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Stabilities of Gallium(III), Iron(III), and Indium(III) Chelates of Hydroxyaromatic Ligands with Different Overall Charges

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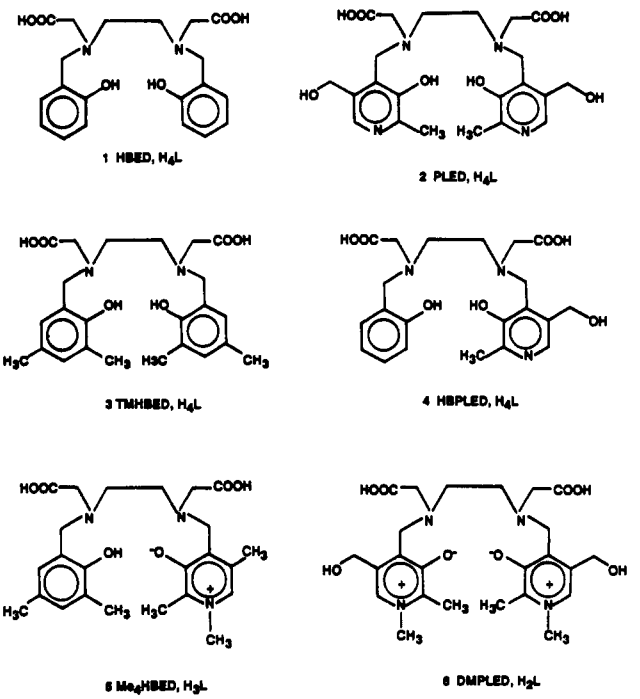
The stability constants of the Ga(III), In(III), and Fe(III) chelates of three new ligands, *N*-(2-hydroxybenzyl)-*N'*-(pyridoxyl)-ethylenediamine-*N,N'*-diacetic acid (HBPLED), *N*-(2-hydroxy-3,5-dimethylbenzyl)-*N'*-((3-hydroxy-1,2,5-trimethyl-4-pyridiniumyl)methyl)ethylenediamine-*N,N'*-diacetic acid (Me_4HBPLED), and *N,N'*-bis((1,2-dimethyl-3-hydroxy-5-(hydroxymethyl)-4-pyridiniumyl)methyl)ethylenediamine-*N,N'*-diacetic acid (DMPLED) are reported and compared with those of the parent ligands *N,N'*-bis(2-hydroxybenzyl)ethylenediamine-*N,N'*-diacetic acid (HBED), *N,N'*-bis(2-hydroxy-3,5-dimethylbenzyl)-ethylenediamine-*N,N'*-diacetic acid (TMHBED), and *N,N'*-bis(pyridoxyl)ethylenediamine-*N,N'*-diacetic acid (PLED). This comparison shows the effect on chelate stability of the methylation of the pyridine nitrogens, which increases the overall charges of the complex molecules without changing the donor atoms in the coordination sphere. The data show that quaternization of the pyridine nitrogen decreases the stability constants of the Fe(III) and Ga(III) chelates by about 2 orders of magnitude. A perturbation in the trends is seen in the chelates of Me_4HBPLED, whereby methylation of the benzene ring tends to increase the metal chelate stabilities. The log *K* values reported are as follows: For HBPLED: Ga, 31.02; Fe, 31.01; In, 28.97. For Me_4HBPLED: Ga, 31.85; Fe, 32.97; In, 27.82. For DMPLED: Ga, 27.27; Fe, 27.20; In, 21.47.

