic depurination that the observed rate constant does not pass through a minimum. Tentatively one might assume that coordination of a third $(dien)Pd^{2+}$ ion to sterically hindered N3 position could markedly destabilize the N-glycosidic bond. For comparison, N3 alkylated purine nucleosides are much more unstable than their N1 or N7 alkylated counterparts.[23] Most probably the 6-amino group is not involved, since a similar rate-acceleration was observed with 9-(2-deoxy-β-D-erythro-pentofuranosyl)purine at pH 3.0.

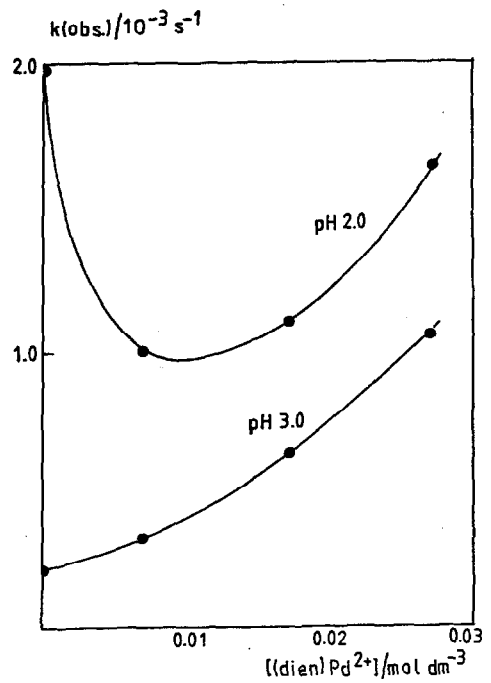


Fig.2: First-order rate constants for the depurination of 2'-deoxyadenosine in the presence of (dien)Pd(II) ions at 333.2 K. I = 1.0 mol dm^{-3} ($NaNO_3$).

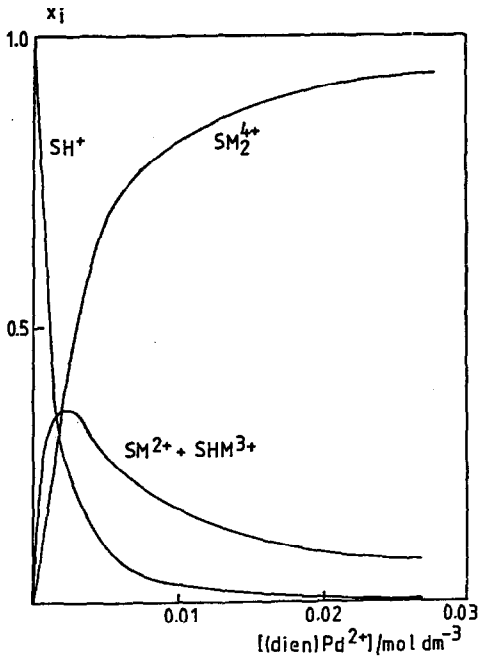


Fig.3: Distribution of (dien)Pd(II) complexes of adenosine at 307 K. I = 0.5 mol dm^{-3} (KNO_3). Calculated from the data of Kim and Martin.[20,22]

In summary, complexing of 2'-deoxyguanosine and 2'-deoxyadenosine with (dien)Pd(II) ion exerts completely different effects on their acidic depurination; while the depurination of 2'-deoxyguanosine is retarded by almost two orders of magnitude, that of 2'-deoxyadenosine is moderately accelerated.

Effect of Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) ions on acidic depurination. Table 1 summarizes the rate constants obtained for the hydrolysis of 2'-deoxyadenosine and 2'-deoxyguanosine in the presence of various divalent metal ions. Each metal ion retards more markedly the depurination of 2'-deoxyguanosine than that of 2'-deoxyadenosine. The main reason for this difference is that metal ions compete more efficiently with

Table 1: The influence of divalent metal ions on the first-order rate constants of acidic hydrolysis of 2'-deoxyadenosine and 2'-deoxyguanosine at 333.2 K.[a]

M^{2+}	pH	$[M^{2+}]$/mol dm^{-3}	$k_M(A)/k_H(A)$	$k_M(G)/k_H(G)$	$k_M(A)/k_M(G)$
Co^{2+}	2.0	0.050	1.07(1.00)[b]	0.88(0.86)[b]	1.4
		0.10	1.03(1.00)	0.78(0.76)	1.6
		0.20	1.03(0.99)	0.64(0.61)	1.9
	3.5	0.050	0.98(0.98)	0.58(0.68)	1.7
		0.10	0.96(0.95)	0.49(0.52)	2.0
		0.20	1.00(0.91)	0.40(0.35)	2.5
Ni^{2+}	2.0	0.10	0.95(1.00)	0.52(0.56)	2.2
		0.20	0.91(0.99)	0.34(0.39)	3.2
	3.5	0.10	0.90(0.93)	0.33(0.30)	2.7
		0.20	0.86(0.87)	0.23(0.18)	3.7
Cu^{2+}	2.0	0.050	1.02(0.99)	0.49(0.44)	2.5
		0.10	1.06(0.99)	0.33(0.28)	3.8
		0.20	0.96(0.98)	0.24(0.17)	4.7
	3.5	0.050	1.05(0.88)	0.22(0.21)	4.8
		0.10	0.90(0.78)	0.085(0.12)	11
		0.20	0.85(0.64)	0.055(0.063)	15
Zn^{2+}	2.0	0.050	1.01(1.00)	0.91(0.91)	1.3
		0.10	0.94(1.00)	0.82(0.83)	1.4
		0.20	0.91(0.99)	0.69(0.71)	1.6
	3.5	0.050	1.04(0.98)	0.73(0.77)	1.4
		0.10	0.99(0.95)	0.62(0.63)	1.6
		0.20	0.93(0.91)	0.58(0.46)	1.6
Cd^{2+}	2.0	0.050	0.98	0.83	1.4
		0.10	0.93	0.62	1.8
		0.20	0.97	0.53	2.2
	3.5	0.050	0.98	0.52	1.9
		0.10	0.83	0.33	2.5
		0.20	0.79	0.28	2.8

