to 25% at pH 3. At higher concentrations, CH_3OH competes with Cr^{2+} for R• (eq 20),[17] which results in the formation of some $CrCH_2OH^{2+}$.

$$CH_3OH + R^\bullet \rightarrow RH + {}^\bullet CH_2OH \quad (20)$$

While pure aqueous or mixed CH_3CN/H_2O solutions yield no organochromium ions at pH 0, solutions containing alcohols (CH_3OH, C_2H_5OH, and 2-C_3H_7OH) yield the (hydroxyalkyl)-chromium complexes derived from the solvent alcohol. This is consistent with the proposed reaction mechanism. At pH 0, all the hydrated electrons are scavenged by H^+ to yield H• (eq 19). In purely aqueous solutions, H• reacts with Cr^{2+} and yields CrH^{2+}, but in mixed H_2O/alcohol solutions, H• is scavenged by alcohols and yields α-hydroxyalkyl radicals (eq 21). Colligation with Cr^{2+} produces $CrCR^1R^2OH^{2+}$.

$$H^\bullet + R^1R^2CH_2OH \rightarrow R^1R^2\dot{C}OH + H_2 \quad (21)$$

The *tert*-butylchromium ion, $CrC(CH_3)_3^{2+}$, was prepared and characterized for the first time in the course of this work. Its identity was established by a number of tests. The same complex is produced by two different methods, photochemically from $(CH_3)_3CBr$ and thermally from the hydroperoxide. In the presence of excess Cr^{2+}, the only gaseous decomposition product is isobutane, formed by acidolysis. The other decomposition mode is homolysis. This was clearly established by the inhibiting effect of Cr^{2+} and by the invariability of the rate constant k_h (0.60 s^{-1}) with the concentration and nature of the scavenger ($(NH_3)_5CoCl^{2+}$ and *t*-BuOOH). The rate constant k_h is the largest measured for an organochromium complex, in full accord with the known steric effect of the alkyl group on homolysis.[18,19]

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Registry No. Cr^{2+}, 7440-47-3; C_2H_5Br, 74-96-4; C_2H_5Cl, 75-00-3; 1-C_3H_7Br, 106-94-5; 2-C_3H_7Br, 75-26-3; $(CH_3)_3CBr$, 507-19-7; $(CH_3)_3CCH_2Br$, 630-17-1; $NC(CH_2)_4Br$, 5332-06-9; $(H_2O)_5CrC_2H_5^{2+}$, 52653-39-1; $(H_2O)_5CrC_3H_7^{2+}$, 52653-40-4; $(H_2O)_5CrCH(CH_3)_2^{2+}$, 60764-48-9; $(H_2O)_5CrC(CH_3)_2^{2+}$, 138666-90-7; $(H_2O)_5CrCH_2C(CH_3)_3^{2+}$, 52653-41-5; $(H_2O)_5Cr(CH_2)_4CN^{2+}$, 138693-66-0; $(H_2O)_5C(CH_3)_3C(CH_3)_2OOH$, 62696-04-2.

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Kinetic Study of the Interaction of Aquated Palladium(II) Complexes with Purine 5′-Nucleoside Monophosphates and Ribose 5′-Monophosphate in Aqueous Solution. Effects of Steric Hindrance and Phosphate-Induced Reactivity

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The complex formation reactions of a series of complexes of the type $Pd(R_4en)(H_2O)_2^{2+}$ (R_4en = N-substituted ethylenediamine, R = H, Me, Et) with ribose, adenosine, inosine, and guanosine 5′-monophosphate were investigated as a function of monophosphate concentration and temperature in the pH range 4–5. In all cases the complex formation with 5′-XMP (X = R, A, I, G) occurs in two consecutive steps for which the pseudo-first-order rate constants fit the equation $k_{obs} = k_a + k_b[XMP]$. The experiments with ribose monophosphate revealed complex formation rate constants significantly smaller than those reported before for inosine, but larger than those found for adenosine. This trend is also observed for the nucleoside monophosphates for which the rate constants follow the sequence AMP < RMP < IMP < GMP. In addition, all nucleotides react significantly faster than the corresponding nucleosides, demonstrating a significant transition state stabilization effect by the monophosphate group during the complex formation reactions. The systematic variation of the substituents on the en ligand decreases the formation rate constant by as much as 3 orders of magnitude in going from the unsubstituted (R = H) to the most sterically hindered species (R = Et). The complex formation reactions all proceed according to an associative substitution mechanism and are accompanied by significantly negative ΔS^* values. The results are discussed in reference to data available for the corresponding nucleosides and structural information on the final reaction products reported in the literature.

