Kinetic Recognition of the 5'-Phosphate Group in the Attack of Cisplatin-type Systems on DNA

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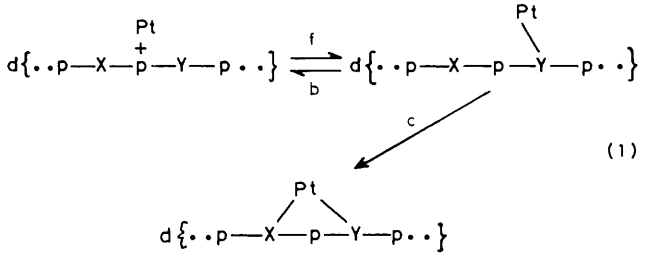
Mathematical models have been constructed to describe the kinetics of formation of *cis*-[Pt(NH$_3$)$_2$\{d(. . pApGp . .)\}] and *cis*-[Pt(NH$_3$)$_2$\{d(. . pGpGp . .)\}] from DNA and cisplatin-type systems; the rate constant and entropy of activation of the initial reaction with G indicate that the 5'-phosphate is very important kinetically.

While there is a considerable amount of information available on the rates of reaction of cisplatin-type systems with mononucleotides,[1—4] a question of more biochemical importance is the attack of these species on DNA. Here, values for rate constants and activation parameters relating to the attack on DNA and to the production of platinated d(GpG) and d(ApG) units are estimated from experiments of Reedijk's group[5] in which salmon sperm DNA was treated with cisplatin and the platinated DNA so produced degraded enzymically.† Striking results of this work are that there are only two main products, *cis*-[Pt(NH$_3$)$_2$\{d(p*G*p*G*)\}] and *cis*-[Pt(NH$_3$)$_2$-\{d(p*A*p*G*)\}], and that these are formed not in equal amounts but in the approximate ratio 4 : 1. {Bonding is at N(7) in both dpG and dpA. Small quantities of *cis*-[Pt(NH$_3$)$_2$(dpG)$_2$] and [Pt(NH$_3$)$_3$(dpG)] are also produced and are ignored here.}

In the formation of the binucleotides, there must be two crucial steps, the initial reaction with the first base and the subsequent cyclisation process; see reaction (1) in which platinum(II) attacks the nucleobases, X and Y, of a DNA chain. The initial reaction is probably reversible since strong nucleophiles such as cyanide ion can displace the purine and pyrimidine bases, *N* and *N'*, from complexes of type *cis*-[Pt(NH$_3$)$_2$(*N*)*N'*].[6] In Reedijk's work,[5] the facts that only complexes containing dG are produced and that of these only Pt\{d(p*G*p*G*)\} and Pt\{d(p*A*p*G*)\} species are formed, indicate considerable selectivity during reaction (1).



(1)

We have considered three models, in which selectivity is introduced (A) in the cyclisation process (c), or (B) in the initial reaction forwards (f), or (C) in the backward step (b) of the initial reaction. These models represent limiting cases, and selectivity could well be present at all these stages. However, as will be seen later, there is a remarkable constancy in the values of k_f\{d(*GG*)\} and the corresponding $\Delta H_f^{\ddagger}$ and $\Delta S_f^{\ddagger}$ values whichever model is used. In the models, Runge–Kutta calculations were performed on the simultaneous differential equations implied by reaction (1). Values of k_f, k_b, and k_c were varied until good fits were obtained with the concentrations of *cis*-[Pt(NH$_3$)$_2$\{d(p*G*p*G*)\}], and *cis*-[Pt(NH$_3$)$_2$-\{d(p*A*p*G*)\}] recorded after varying intervals by Reedijk's team.[5] Normally there is a plausible spread of 10% in the rate constants obtained from the simulations. The mole fractions of C and G *versus* A and T units in salmon sperm DNA are taken to be 0.19 to 0.31.[7]

There are several features common to all three models. First, there is no evidence for cisplatin-type compounds attacking thymine derivatives;[1] therefore no reactions involving this base are included. Secondly, since no Pt\{d(p*C*p*G*)\}- nor Pt\{d(p*G*p*C*)\}-containing products are formed, it is assumed that k_c\{d($\overleftarrow{CG}$)\} and k_c\{d($\overrightarrow{GC}$)\} are zero. (Inagaki and Kidura[8] have evidence that cyclisation does not occur in the analogous deoxydinucleotide systems.) Similarly the absence of any dinucleotide products not containing G leads to the ignoring of all cyclisation reactions involving just A and/or C. Thirdly, the fact that a Pt\{d(p*A*p*G*)\}-containing unit is formed but one with Pt\{d(p*G*p*A*)\} is not, shows that in the DNA chain Pt\{d(pAp*G*pA)\} can cyclize to form a Pt–dA link with the preceding but not the succeeding base. In the models therefore k_c\{d($\overrightarrow{GA}$)\} is set equal to zero, as are the remaining k_c terms, k_c\{d($\overrightarrow{GG}$)\} and k_c\{d($\overrightarrow{AG}$)\}, by analogy. Fourthly, as a succeeding A cannot induce cyclisation in Pt\{d(p*G*pA)\}, it seems unlikely that it will influence strongly k_f\{d(*GA*)\} or k_b\{d(*GA*)\}. Therefore, it is assumed that a succeeding base, Z, in a chain, XYZ, exerts no selectivity in the initial attack on Y, so that k_f\{d(*YZ*)\} and k_b\{d(*YZ*)\} are independent of Z. [In contrast in models B and C, k_f\{d(*XY*)\} and k_b\{d(*XY*)\}, respectively, are dependent on X.]