[a]At ionic strength of 1.0 mol dm^{-3} adjusted with $NaClO_4$.
[b]Rate-retardations given in parantheses are calculated by eqn. (2) using the equilibrium data reported[24,25] for adenosine and guanosine at 298.2 K.

protons for 2'-deoxyguanosine than for 2'-deoxyadenosine. In other words, the ratio of the formation constants, K_H/K_M of proton and metal ion complexes is with 2'-deoxyadenosine considerably larger than with 2'-deoxyguanosine. For example, with Cu(II) ion the values of this ratio are 790 and 2.7 for adenosine and guanosine, respectively.[24,25] In acidic solutions the metal ions listed above most probably compete with protons for the N7 site of

guanine base.[2] If it is further assumed that the N7 metallated 2'-deoxyguanosine undergoes depurination much less readily than the N7 protonated species, which is the case with (dien)Pt(II) and (dien)Pd(II) complexes, the ratio of rate constants, k_M/k_H, obtained at a fixed pH in the presence and absence of metal ions may be expressed by eqn. (2).[8]

$$k_M/k_H = (1 + K_H[H^+])/(1 + K_H[H^+] + K_M[M^{z+}]) \quad (2)$$

Substitution of the formation constants determined earlier[25] for guanosine (298.2 K, I = 1.0 mol dm^{-3}) gave the values of k_M/k_H listed in parantheses in Table 1. A fairly good agreement between the calculated and experimental values lends considerable support to the suggested mode of participation of metal ions in the depurination of 2'-deoxyguanosine. It should be noted that the equilibrium data employed refer to guanosine at a 35 K lower temperature than used in the kinetic measurements. This fact, however, seems to be of minor importance, since the basicity and complexing efficiency of 2'-deoxyguanosine do not differ markedly from those of guanosine, and K_H and K_M exhibit with all likelihood rather similar temperature dependences. Accordingly, the rate constant ratio, k_M/k_H, may be expected to be comparable with guanosine and its 2'-deoxy derivative, and quite insensitive to changes in temperature.

The influences that metal ions exert on the depurination of 2'-deoxyadenosine are smaller than expected on the basis of eqn. (2). In 2'-deoxyadenosine the preferred site of coordination is N7,[26-29] while protonation takes place at N1.[13,14] As shown above with (dien)Pt(II), N7 coordinated complexes of 2'-deoxyadenosine may be expected to undergo depurination at a fixed pH almost as readily as the uncomplexed substrate. Accordingly, eqn. (2) is not valid in the case of 2'-deoxyadenosine, since the hydrolysis of complexed substrate cannot be neglected.

In conclusion, the preceding data clearly show that metal ions can be used to protect 2'-deoxyguanosine selectively against acidic depurination. In this respect aquo ions of $(dien)Pt^{2+}$ and $(dien)Pd^{2+}$ are by far the most useful ones. In considering the applicability of (dien)Pt(II) ion it should be remembered that not only depurination, but also formation of (dien)Pt(II) complexes show a marked kinetic selectivity.[30] Accordingly, under kinetically controlled conditions guanine residues are mainly platinated, and thus converted resistant to depurination. Possible side reactions leading to platination of adenine residues at N1 or N7 are not actually harmful, since these complexes undergo depurination almost as rapidly as free residues. Among the other metal ions studied Cu(II) ion appears to be the only one that is coordinated strongly enough to result in some selectivity in the

hydrolysis of purine 2'-deoxyribonucleosides, but the influence is considerably smaller than that of (dien)Pt(II) or (dien)Pd(II) ions.

EXPERIMENTAL

Materials. [(dien)PdJ]J and [(dien)PtJ]J were prepared as described earlier,[31] and converted to the corresponding aquo ions by treating the salts in dark with 1.98 equivalent of $AgNO_3$. The metal perchlorates employed were commercial products of G. Frederick Smith Chemical Company. 2'-deoxyadenosine and 2'-deoxyguanosine were purchased from Sigma Chemical Company. The preparation of their (dien)Pt(II) complexes,[32] as well as that of 9-(2-deoxy-β-D-erythro-pentofuranosyl)purine,[14] has been described previously.

Kinetic measurements. The rate constants for the depurination reactions were obtained by HPLC technique described earlier.[15]

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