Introduction

We recently reported a detailed kinetic and mechanistic study of the complex formation reactions of $Pd(en)(H_2O)_2^{2+}$ and $Pd(Et_4en)(H_2O)_2^{2+}$ (en = ethylenediamine) with the purine nucleosides adenoside and inosine[2] as a model reference system for the more inert *cis*-$Pt(NH_3)_2(H_2O)_2^{2+}$ antitumor complex. The introduction of steric hindrance on the en ligand enables a kinetic tuning of the lability of the diaqua complex and allows an extrapolation of the data to the less labile Pt(II) complex. The quoted Pd(II) and Pt(II) complexes exhibit very similar thermodynamic properties in terms of complex formation and acid dissociation constants, although their reactivity differs by 5 orders of magnitude.[3,4] Similar results were previously reported for a series of sterically hindered diethylenetriamine–Pd(II) complexes.[5,6] We have now extended our studies to the 5′-monophosphates of the purine nucleosides, adenosine (AMP), inosine (IMP), and guanosine (GMP) and of ribose (RMP) itself. These substrates represent simple models for the interaction of DNA with metal ions and complexes, which is widely accepted to be an important step in the reaction mechanism of the cis Pt(II) antitumor drugs.[7]

Structural studies on reaction products using NMR and X-ray techniques have contributed significantly to resolve the most frequently found binding sites in DNA and DNA constituents.[7] In this respect it has been shown that oxo purines such as guanine and adenine show a strong preference for the N7 binding site, and that a cis Pt(II) complex prefers adjacent guanine units in DNA for complexation.[7] However, there are cases known where N1 of the purine ring is involved in coordination to the platinum complex, especially in those cases where the nucleic base bridges two metal centers.[8] Since DNA also contains a phosphate

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backbone, possible phosphate interaction or coordination during complex formation with metal ions should be taken into account. Bose et al.[9] proposed the formation of nucleotide complexes between *cis*-$Pt(NH_3)_2Cl_2$ and AMP, ADP, and ATP coordinated through both a phosphate group and N7 of the purine ring based on ^{31}P, ^{13}C, and ^1H NMR data. Marzilli and co-workers[10-12] criticized this work and ascribed the observed signals to mainly base coordination via N7. However, they did report evidence for the formation of N7,PO chelates between aquated cis Pt(II) complexes and GMP, and N7,N7 coordination in the reaction with AMP. It was also suggested that the often discussed formation of N7,O6 chelates may in fact be due to N7,PO interaction. Similar work on the Pd(en) system indicates the easy formation of 1:2 complexes with the oxopurines where only N7 is coordinated in both ligands.[13] Experiments with oligonucleotides confirmed the strong affinity of cis Pt(II) complexes for the d(GpG) sites,[7,14] and it was concluded[14] that the transition-state structure for the first platination step of a guanine in DNA is determined by hydrogen bonding between the 5′-phosphate and both the leaving H_2O and axial NH_3 ligands. Such stabilization of the transition state by the phosphate group was also suggested[15] to be very important from a kinetic point of view.

The outlined structural information suggests that a significantly different kinetic behavior can be expected for reactions of Pd$(R_4en)(H_2O)_2^{2+}$ (R = H, Me, Et) with monophosphate nucleosides than for the corresponding nucleosides if the phosphate group plays an important role in the formation of the transition state during such complex formation reactions. The results reported in this paper clearly demonstrate the validity of this suggestion.

Experimental Section

Materials. Complexes of the type Pd$(R_4en)Cl_2$ (R = H, Me, Et) were prepared according to the general procedure published before.[3] For the substituted en complexes, *N,N,N′,N′*-tetramethyl- and *N,N,N′,N′*-tetraethylethylenediamine were used. Chemical analyses[16] confirmed the purity of the isolated complexes. The dichloro complexes were converted in solution to the diaqua species by treatment with $AgClO_4$ and precipitation of AgCl as described before.[2] Any excess of Ag^+ in solution was removed by adjustment of the pH to 12–13 and filtration of Ag_2O. The diaqua stock solutions were stored at pH ≈ 3 to prevent hydrolysis and formation of dimeric species. The nucleotides AMP, IMP, and GMP were used as sodium or disodium salts (Sigma) without further purification. Ribose 5′-monophosphate was used as the barium salt (Sigma). The pH of the test solutions was adjusted with $HClO_4$ and NaOH and measured before and after the reactions. Samples used for pH measurements were rejected in order to prevent any contamination by Cl^-. The reference electrode of the pH meter was filled with NaCl instead of KCl to prevent the precipitation of $KClO_4$ since $NaClO_4$ was used to adjust the ionic strength of all test solutions to 0.10 M. Millipore water was used in the preparation of all solutions.

