Kinetics of Binding of DNA Nucleotides to Antitumour Type Complexes

Rina Jacobs, Frans Prinsloo and Ernst Breet*

Research Unit for Chemical Kinetics, Potchefstroom University for Christian Higher Education, 2520 Potchefstroom, Republic of South Africa

The binding of DNA nucleotides to a platinum group metal complex concomitantly through the purine/pyrimidine ring and phosphate residue sites is described for the first time by a unified rate law based on the results of a comprehensive kinetic study.

The evidence for phosphate binding in addition to normal base binding on treating platinum group metal complexes with DNA nucleotides has largely resulted from structure-related studies.[1–3] The few available kinetic studies[4,5] on the subject neither present a mechanism reflecting the relationship between phosphate and base binding nor a rate law for the entire complexation process. This paper is the first to address these shortcomings by virtue of a comprehensive kinetic study. It represents the results obtained with one nucleotide (cytidine-5′-monophosphate) only, since it serves as an introduction to a more comprehensive paper[6] covering all of the nucleotides of DNA.

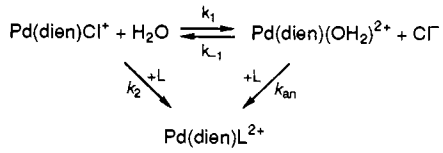
The general mechanism in Scheme 1 for the substitution reactions at the square-planar Pd(dien)Cl^+ and Pd(dien)-$(OH_2)^{2+}$ (dien = diethylenetriamine) centres, which have been selected as a labile reference system for the interaction of antitumour complexes with DNA, is such that for L = strong nucleophile the two-term rate law $k_{obs} = k_1 + k_2[L]$ applies and k_{an} is a non-rate-determining step. It was shown previously,[7] however, that with L = free bases, nucleosides and 5′-nucleotides of DNA, the anation step (k_{an}) is slowed to such an extent that it becomes rate determining and renders the aqua complex as the *only* entity reacting with DNA.

The selected pseudo-first-order rate constants k_{obs} in Table 1 for the free base (cytosine) and nucleoside (C) are totally different from those for the nucleotide (CMP) at neutral pH, whereas at pH = 3 the values for the three entities are very similar. This indicates that the phosphate residue of the nucleotide, in view of its $pK_a \sim 6$,[8] serves as an additional coordination site to the normal pyrimidine ring nitrogen N(3) at neutral pH.

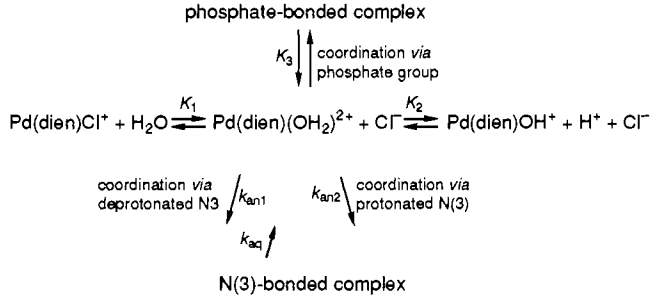
The effect of phosphate binding is to lower, through preequilibrium K_3 in Scheme 2, the fraction of aqua complex available to react with the pyrimidine N(3) atom, in much the same way as the fraction of aqua complex is diminished through preequilibrium K_1 by the addition of chloride ion (k_{obs} decreases with increasing $[Cl]_T$ in Table 1). The instability constant for the phosphate-bonded complex was determined to be $K_3 = 18.4\ s^{-1}/1.2 \times 10^4\ dm^3\ mol^{-1}\ s^{-1} = 1.53 \times 10^{-3}$ mol dm^{-3}, in good agreement with the average

Table 1 Selected (pH = 3 and 7 only) rate constants (measured stopped-flow spectrophotometrically at λ = 350 nm) for the reaction of Pd(dien)$(OH_2)^{2+}$ ($[Cl]_T = 0$) and Pd(dien)Cl^+ ($1 \times 10^{-3} \leqslant [Cl]_T \leqslant 5 \times 10^{-3}$ mol dm^{-3}) with cytosine, C and CMP. [Complex] = 1×10^{-3} mol dm^{-3}; ionic strength = 1×10^{-1} mol dm^{-3}; T = 25 °C.