Introduction

Highly stable chelates of Ga(III), In(III), and Fe(III) are of considerable interest because the radioactive isotopes ^{67,68}Ga and ^{111}In may be employed as radiopharmaceuticals for diagnostic imaging applications,[1-5] and the Fe(III) complexes have possible applications as paramagnetic contrast agents for magnetic resonance imaging (MRI).[6,7] The chelates of these metal ions with ligands containing hydroxyaromatic donor groups such as *N,N'*-bis(2-hydroxybenzyl)ethylenediamine-*N,N'*-diacetic acid (HBED) (**1**) and *N,N'*-bis(pyridoxyl)ethylenediamine-*N,N'*-diacetic acid (PLED) (**2**) have received attention from several groups because their very high thermodynamic stabilities minimize the potential for ligand-exchange reactions with plasma transferrin following intravenous injection. In addition, the extremely high thermodynamic stabilities assure kinetic inertness of these chelates, so that they may retain their identities in contact with the many metal ion carriers encountered in the biological milieu.[3,5,8,9] It has been found that halogenation and alkylation of the aromatic rings in HBED, i.e., as in the tetramethyl HBED derivative *N,N'*-bis(2-hydroxy-3,5-dimethylbenzyl)ethylenediamine-*N,N'*-diacetic acid (TMHBED) (**3**) can substantially alter the clearance from a renal to a hepatic pathway.[3,10] In addition to this type of lipophilicity, the overall charge of the molecule or complex has frequently been considered important in the control of membrane permeability and clearance mechanisms, with a zero charge being considered most effective for membrane penetration. While the trivalent metal chelates of HBED and PLED have single negative charges, the pyridine nitrogens of PLED offer the possibility of adding positive charges, one at a time, by quaternization of the aromatic nitrogens, thus changing the overall charges of the chelates from −1 to 0 and +1. This paper reports the stabilities of the Ga(III), Fe(III), and In(III) chelates of three new ligands related to HBED and PLED: a "hybrid" between these two ligands, *N*-(2-hydroxybenzyl)-*N'*-(pyridoxyl)ethylenediamine-*N,N'*-diacetic acid (HBPLED) (**4**); a HBED–PLED analogue containing one methylpyridinium group, *N*-(2-hydroxy-3,5-dimethylbenzyl)-*N'*-((3-hydroxy-1,2,5-trimethyl-4-pyridiniumyl)-methyl)ethylenediamine-*N,N'*-diacetic acid (Me_4HBPLED) (**5**); and a PLED derivative with two methylpyridinium groups, *N,N'*-bis((1,2-dimethyl-3-hydroxy-5-(hydroxymethyl)-4-pyridiniumyl)methyl)ethylenediamine-*N,N'*-diacetic acid (DMPLED) (**6**).

Experimental Section

Materials. The new ligands *N*-(2-hydroxybenzyl)-*N'*-(pyridoxyl)-ethylenediamine-*N,N'*-diacetic acid (HBPLED), *N*-(2-hydroxy-3,5-dimethylbenzyl)-*N'*-((3-hydroxy-1,2,5-trimethyl-4-pyridiniumyl)methyl)-ethylenediamine-*N,N'*-diacetic acid (Me_4HBPLED), and *N,N'*-bis((1,2-dimethyl-3-hydroxy-5-(hydroxymethyl)-4-pyridiniumyl)methyl)-ethylenediamine-*N,N'*-diacetic acid (DMPLED) were synthesized and characterized as reported elsewhere.[11]



Potentiometric Studies. The procedures employed for the potentiometric pH measurements have been described in detail.[12] The Corning

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Table I. Ligand Protonation Constants and Trivalent Metal Ion Stability Constants[a]

ligand	log K [HL]/[H][L]	[H$_2$L]/[H][HL]	[H$_3$L]/[H][H$_2$L]	[H$_4$L]/[H][H$_3$L]	[H$_5$L]/[H][H$_4$L]	[GaL]/[Ga][L]	[FeL]/[Fe][L]	[InL]/[In][L]
HBED, H$_4$L[b,c]	12.35	12.08	8.45	4.76	2.18	37.73	36.74	27.90
TMHBED, H$_4$L[d]	13.12	12.99	7.94	4.09	1.4	34.19	37.41	30.72
PLED, H$_4$L[e,f]	10.89	10.28	7.20	5.73	3.26	32.31	30.78	26.54
HBPLED, H$_4$L[g]	12.10 (3)	10.73 (1)	8.25 (1)	5.71 (1)	2.85 (1)	31.0 (2)	31.01 (6)	28.97 (9)
Me$_4$HBPLED, H$_3$L[h]	12.07 (3)	9.72 (1)	5.99 (1)	2.96 (1)	1.1 (1)	31.85 (8)	32.97 (3)	27.82 (3)
DMPLED, H$_2$L[i]	8.95 (1)	6.85 (1)	3.30 (1)	2.24 (1)	1.3 (1)	27.3 (2)	27.2 (2)	21.47 (3)

[a] Values in parentheses are expressed as variation of last significant digit shown. [b] log ([H$_6$L]/[H][H$_5$L]) = 1.17. [c] Reference 16. [d] Reference 13. [e] Reference 15. [f] log ([H$_6$L]/[H][H$_5$L]) = 2.31. [g] Protonation constant σ_{fit} = 0.013. [h] Protonation constant σ_{fit} = 0.004. [i] Protonation constant σ_{fit} = 0.009.