Measurements. UV–vis spectra were recorded on Shimadzu UV 250 and Hitachi U3200 spectrophotometers. Kinetic measurements were performed on a Durrum D110 stopped-flow instrument attached to an on-line data acquisition system[17] with which the kinetic traces were evaluated, using the OLIS KINFIT (Jefferson, GA) set of programs. All measurements were performed under pseudo-first-order conditions, i.e., at at least a 10-fold excess of the monophosphate species. More details on the data-fitting procedures are given in the following section.

Results and Discussion

General Considerations. The experimental conditions in this study were selected in such a way as to minimize the complexity of the investigated reactions. Complexes of the type Pd$(R_4en)(H_2O)_2^{2+}$ closely resemble the coordination geometry of *cis*-$Pt(NH_3)_2(H_2O)_2^{2+}$ and exhibit pK_a values of 5.6, 5.4, and 5.9 for R = H, Me, and Et, respectively.[4] It follows that at the selected pH between 4.0 and 4.8 less than 10% of the diaqua complexes will be present as aquahydroxo species. The 5′-monophosphates (AMP, IMP, GMP, and RMP) exhibit in addition to the protonation equilibria of the nucleic bases,[2,7,18] protonation of the dianionic phosphate moiety at $pK_a \approx 6$[18] such that a monoanionic phosphate group will exist under the selected experimental conditions. A further complication is the solubility of the nucleotides and the possible formation of polymeric species at higher concentrations, which restricted the concentration range to 0.02 M. All efforts to isolate reaction products under the experimental conditions selected for the kinetic measurements were unsuccessful mainly due to the high solubility of the products. In addition, NMR techniques revealed little information on the structure of the product species due to the relatively low concentration of the Pd(II) complexes and the large excess of free nucleotide. The kinetic results of this investigation can therefore only be interpreted on the basis of structural information reported in the literature[7-15] for these and related systems.

Complex Formation Reactions. The UV–vis spectra recorded for the reaction of the diaqua complexes with the 5′-monophosphates are very similar to those found for the reactions with the corresponding nucleosides.[2] The reactions are accompanied by a significant decrease in absorbance around 350 nm and an increase in absorbance around 300 nm for the nucleotides and around 260 nm for RMP. When Pd$(R_4en)(H_2O)_2^{2+}$ is mixed with XMP (X = A, I, G, R) in the stopped-flow system, the absorbance–time traces clearly indicate two consecutive reaction steps for all investigated systems, similar to those observed before.[2] (Detailed examples of typical kinetic traces and the employed data fitting are given in ref 2.) The reactions are such that they can be separated quite easily in most cases using a two-exponential fit, and the pseudo-first-order rate constants exhibit a dependence on the XMP concentration for both reaction steps. In addition, only the second reaction step is observed when a 1:1 mixture of the diaqua complex and XMP is treated with an excess of XMP. These observations indicate that we are dealing with two subsequent complex formation steps, as outlined in (1). k_{obs} for each

$$Pd(R_4en)(H_2O)_2^{2+} + XMP^- \underset{k_2}{\overset{k_1}{\rightleftharpoons}} Pd(R_4en)(XMP)(H_2O)^+ + H_2O$$

$$Pd(R_4en)(XMP)(H_2O)^+ + XMP^- \underset{k_4}{\overset{k_3}{\rightleftharpoons}} Pd(R_4en)(XMP)_2 + H_2O \quad (1)$$

step can be expressed by the well-known two-term rate law (2),

$$k_{obs} = k_a + k_b[XMP] \quad (2)$$

where k_b represents the forward rate constant k_1 or k_3 and k_a represents k_2 for the first reaction and k_4, k_2, $k_2 + k_4$ for the second reaction step.[3] A summary of all the observed rate constants along with the values of k_a and k_b is given in Table I.

In an effort to determine the possible influence of the phosphate group on the complex formation reactions of the nucleotides, the reactions in (1) were first studied for ribose 5′-monophosphate, i.e., RMP. The selected pH of ca. 4 is such that the monoanionic species will be the major reactant. The reactions clearly exhibited evidence for two consecutive steps, although the small absorbance change associated with the second step did not allow the acquisition of accurate kinetic data. Preliminary data demonstrated that the second step exhibited a definite dependence on the RMP concentration, although the exact nature of this dependence, i.e., the