In model A the selectivity is introduced at the cyclisation stage (c). It is assumed that there is no selectivity in the initial reaction (i), nor in the corresponding backward process (b); thus k_f\{d(*XG*)\} and k_b\{d(*XG*)\} and hence the equilibrium constant *K*\{d(*XG*)\} are all independent of X. The greater production of *cis*-[Pt(NH$_3$)$_2$\{d(p*G*p*G*)\}] than of *cis*-[Pt(NH$_3$)$_2$\{d(p*A*p*G*)\}] depends simply on the fact that k_c\{d($\overleftarrow{GG}$)\} is greater than k_c\{d($\overleftarrow{AG}$)\}. Data are given in Table 1. In Table 2 are kinetic parameters for various mononucleotides in reaction (2), which is analogous to (f) in reaction (1). (BH^- represents the singly deprotonated phosphoric acids.)

cis-[Pt(NH$_3$)$_2$(OH$_2$)(OH)]$^+$ + BH^- ⇌ *cis*-[Pt(NH$_3$)$_2$(BH)(OH$_2$)]$^+$ + OH^- (2)

† Notation: A = adenosine, C = cytidine, G = guanosine, T = thymidine; p = phosphate; in the formulae of bi- and poly-nucleotide chains the letters in italics denote bonding to Pt, *e.g.* in Pt(Ag*G*) and Pt(*GpG*), the G are bonded to Pt. The symbols k_f, k_b, and k_c denote rate constants as in equation (1); in the designation of these rate constants italics are used to indicate which base is bonded to the platinum at the end of reaction [for brevity, phosphates (p) are omitted] *e.g.* k_f(*AG*) refers to an initial forward step in which Pt attacks ApG bonding to G but not to A; an arrow indicates the direction of cyclisation, *e.g.* $k_c(\overleftarrow{AG})$ indicates the cyclisation step Pt(ApG)→Pt(*A*p*G*); d = 2'-deoxy; preceding and following p are 5'- and 3'-bonded respectively, *e.g.* dpG is 2'-deoxyguanosine 5'-monophosphate.

Table 1. Simulated rate constants.

	k_f/dm^3 mol^{-1} s^{-1}				k_b/s^{-1}				k_c/s^{-1}	
	GG	*AG*	*CG*	*TG*	*CG*	*AG*	*CG*	*TG*	$\overleftarrow{GG}$	$\overleftarrow{AG}$
Model A										
37 °C		0.71				4.25×10^{-4}			4.0×10^{-3}	7.1×10^{-5}
50 °C		1.51				1.2×10^{-3}			7.0×10^{-3}	2.4×10^{-4}
Model B										
37 °C	0.95	0.14	$\leq 1.0 \times 10^{-3}$			Small			Large	
50 °C	1.65	0.31	$\leq 1.3 \times 10^{-3}$			Small			Large	
Model C										
37 °C		0.89			3×10^{-9}	9.8×10^{-4}	Small		1.0×10^{-4}	
50 °C		1.48			10×10^{-9}	14×10^{-4}	Small		2.9×10^{-4}	

Table 2. Parameters for step (f) of reaction (1) for d(. . Gp*G* . .) units in DNA and for Ap*G*,[a] and of reaction (2) for mononucleotides.[b]

Model or cpd.		k (37 °C) /dm^3 mol^{-1} s^{-1}	$\Delta H^{\ddagger c}$ /kJ mol^{-1}	$\Delta S^{\ddagger c}$ /J K^{-1} mol^{-1}
d(.. pGpGp . .)	A	0.71	46.6 [5.6]	−98 [17]
	B	0.95	31.0 [6.6]	−146 [21]
	C	0.89	31.0 [5.3]	−149 [16]
pG		13.0	37.5 (2.3)	−103 (8)
dpG		7.4	34.0 (1.0)	−119 (3)
pA		0.27	34.1 (5.5)	−146 (19)
Gp		0.087	20.8 (5.6)	−199 (19)
ApG		1.96 (25 °C)		

[a] Ref. 4. [b] Ref. 4 and unpublished work (S. S. Eapen, D. J. Evans, and M. Green); the reactions are of *cis*-$[Pt(NH_3)_2(OH_2)(OH)]^+$ with the sodium salts of the mononucleotides, the procedures being similar to those used in refs. 1—3 to study the kinetics of reaction of the mononucleotide acids. [c] Square brackets [] reflect a 10% error in the simulated rate constants; parentheses () enclose standard errors.