Model 150 pH meter employed, fitted with glass and calomel electrodes, was calibrated with strong acid and strong base at 25.0 °C and 0.100 M ionic strength (adjusted with KCl as supporting electrolyte) so as to read −log [H$^+$], designated as p[H], directly. For comparative purposes: p[H] = pH − 0.07. The ion product constant of the experimental solution, log ([H$^+$][OH$^-$]), under these conditions was found to be −13.78. Equilibrium constants (dissociation constants) involving the ligands and their trivalent metal chelates were calculated from potentiometric data as described.[12]

Spectrophotometric Studies. The first protonation constants of HBPLED and Me$_4$HBPLED were determined at approximately 5 × 10^{-5} M concentrations of the ligand in the UV region using a Perkin-Elmer Model 553 spectrophotometer equipped with 1.00 cm thermostated cells. The p[H] of the test solutions was determined from the stoichiometry of added KOH, with [KOH] + [KCl] = 0.100 M to keep the ionic strength constant.

All metal complexes studied in this investigation undergo partial dissociation in acid solution according to the reaction

$$M^{3+} + H_nL^{(n-m)+} \rightleftharpoons ML^{(3-m)+} + nH^+$$

where m is the number of hydrogen ions bound to the neutral ligand and n is the degree of protonation of the free ligand at the p[H] of the equilibrium measurements; n varied from 4 to 5, depending on the value of the stability constant K_{ML} ([ML$^{(3-m)+}$]/[M^{3+}][L^{m-}]).

A stock solution containing known ligand and metal ion concentration (~1 × 10^{-3} M) was prepared. To prepare a spectrophotometric test solution, a 1.00-mL aliquot was taken, together with varying volumes of 1.00 M HCl and a suitable quantity of 1.00 M KCl to adjust the ionic strength to 0.100 upon dilution to 10.0 mL. The exact volumes were measured usually by means of a Gilmont 2-mL microburette. Values of p[H] below 2.0 were calculated from the large excess of added HCl present. The spectra were obtained in 1.00-cm matched quartz cells on a Fast Scan Perkin-Elmer Model 503 spectrophotometer equipped with a thermostated cell compartment maintained at 25.0 °C with a refrigerated constant-temperature bath.

Achievement of equilibrium was assured by repetitive scanning until spectra were identical for a period of time. This was judged from rates of equilibration which varied with the ligand being investigated, and with the metal ion. A satisfactory equilibrium position was obtained rapidly with the In(III) complexes, but those of Fe(III) and Ga(III) reached equilibrium very slowly. Because the equilibrium position (degree of dissociation) varied approximately with the fifth power of [H$^+$], finding a satisfactory p[H] for partial dissociation required very fine adjustment of the quantity of HCl added to the solution. Once partial dissociation was achieved the equilibrium constant could be readily calculated from the absorbance spectrum mass balance equations and known parameters, because of the characteristic differences in the λ_{max} values of the metal complex (~315 nm) and of the free ligand (290 nm). A typical example of a determination is shown in Figure 1. Visible spectra were employed with iron(III) solutions.

Results

The protonation constants of the new ligands investigated in this research, and their Ga(III), Fe(III), and In(III) stability constants are listed in Table I, together with the corresponding values of the parent ligands HBED[13] and PLED[14,15] and a tetramethyl-substituted derivative of HBED, TMHBED.[13] The ligand protonation constants were determined potentiometrically from the p[H] profiles as described previously,[12] while the Ga(III), Fe(III), and In(III) stability constants were calculated from the absorbance spectra of the partially dissociated complexes at equilibrium at low p[H] by the procedure described above.