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Table I. Kinetic Data for the Reaction of $Pd(R_4en)(H_2O)_2^{2+}$ with a Series of 5′-Monophosphates[a]

						first step			second step		
XMP	R	pH	λ, nm	T, °C	[XMP], M	k_{obs},[b] s^{-1}	k_a, s^{-1}	k_b, M^{-1} s^{-1}	k_{obs},[b] s^{-1}	k_a, s^{-1}	k_b, M^{-1} s^{-1}
RMP	H	4.3	335	25.0	0.0075	36 ± 4	≈0	48000 ± 40			
			295		0.010	48 ± 10					
					0.015	71 ± 8					
					0.020	95 ± 5					
				12.1	0.020	35 ± 4		1750 ± 200			
				18.2	0.020	47 ± 2		2350 ± 100			
				32.0	0.020	126 ± 7		6300 ± 350			
	Me	4.3	335	12.0	0.005	8.5 ± 1.0	6.74 ± 0.50	355 ± 36			
			295		0.010	10.4 ± 1.5					
					0.020	14 ± 2					
				25.0	0.005	20 ± 2	15.2 ± 1.3	1024 ± 86			
					0.010	26 ± 2					
					0.012	28 ± 2					
					0.020	36 ± 4					
				38.7	0.005	44 ± 3	30.2 ± 0.1	2849 ± 4			
					0.010	59 ± 6					
					0.020	87 ± 10					
	Et	4.0	270	15.5	0.005	1.20 ± 0.12	1.04 ± 0.05	26.6 ± 4.4			
					0.010	1.34 ± 0.04					
					0.015	1.39 ± 0.03					
					0.020	1.63 ± 0.03					
				25.0	0.005	2.5 ± 0.2	1.96 ± 0.25	99 ± 18			
					0.010	2.9 ± 0.3					
					0.015	3.2 ± 0.2					
					0.020	4.1 ± 0.3					
				34.4	0.005	6.0 ± 0.3	4.6 ± 0.5	262 ± 37			
					0.010	6.8 ± 0.8					
					0.015	8.9 ± 0.7					
					0.020	9.7 ± 0.5					
AMP	H	4.0–4.3	300	25.0	0.0050	61 ± 4	≈0	12190 ± 100	10.1 ± 1.0	6.4 ± 0.4	710 ± 55
					0.0060	73 ± 8			10.5 ± 1.1		
					0.0080	77 ± 11			11.9 ± 1.2		
					0.010	122 ± 20			13.6 ± 1.3		
	Me	4.0	370	14.7	0.005	1.9 ± 0.1	0.1 ± 0.7	339 ± 52	0.046 ± 0.001	0.030 ± 0.001	3.10 ± 0.01
					0.010	3.1 ± 0.3			0.061 ± 0.001		
					0.015	5.9 ± 0.4			0.077 ± 0.001		
					0.020	6.6 ± 0.6					
				25.0	0.005	2.79 ± 0.02	0.2 ± 0.2	515 ± 1	0.140 ± 0.005	0.11 ± 0.02	6.61 ± 0.23
					0.010	5.34 ± 0.02			0.171 ± 0.005		
					0.015	7.94 ± 0.08			0.206 ± 0.006		
					0.020	10.5 ± 0.1					
				35.6	0.005	3.9 ± 0.3	0.9 ± 0.5	673 ± 35	0.368 ± 0.010	0.30 ± 0.04	12.9 ± 0.4
					0.010	7.8 ± 0.3			0.436 ± 0.012		
					0.015	11.2 ± 0.4			0.497 ± 0.012		
					0.020	14.4 ± 0.8					
	Et	4.8	420	14.0	0.0030	0.69 ± 0.06	0.54 ± 0.08	37 ± 7			
					0.0092	0.83 ± 0.12					
					0.015	1.12 ± 0.13					
				25.0	0.0026	0.85 ± 0.08	0.76 ± 0.06	57 ± 4			
					0.0030	0.95 ± 0.08					
					0.0092	1.28 ± 0.04					
					0.015	1.52 ± 0.04					
				35.8	0.0030	1.57 ± 0.10	1.20 ± 0.08	122 ± 11			
					0.0092	2.33 ± 0.07					
					0.015	3.11 ± 0.10					
AMP	Et	4.8	420	12.9	0.0025				0.0264 ± 0.0015	0.021 ± 0.001	2.15 ± 0.07
					0.010				0.0425 ± 0.0010		
					0.015				0.0533 ± 0.006		
				25.0	0.0026				0.054 ± 0.001	0.037 ± 0.001	7.01 ± 0.14
					0.0030				0.059 ± 0.003		
					0.010				0.075 ± 0.002		
					0.015				0.143 ± 0.003		
				35.3	0.0025				0.107 ± 0.003	0.076 ± 0.007	16.1 ± 0.7
					0.0075				0.211 ± 0.004		
					0.015				0.312 ± 0.004		
IMP	H	4.0	360	25.0	0.0040				56 ± 4	≈0	14200 ± 260
			300		0.0050				72 ± 2		
					0.0060				87 ± 5		
					0.010				142 ± 10		
				15.3	0.010				97 ± 6		9738 ± 600
				29.9	0.010				173 ± 8		17285 ± 800
				35.2	0.010				202 ± 10		20245 ± 1000
IMP	Me	4.0	370	25.0	0.0040	154 ± 3	≈0	38500 ± 1000			
			300		0.0060	214 ± 8					
					0.010	385 ± 10					