The selectivity in the cyclisation step towards dG as opposed to dA, that is $k_c\{d(\overleftarrow{GG})\}/k_c\{d(\overleftarrow{AG})\}$, is 56 at 37 °C, which is similar to that shown by *cis*-$[Pt(NH_3)_2(OH_2)(OH)]^+$ towards the mononucleotides pG and pA, where $k_f(pG)/k_f(pA)$ is 48 (see Table 2). On this basis, model A seems reasonable. In addition, reaction (1) for the dinucleotide, ApG, and *cis*-$[Pt(NH_3)_2(OH_2)(OH)]^+$ has been studied at 25 °C, and $k_c(\overleftarrow{AG})$ found to be 5.5×10^{-5} s^{-1} (ref. 9), compatible with the values for $k_c\{d(\overleftarrow{AG})\}$ in model A.

Model B is, in some respects, an opposite of A. The selectively lies entirely in the initial forward step. The cyclisation process is fast, so that the initial process is in effect irreversible. Thus $k_f\{d(XG)\}$ is dependent on X, $k_b\{d(XG)\}$ is made zero, and $k_c\{d(\overleftarrow{XG})\}$ is large. It follows that $k_f\{d(XG)\}$ has to be small if X is C or T. The value of $k_f\{d(GG)\}/k_f\{d(AG)\}$ at 37 °C is 6.8. At first sight this seems large since it is the G units which are attacked in each case. However N(7) of the base which is not formally attacked, *i.e.* the left-hand G or A, can probably come within about 3 Å of the platinum,[10] which is not an unreasonable figure for weak co-ordinaton (also see later). However, a serious criticism of model B is that the simulated value of $k_c(\overleftarrow{AG})$ is taken to be 'large' which the actual value of 5.5×10^{-5} s^{-1} (at 25 °C) is not.[9]

In model C, selectivity is introduced by altering the degree of reversibility of the initial step. Thus, while $k_f\{d(XG)\}$ is constant, $k_b\{d(XG)\}$ is dependent on X = G or A, and small for X = C or T. With the possible exception of guanosine-3′-monophosphoric acid,[2] studies on reaction (2) indicate equilibrium constants, k_f/k_b, for guanine-containing systems to be greater than 10^3 dm^3 mol^{-1} (ref. 1). Thus while the value at 37 °C of 9.4×10^4 dm^3 mol^{-1} for $k_f\{d(GG)\}/k_b\{(GG)\}$ is reasonable, that of 269 dm^{-3} mol^{-1} for $k_f\{d(AG)\}/k_b\{d(AG)\}$ is not. However the values of $k_c\{d(\overleftarrow{AG})\}$ agree reasonably with the experimental value of $k_c(\overleftarrow{AG})$ of 5.5×10^{-5} s^{-1} at 25 °C.[9]

Thus there are serious flaws in models B and C. Moreover in model A it is too drastic to assume that $k_f\{d(XG)\}$ and $k_b\{d(XG)\}$ are independent of X, since Chottard's group[11] have observed that this is not so for various dinucleotides, *e.g.* for GpG and ApG, $k_f(GG)/k_f(AG) = 3.3$ (at 20 °C). However, all three models are extremes in that selectivity is introduced in only one reaction step. In the full paper we will consider intermediate models. Nevertheless at this stage two observations can be based on the present three models. First, a parameter that varies little between the models is $k_f\{d(GpG)\}$. In Table 2 these simulated values are compared with experimental values of the corresponding k_f for mononucleotides in reaction (2). Values decrease in the order pG > dpG > Ap*G* > d(Gp*G*) (in DNA) > Gp. Thus the rate constants fall as the bulk around pG (the 5′-bonded phosphate group) increases, as might be expected on steric grounds. However, the presence and the positioning of the phosphate are also important, since despite the bulk of the DNA the d(Gp*G*) unit in it reacts faster than Gp (the 3′-phosphate). Within the last two years, the 5′-phosphate group has been shown to be very important in determining the shape of DNA chains containing platinated d(pGpG) units.[12] This work indicates that the 5′-phosphate group is also very important form a kinetic point of view. This conclusion leads to the second observation, namely that the simulated values of the

entropy of activation for the d(Gp*G*) unit in DNA lie closer to those of pG and dpG than to that of Gp (Table 2). The data indicate that in the initial attack of Pt on the second G in a d(. . pGpGpZp . .) unit, the platinum 'recognises' the second but not the third phosphate group. It must be concluded that attack on a d(. . pGp . .) unit is controlled in a similar way and that there is an interaction of considerable significance between the 5'-phosphate and the platinum moiety, which accelerates reaction (f).

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