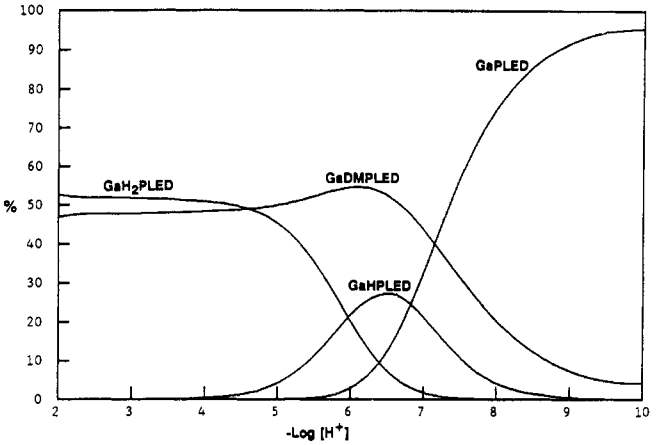

Figure 1. Species distribution curves showing competition for Ga(III) by two ligands as a function of pH. Total concentrations of PLED, DMPLED, and Ga(III) are 0.0020 M in each case. All gallium(III) containing species are shown.

Table II. Protonation Constants of Metal Complexes (μ = 0.100 M (KCl); t = 25.0 °C)

ligand	quotient	log K^H_{ML} Ga(III)	Fe(III)	In(III)
PLED[a]	[MHL]/[H][ML]	7.10	6.93	7.15
	[MH$_2$L]/[H][MHL]	6.2	6.02	6.34
HBPLED	[MHL]/[H][ML]	6.88[b]	6.86[c]	6.21[d]
	[MH$_2$L]/[H][MHL]	3.63[b]	3.38[c]	2.89[d]

[a] From ref 15. [b] σ_{fit} for both constants 0.006. [c] σ_{fit} for chelate protonations 0.021. [d] σ_{fit} for both constants 0.014.

Trivalent metal complexes of the ligands PLED and HBPLED which contain pyridine rings undergo protonation reactions at low pH, the first near pH 7, the second lower. The corresponding protonation constants, listed in Table II, were determined from the p[H] profiles of the 1:1 metal chelate systems by the use of program BEST.[12] For the trivalent metal complexes investigated with the remaining ligands, no protonated metal complex intermediates were detected at low pH as the result of protonation of the hydroxyaromatic groups. Such protonation reactions had been observed in the coordination studies of both HBED and PLED with divalent metal ions.[16,17]

Discussion

Ligand Protonation Constants. The protonation constants of the parent ligand HBPLED and PLED show the trends described

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Table III. pM Values[a] for Metal Complexes at Physiological pH

ligand	Fe(III) log K_{ML}	Fe(III) pM	Ga(III) log K_{ML}	Ga(III) pM	In(III) log K_{ML}	In(III) pM
HBED[b]	36.74	26.73	37.73	27.7	27.90	17.9
PLED[c]	30.78	24.2	32.31	25.7	26.54	20.0
HBPLED[d]	31.01	21.8	31.0	21.8	28.97	19.7
Me_4HBPLED[d]	32.97	25.8	31.85	24.6	27.82	20.6
DMPLED[d]	27.20	25.4	27.27	25.5	21.47	19.7
transferrin[e]	20.67		20.3		19.2	
		20.7		20.4		18.9
	19.38		19.3		18.1	

[a] Under conditions of 100% excess ligand at p[H] 7.3. t = 25.0 °C; μ = 0.100 M. [b] Reference 16. [c] Reference 15. [d] This work. [e] Calculated for 0.2 mM $NaHCO_3$.[19]

previously,[13,15-17] with the highest values corresponding to the protonation of the aliphatic tertiary nitrogens and with partial participation of the hydroxyaromatic oxygens. The increase of basicity resulting from methylation of the aromatic rings is seen in the higher protonation constants for TMHBED relative to those of HBED. The significantly lower protonation constants of PLED reflect the electron-withdrawing influence of the pyridine nitrogens. In fact, the latter ligand shows a reversal of protonation sites,[18] with the pyridine nitrogen being more basic than the oxyaromatic donor atoms.

The high basicities of the first few protonation sites of HBPLED and Me_4HBPLED relative to those of DMPLED reflect the participation of the highly basic phenolate oxygen, along with the aliphatic tertiary amino groups. In HBPLED, the first three protonation constants involve these functional groups, while the fourth is probably due mainly to the pyridine nitrogen. In Me_4HBPLED, which does not have a pyridine nitrogen available for protonation, there is a considerable drop for the fourth protonation constant, indicating that the poorly basic hydroxypyridinate group is involved. This conclusion is reinforced by the low values of the third and fourth protonation constants of DMPLED, which also involve hydroxypyridinate protonation, subject to the electron-withdrawing effects of the positive quaternary methylated aromatic nitrogens. Since this effect would be similar in magnitude to that produced by protonation of the pyridine nitrogens, it seems that one might therefore expect that oxyaromatic oxygens meta to a protonated pyridine would be expected to have log protonation constants in the range 2–4. The first two log K's for protonation of DMPLED are considerably lower than those of ethylenediamine, and even of EDTA, showing that hydrogen bonding to the oxyaromatic oxygens is very weak, if it exists at all, and that the electron-withdrawing effects of the positive pyridinium nitrogens are transmitted through the methylene linkages to the aliphatic amino groups.