Table I (Continued)

XMP	R	pH	λ, nm	T, °C	[XMP], M	first step k_{obs},[b] s^{-1}	first step k_a, s^{-1}	first step k_b, M^{-1} s^{-1}	second step k_{obs},[b] s^{-1}	second step k_a, s^{-1}	second step k_b, M^{-1} s^{-1}
				25.0	0.0080				2.17 ± 0.02	≈0	265 ± 1
					0.010				2.70 ± 0.02		
					0.012				3.23 ± 0.04		
				14.4	0.010				2.41 ± 0.08		241 ± 8
				33.9	0.010				3.21 ± 0.10		321 ± 10
	Et	4.8	370	25.0	0.0010	0.08 ± 0.09	≈0	860 ± 25			
			295		0.050	4.50 ± 0.04					
					0.0092	8.10 ± 0.04					
				14.9	0.010	5.88 ± 0.15		588 ± 15			
				35.6	0.010	15.1 ± 0.4		1510 ± 40			
				25.0	0.00375				0.038 ± 0.002	≈0	9.73 ± 0.02
					0.0075				0.075 ± 0.003		
					0.015				0.148 ± 0.006		
				15.0	0.015				0.092 ± 0.004		6.1 ± 0.2
				17.1	0.015				0.101 ± 0.005		6.7 ± 0.3
				30.1	0.015				0.183 ± 0.003		12.2 ± 0.2
				38.3	0.015				0.260 ± 0.004		17.3 ± 0.2
GMP	H	4.0	360	12.2	0.0035				49 ± 1	≈0	14550 ± 190
			300		0.0040				57 ± 1		
					0.0050				71 ± 3		
					0.0060				85 ± 6		
				16.0	0.0035				67 ± 3	≈0	18637 ± 605
					0.0040				75 ± 5		
					0.0050				89 ± 4		
					0.0060				112 ± 8		
				25.0	0.0040				130 ± 8	≈0	32440 ± 2000
	Me	4.0	360	25.0	0.0025				1.16 ± 0.04	≈0	547 ± 38
			300		0.0035				1.76 ± 0.02		
					0.0060				3.00 ± 0.25		
					0.0120				6.37 ± 0.25		
				14.6	0.0050				2.3 ± 0.1		462 ± 20
				32.7	0.0050				3.3 ± 0.2		660 ± 40
	Et	4.9	370	25.0	0.0026	5.3 ± 0.7	≈0	2210 ± 90	0.062 ± 0.009	≈0	25.5 ± 0.7
					0.0052	11.5 ± 0.6			0.130 ± 0.004		
					0.0090	19.4 ± 1.3			0.225 ± 0.004		
				17.1	0.0060	10.4 ± 0.1		1730 ± 10	0.081 ± 0.001		13.5 ± 0.2
				36.8	0.0060	25.7 ± 1.4		4239 ± 240	0.223 ± 0.012		37.2 ± 2.0

[a] Experimental conditions: [Pd(II)] was varied to fulfill pseudo-first-order requirements; ionic strength = 0.1 M. [b] Mean value of at least four kinetic runs.

occurrence of an intercept or not, could not be resolved due to the poor quality of the data. For this reason only kinetic data related to the first reaction step are summarized in Table I. Plots of k_{obs} versus [RMP] exhibit meaningful intercepts for R = Me and Et, but not for the unsubstituted complex. The values of k_b decrease significantly with increasing steric hindrance on the en ligand, which is accompanied by a general increase in the associated $\Delta H^{\ddagger}$ values especially observed for k_1. A comparison with the corresponding rate constants for complex formation with adenosine and inosine[2] (see Table II) indicates that the reaction with RMP is significantly faster than with adenosine, but significantly slower than with inosine. Thus coordination via the monophosphate group (O donor) is expected to play an important role in the complex formation reactions with the nucleotides in terms of either a transition state or a final product state stabilization effect. The product complexes are not as stable as for instance in the case of the nucleosides, as reflected by the values for k_1/k_2 of 67 and 50 M^{-1} for R = Me and Et, respectively.