L	pH	$[L]_T$/ mol dm^{-3}	k_{obs}/s^{-1}			
			$[Cl]_T = 0$	$[Cl]_T = 0.001$	$[Cl]_T = 0.002$	$[Cl]_T = 0.005$
Cytosine, C	3	0.010	2.14 ± 0.15	1.43 ± 0.15	0.96 ± 0.02	0.53 ± 0.02
CMP	3	0.010	2.08 ± 0.09	1.59 ± 0.03	1.03 ± 0.07	0.53 ± 0.01
Cytosine, C	7	0.010	32.7 ± 0.6	17.2 ± 0.5	12.7 ± 0.9	6.8 ± 0.5
CMP	7	0.010	7.3 ± 0.1	7.4 ± 0.2	7.3 ± 0.2	5.5 ± 0.2



Scheme 1



Scheme 2

value $K = 1.43 \times 10^{-3}$ mol dm^{-3} extracted from the literature[9] for comparable phosphato complexes. This value is of the same order of magnitude as the instability constant $K_1 = 1.13 \times 10^{-3}$ mol dm^{-3} [10] for the chloro complex, implying that the fraction of aqua complex available for coordination with the pyrimidine N(3) atom is decreased to almost the same extent by the phosphate group and chloride ion (k_{obs} shows no $[Cl]_T$ dependence for CMP at neutral pH in Table 1).

The pyrimidine ring site N(3) ($pK_L = 4.45$)[11] binds to the central metal atom by displacement of the water molecule of the aqua complex, the available fraction of which is determined by equilibria K_1 and K_2 in the case of the free base and nucleoside and K_1, K_2 and K_3 in the case of nucleotide. The observed second-order rate constant k_{an} for the formation of the N(3)-bonded complex was determined at each pH in the range $2 \leqslant$ pH $\leqslant 8$ from the slope of a plot of k_{obs} *versus* $f_{aqua}[L]_T$, with the value of f_{aqua} calculated by using eqn. (1)

$$f_{aqua} = K_1[H^+]/([H^+][Cl^-] + K_1[H^+] + K_1K_2 + K_1K_3^{-1}[H^+][\text{phosphate}]) \quad (1)$$

without the last term in the denominator for the free base and nucleoside and *with* the last term in the denominator for the nucleotide. For such calculations, the values $K_1 = 10^{-2.95}$, $K_2 = 10^{-7.38}$, $K_3 = 10^{-2.82}$ were either measured or taken from the literature,[10-12] [phosphate] $= [L]_T K_a/(K_a + [H^+])$ was calculated using $K_a = 10^{-6.08}$ and $[Cl^-]$ was determined by computer simulation of the equilibrium concerned. The intercepts of the plots of k_{obs} *versus* $f_{aqua}[L]_T$ are always zero for $2 \leqslant$ pH < 5, but a distinct intercept is observed for the nucleotide at pH $\geqslant 5$. This may be attributed to a reverse aquation reaction of the N(3)-bonded complex with rate constant k_{aq} (*cf.* Scheme 2) in view of the relatively low percentage (*e.g.* 16.4% of the nucleotide as opposed to 99.7% of the nucleoside/free base at pH = 7) of the incoming ligand available for N(3) coordination under the conditions concerned.