Stability Constants. The stability constants listed in Table I for the Ga(III), Fe(III), and In(III) chelates of the three new ligands show the effect of the positive quaternary nitrogens on lowering metal ion affinity. This factor is especially well developed in DMPLED, with two positive methyl pyridinium groups, and reductions in log K relative to PLED of 3.5–5 orders of magnitude, depending on the metal ion. The effects are not so clear for Me_4HBPLED, which has methyl substituents that increase the basicity of the phenolic donor group and possibly metal binding affinity as well. However, the high degree of methylation of the aromatic rings could also produce adverse steric effects for metal ion coordination.

Replacing one pyridoxyl group of PLED by hydroxybenzyl to give HBPLED results in little or no increase in metal ion affinity (except for In(III), which shows a small increase) even though the overall basicity of the ligand, as judged by log $K^{H}_{H_5L}$, is increased by ~3 orders of magnitude. The reason for this behavior is not obvious, but may be steric in nature involving the methyl group. The metal ion most affected by the decreased ligand basicity resulting from the methylpyridinium electron-withdrawing groups is In(III), which also has considerably lower affinity for other oxyaromatic-containing ligands than the considerably smaller metal ions Fe(III) and Ga(III).[13] In spite of the reduced values of the log K's of the trivalent metal ion complexes of the ligands containing methylpyridinium groups, the stabilities of most of these complexes are sufficiently high to avoid interference by transferrin. In(III) complexes and especially In(III)–DMPLED may be exceptions. In this case, the stability of the complex is too low to resist exchange with transferrin. This is best seen in Table III, which gives the pM values of the complexes of interest, including those of transferrin, at physiological pH. Exchange is considered likely if the pM value for a particular metal ion is close to (or lower than) that of transferrin. The pM values listed for the complexes of HBPLED for all three metal ions are only marginally higher than those of transferrin. This is due to strong hydrogen ion competition for the ligand (i.e., high protonation constants) without compensating increases in the stability constants.

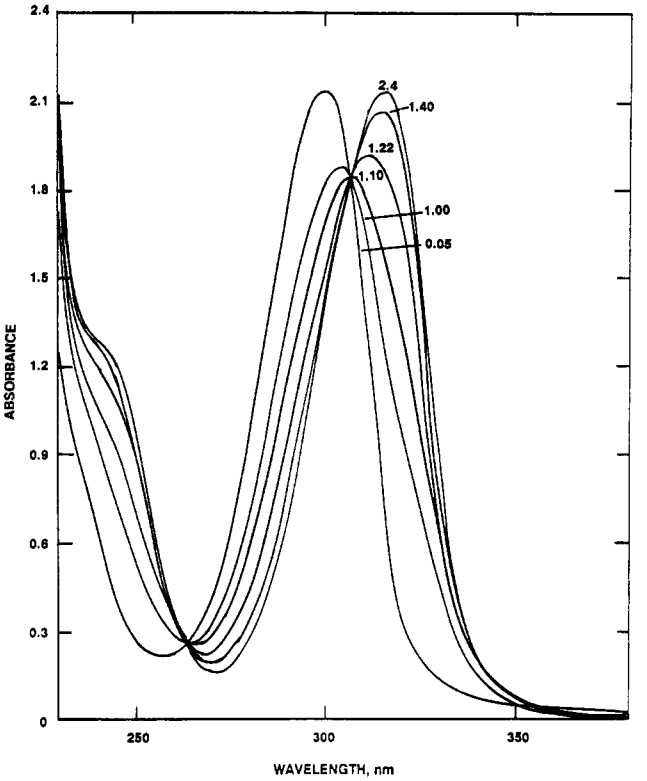

Figure 2. Ultraviolet absorbance spectra as a function of p[H] of the 1:1 DMPLED–In(III) system. Concentration of each component is 1.58×10^{-4} M. The two spectra at the extreme p[H] values were used to obtain the extinction coefficients of InL^+ and H_5L^{3+} at 315 nm, while the intermediate curves were used to obtain the degree of complex formation and the equilibrium constant of the reaction: $M + H_5L \rightleftharpoons ML + 5H^+$. Numbers indicated on the curves are p[H] values.