The reaction of AMP and $Pd(en)(H_2O)_2^{2+}$ is very fast, and it is difficult to separate the first reaction step from the second due to the small absorbance changes associated with the first step. Rate constants for the first step are subjected to fairly large error limits, also partly due to the interference of the second reaction especially at low [AMP]. Various data fitting procedures were adopted (OLIS KINFIT package), but similar results were obtained. The reactions of AMP with $Pd(Me_4en)(H_2O)_2^{2+}$ and $Pd(Et_4en)(H_2O)_2^{2+}$ are significantly slower and both reaction steps can be measured rather accurately. The observed reactions are throughout significantly faster than reported for adenosine[2], which must be related to the influence of the monophosphate group as predicted above. In the reaction of $Pd(en)(H_2O)_2^{2+}$ with IMP, only one reaction step can be observed, which was assigned to the second complex formation step. This was based on the observation that the absorbance change observed on the stopped-flow instrument was significantly smaller than recorded spectrophotometrically for the overall reaction. Furthermore, identical kinetic traces and data were obtained when a 1:1 mixture of the diaqua complex and IMP was used instead of only the diaqua complex. These observations demonstrate that a rapid complex formation step to produce the 1:1 complex must occur within the mixing time of the stopped-flow instrument. The reactions with the Me- and Et-substituted complexes are significantly slower, and both complex formation reactions could be observed. A similar trend was observed for the reactions with GMP. In that case the first reaction for both the en and Me_4en complexes is too fast to be measured, and only the second complex formation step could be studied. For the more sterically hindered Et_4en complex, both reaction steps could be measured without any complication.

A summary of all available kinetic and thermodynamic data for the complex formation reactions of $Pd(R_4en)(H_2O)_2^{2+}$ with chloride, nucleosides, and nucleotides is given in Table II. Rate constants, activation parameters and overall equilibrium constants for the various reactions can now be compared as a function of the steric hindrance on the en ligand. A comparison of the k_1 and k_3 values for the series of L demonstrates a steady decrease in rate constants followed by a steady increase along the series of L. The nucleosides are less reactive than chloride, whereas the 5′-monophosphates increase in their reactivity along the series RMP < AMP < IMP < GMP. This trend also determines the sequence of the K_1 values, where data are available. Important to note is that k_1 for the reaction with RMP is slower than that for the reaction with inosine, but faster than for the reaction with adenosine. Furthermore, the introduction of a monophosphate group on the nucleoside causes a remarkable increase in both k_1

Table II. Summary of Kinetic and Thermodynamic Data for Complex Formation Reactions of $Pd(R_4en)(H_2O)_2^{2+}$ According to the General Scheme[a]

$$Pd(R_4en)(H_2O)_2^{2+} + L^{n-} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} Pd(R_4en)(H_2O)L^{(2-n)+} + H_2O$$

$$Pd(R_4en)(H_2O)L^{(2-n)+} + L^{n-} \underset{k_4}{\overset{k_3}{\rightleftharpoons}} Pd(R_4en)L_2^{(2-2n)+} + H_2O$$