The observed second-order rate constant k_{an} is related to k_{an1} and k_{an2}, the absolute (*i.e.* pH independent) rate constants for the reaction with the unprotonated and protonated forms of the ligand, by eqn. (2) with $K_L = 10^{-4.45}$ and

$$k_{an} = \frac{k_{an1}}{(1 + K_L^{-1}[H^+])(1 + K_2[H^+]^{-1})} + \frac{k_{an2}}{(K_L[H^+]^{-1})(1 + K_2[H^+]^{-1})} \quad (2)$$

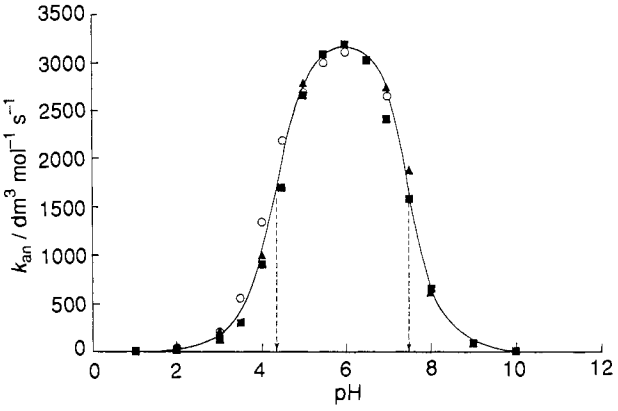


Fig. 1 The pH profile for reaction of $Pd(dien)(OH_2)^{2+}$ with cytosine, C and CMP. ▲ Cytosine, C observed, ○ CMP observed, ■ predicted.

$K_2 = 10^{-7.38}$.[11,12] The values of k_{an} obtained at different pH were fitted to eqn. (2) using a standard non-linear least-squares procedure with k_{an1} and k_{an2} as adjustable parameters. The values turned out to be $k_{an1} = (3416 \pm 100)$ dm^3 mol^{-1} s^{-1} and k_{an2} *ca.* 0, indicating that the DNA components are capable of coordinating in the unprotonated form only. The predicted values of k_{an} derived from the data fit for the three DNA components at various pH are compared with the corresponding observed values by virtue of the pH profile in Fig. 1. The correspondence between observed and predicted rate constants and the realistic pK values (4.4 and 7.4 as compared to 4.45 and 7.38 for the ligand and aqua complex, respectively) shown by the dotted lines at the inflection points, support the proposed mechanism and corresponding rate law. The maximum of the profile corresponds to the pH at which high percentages of unprotonated ligand (97%) and reactive complex species (96%) exist in solution simultaneously. This represents the optimum condition for coordination in the present case and illustrates the biological importance of such pH profiles for complexation processes involving active antitumour complexes.

The authors gratefully acknowledge financial support from the Foundation for Research and Development and the Potchefstroom University.

Received, 19th September 1991; Com. 1/04851A

References

1 M. D. Reily, T. W. Hambley and L. G. Marzilli, *J. Am. Chem. Soc.*, 1988, **110**, 2999.
2 B. A. Cartwright, D. M. L. Goodgame, I. Jeeves and A. C. Skapski, *Biochim. Biophys. Acta*, 1977, **477**, 195.
3 S. S. Massoud and H. Sigel, *Inorg. Chem.*, 1988, **27**, 1447.
4 R. N. Bose, N. Goswami and S. Moghaddas, *Inorg. Chem.*, 1990, **29**, 3461.
5 M. I. Djuran, E. L. M. Lempers and J. Reedijk, *Inorg. Chem.*, 1991, **30**, 2648.
6 F. F. Prinsloo, R. Jacobs and E. L. J. Breet, manuscript in preparation.
7 E. L. J. Breet and R. van Eldik, *Inorg. Chem.*, 1987, **26**, 2517.
8 P. I. Vestues and R. B. Martin, *J. Am. Chem. Soc.*, 1981, **103**, 806.
9 *Metal Ions in Biological Systems*, ed. E. Sigel, vol. 1: Simple Complexes, Marcel Dekker Inc., New York, 1973.
10 M. Kotowski and R. van Eldik, *Inorg. Chem.*, 1984, **23**, 3310.
11 R. B. Martin, *Acc. Chem. Res.*, 1985, **18**, 32.
12 E. L. J. Breet, R. van Eldik and H. Kelm, *Polyhedron*, 1983, **2**, 1181.