parameter	R	$L^{n-} = Cl^-$	inosine	adenosine	RMP	AMP	IMP	GMP
k_1, M^{-1} s^{-1}	H	2.9×10^4	1.4×10^4	1.1×10^3	4.8×10^3	1.2×10^4	*c*	*c*
	Me	1.5×10^4			1.0×10^3	5.1×10^2	3.8×10^4	*c*
	Et	1.6×10^3	2.4×10^2	6.3	99	57	8.6×10^2	2.2×10^3
$\Delta H^{\ddagger}$, kJ mol^{-1}	H	29 ± 2	19 ± 1	65 ± 3	47 ± 6			
	Me	33 ± 2			55.2 ± 0.1	20 ± 2		
	Et	41 ± 3	66 ± 12	48 ± 7	87 ± 6	37 ± 8	31 ± 4	33 ± 6
$\Delta S^{\ddagger}$, J K^{-1} mol^{-1}	H	−61 ± 7	−103 ± 9	+33 ± 9	−17 ± 21			
	Me	−55 ± 7			−2.2 ± 0.4	−126 ± 8		
	Et	−45 ± 10	+21 ± 42	−69 ± 22	−84 ± 20	−84 ± 25	−84 ± 13	−71 ± 19
k_2, s^{-1}	H	7.5	≈0	9.3	≈0	≈0	*c*	*c*
	Me	≈0			15.2	0.2	≈0	*c*
	Et	≈0	1.3	1.3×10^{-2}	2.0	0.8	≈0	≈0
$\Delta H^{\ddagger}$, kJ mol^{-1}	H			71 ± 5				
	Me				39 ± 2			
	Et		49 ± 5	42 ± 7	55 ± 6	26 ± 4		
$\Delta S^{\ddagger}$, J K^{-1} mol^{-1}	H			+12 ± 18				
	Me				−91 ± 5			
	Et		−77 ± 17	−139 ± 25	−53 ± 20	−161 ± 13		
$K_1 = k_1/k_2$, M^{-1}	H	4.0×10^3		1.2×10^2				
	Me	3.2×10^3			66	2.5×10^3		
	Et	2.8×10^3	1.8×10^2	4.8×10^2	50	71		
k_3, M^{-1} s^{-1}	H	1.3×10^3	9.3×10^2	3.7×10^2		7.1×10^2	1.4×10^4	3.2×10^4
	Me	2.9×10^2				6.6	2.6×10^2	5.5×10^2
	Et	13.3	12.4	4.7×10^2		7.0	9.7	25.5
$\Delta H^{\ddagger}$, kJ mol^{-1}	H	52 ± 1	50 ± 8	68 ± 6			25 ± 1	41.9 ± 0.2
	Me	42 ± 1				48 ± 1	8 ± 3	12 ± 2
	Et	47 ± 4	58 ± 1			64 ± 2	31.0 ± 0.2	35 ± 9
$\Delta S^{\ddagger}$, J K^{-1} mol^{-1}	H	−10 ± 2	−21 ± 28	+33 ± 21			−81 ± 3	−18 ± 1
	Me	−57 ± 4				−69 ± 4	−171 ± 9	−153 ± 6
	Et	−66 ± 15	−30 ± 3			−16 ± 7	−123 ± 1	−101 ± 31
k_4, s^{-1}	H	9.7[b]	8.6	1.5		6.4	≈0	≈0
	Me	2.8[b]				0.11	≈0	≈0
	Et	0.29[b]	≈0	≈0		0.037	≈0	≈0
$\Delta H^{\ddagger}$, kJ mol^{-1}	H	48 ± 1	81 ± 7	64 ● 4				
	Me	55 ± 4				79 ± 3		
	Et	40 ± 4				39 ± 5		
$\Delta S^{\ddagger}$, J K^{-1} mol^{-1}	H	−59 ± 2	+45 ± 22	−27 ± 12				
	Me	−50 ± 14				3 ± 10		
	Et	−120 ± 13				−102 ± 18		
$K_2 = k_3/k_4$, M^{-1}	H	1.4×10^2	1.1×10^2	2.5×10^2		1.1×10^2		
	Me	1.0×10^2				60		
	Et	0.5×10^2				190		

[a] Data reported at 25 °C, pH = 4–5, and 0.10 M ionic strength in this study or in refs 2–4. [b] From base hydrolysis reaction.[4] [c] Too fast to measure on stopped-flow instrument.

and k_3 (compare data for inosine and adenosine with IMP and AMP, respectively). This clearly demonstrates that the monophosphate group has a significant transition state stabilization effect in order to favor the complex formation reactions. This is in agreement with earlier observations and could be in line with the suggestion that the phosphate group interacts via hydrogen bonding with the leaving solvent molecule and the axial amine group.[7,14,15,19,20] All the studied reactions exhibit a significant decrease in rate constants with increasing steric hindrance. This can also be clearly seen in the trend that k_3 increases less along the series AMP, IMP, and GMP than k_1, especially for the more hindered complexes, since the presence of one nucleotide in the coordination sphere hinders the entrance of the second. These trends are all in agreement with that expected for an associative substitution mode. The reported values of $\Delta S^{\ddagger}$ are in most cases significantly negative and the available $\Delta V^{\ddagger}$ data[2] are also negative, which further support the operation of an associative mechanism. In addition, the transition-state stabilization effect referred to above and the hydrogen-bonding interaction of the phosphate group with the leaving ligand and axial amine groups will all lead to a more ordered transition state i.e., significantly negative $\Delta S^{\ddagger}$ values.

An important result from this study is the observation that of the studied nucleotides GMP is the most reactive one. It is therefore quite understandable that such sites are preferred during the binding of *cis*-$Pt(NH_3)_2Cl_2$ to DNA.[7] The N7 coordination sites are preferred for all the studied nucleotides, whereas some indirect interaction with PO must account for the increased reactivity observed.[14] A realistic extrapolation of the kinetic data now available to biologically relevant conditions will require a systematic study on the effect of Cl^- ions on the various complex formation steps. The formation constants (K_1 and K_2 in Table II) demonstrate that Cl^- will be an effective scavenger of the

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diaqua species. In addition, at biological pH the diaqua species will mainly exist as aqua hydroxo or dihydroxo species,[4] which will drastically influence their substitution behavior. It follows that more systematic work is needed in terms of [Cl^-] and pH dependences to be able to extrapolate to biologically relevant conditions.

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Registry No. AMP, 61-19-8; IMP, 131-99-7; GMP, 85-32-5; Pd(H_4en)$(H_2O)_2^{2+}$, 23709-90-2; Pd(Me_4en)$(H_2O)_2^{2+}$, 106150-16-7; Pd(Et_4en)$(H_2O)_2^{2+}$, 137596-44-2; ribose 5'-monophosphate, 4300-28-1.

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A Kinetic Investigation of the Lanthanide DOTA Chelates. Stability and Rates of Formation and of Dissociation of a Macrocyclic Gadolinium(III) Polyaza Polycarboxylic MRI Contrast Agent

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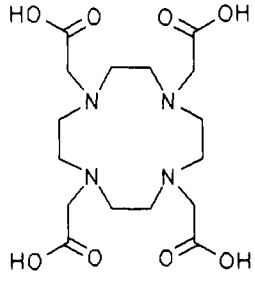
The complexation of the gadolinium(III) ion by DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetracarboxylic acid) proceeds through the formation of an intermediate complex (stability constant $K^* = 6.9 \times 10^3$ M^{-1}) in which the metal ion is incompletely coordinated as shown by NMR spectroscopy. Dyes were used to measure the pH changes that take place during the complexation process. Between pH 4 and 6, the $HDOTA^{3-}$ form is the kinetically active species ($k_{f,HL} = 1.0 \times 10^6$ M^{-1} s^{-1}) despite its low concentration. The dissociation of $GdDOTA^-$ was investigated by scavenging the liberated $^{153}Gd^{3+}$ with an ion exchanger. The dissociation is exceedingly slow even in acidic solutions. It is catalyzed by the H^+ ions ($k_{d,H} = 8.4 \times 10^{-6}$ M^{-1} s^{-1}), and the acid-independent dissociation process can be neglected ($k_{d,0} < 5 \times 10^{-8}$ s^{-1}). The stability constant of $GdDOTA^-$ was computed from the rates of formation and of dissociation (log $K_{ML} = 22.1$). Contrary to earlier findings, this complex does not appear to be unusually stable. However, $GdDOTA^-$ should be a particularly safe MRI contrast agent because of its remarkable kinetic inertness.

Introduction

Nuclear magnetic resonance imaging (MRI) was originally considered a noninvasive radiological technique, but it now appears that contrast agents are often necessary to highlight lesions that otherwise could not have been detected.[1] The contrast of the NMR images depends both on the proton density and on the proton relaxation times T_1 and T_2. The water molecules contribute the most to the NMR signal, and a better contrast between different tissues can be achieved by the addition of various paramagnetic ions that drastically reduce the relaxation times of water. The ion Gd^{3+} appears to be the most useful paramagnetic species because of its high magnetic moment and its long electronic relaxation time.[2] However, this ion is too toxic to be used in vivo and it must be reacted with a chelating agent before being injected in the blood in order to facilitate its rapid excretion through the kidneys. Safe and effective contrast agents containing gadolinium should not dissociate in the body and thus should be highly stable and kinetically inert. High stability is achieved in the case of diethylenetriaminepentaacetic acid (DTPA), and $GdDTPA^{2-}$ is now a commonly used MRI contrast agent. However, it was anticipated that a better kinetic inertness would be achieved with macrocyclic tetracarboxylic ligands such as DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetracarboxylic acid) and its derivatives. Indeed, the steric requirements of the tetraaza cavity of DOTA impart an unusual rigidity to its lanthanide chelates[3] and could be at the origin of the slow rate of formation and of dissociation of these complexes. Preliminary studies[4,5] indicate that $YDOTA^-$ and $GdDOTA^-$ undergo a negligible dissociation in serum at pH 7 over long periods of time. Moreover, a spectrophotometric analysis[6] of the kinetic properties of $CeDOTA^-$ also showed that the metal ion is released very slowly even in acidic media. This unusual kinetics contrasts significantly with the behavior of lanthanide complexes with linear chelating agents which are known to form and to dissociate rapidly. The rate and the mechanism of the formation and of the dissociation of $GdDOTA^-$ are of considerable interest to the design of effective MRI contrast agents[2] or radiolabeled monoclonal antibodies.[4] The primary goal of the present work was thus to study the kinetic properties of $GdDOTA^-$. An important second objective was to deduce the stability constant of $GdDOTA^-$ from kinetic data. Indeed, the very slow rates of complexation and of dissociation of the lanthanide ions by DOTA prevent the reliable measurement of the thermodynamic stability of the $LnDOTA^-$ chelates by classical methods such as potentiometry. It was thus necessary to resort to competition techniques using either a precipitating agent[7] or a dye,[8] but these approaches led to stability constants



DOTA

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