The relative affinities of a metal ion for two ligands can be evaluated only by considering relative degrees of hydrogen ion competition (protonation constants) as well as stability constants. Thus in the case of Ga(III) binding by PLED or DMPLED, the difference of 5 orders of magnitude in the stability constants (in favor of PLED) is balanced by a difference in the protonation constants of these ligands. The differences in Ga(III) binding are illustrated as a function of p[H] by the species distribution curves in Figure 1. It is seen that, because of its lower hydrogen ion affinity, DMPLED is competitive at low p[H], but at higher p[H] values, where the hydrogen ion competition becomes less important, the PLED–Ga(III) complex predominates.

This behavior (Figure 2) is the result of the differences between temporarily protonated and permanently quaternized (methylated) pyridine groups. At low pH, the Ga(III) distribution between the two ligands is fairly equal. As the protons are removed from the H_2PLED–Ga(III) complex, the stability of the complex is

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increased to the extent that above pH 9, the dimethylated complex, which cannot lose its positive charge, loses its Ga(III) in favor of the deprotonated form of Ga(III)-PLED.

It has been shown that the In(III) ion has a preference for carboxylate donors over phenolate or hydroxypyridine donor groups.[13] Thus the stability constant of the DTPA-In(III) complex is greater than those of PLED and DMPLED. Since the summation of the log protonation constants of the latter, even that of DMPLED, is larger than the basicity sum of DTPA, there is no need to make comparisons of the In(III) chelates of these ligands with species distribution curves: a 1:1:1 system of In(III), DMPLED, and DTPA would show only DTPA complexes, and those of DMPLED would not appear at all. In other words there is no competition in this system.

Influence of Charge and Stability of the Complexes on Biodistribution. It is shown in a separate report[11] that although the trivalent metal complexes of HBPLED, Me_4HBPLED and DMPLED have different overall charges (-1, 0, and +1), the biodistributions of the metal complexes in rats are similar and all complexes undergo renal clearance. The ^{111}In and ^{68}Ga complexes resemble the biodistribution and clearance route of the complexes of the parent ligands HBED and PLED. Thus it seems that biodistributions of these complexes are dependent on other factors, which are more important in controlling behavior, than the charge on the complex molecule. It is suggested that the zwitterions in formulas **5** and **6** and corresponding dipolar functional groups in their metal complexes are highly solvated in aqueous solution. This makes both ligands and metal complexes highly hydrophilic and accounts for the observed biodistributions and clearance routes.

When examining a similar complex EHPG (ethylenebis((hydroxyphenyl)glycine),[20] we have found very different in vivo behavior between the iron, indium and gallium complexes. This is not the case for the complexes of HBPLED, Me_4HBPLED, and DMPLED where very similar biodistribution patterns were obtained with indium and gallium and for DMPLED (the only ligand studied) with iron. The similarity in biodistribution is presumably due to the similar pM values of all the complexes and the fact that they are all greater than the corresponding pM value for the metal transferrin complex.

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Metallobiochemistry of Magnesium. Coordination Complexes with Biological Substrates: Site Specificity, Kinetics and Thermodynamics of Binding, and Implications for Activity†

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The association constant (K_a), activation free energy (ΔG^*), and off-rate for magnesium binding (k_{off}) to glucose 1-phosphate (K_a = 15 M^{-1}, ΔG^* = 12.7 kcal mol^{-1}, k_{off} = 2.9 × 10^3 s^{-1}), glucose 6-phosphate (K_a = 8 M^{-1}, ΔG^* = 12.7 kcal mol^{-1}, k_{off} = 3.1 × 10^3 s^{-1}), acetyl phosphate (K_a = 9 M^{-1}, ΔG^* = 13.1 kcal mol^{-1}, k_{off} = 1.5 × 10^3 s^{-1}), AMP^{2-} (K_a = 18 M^{-1}, ΔG^* = 12.7 kcal mol^{-1}, k_{off} = 3.4 × 10^3 s^{-1}), ADP^{3-} (K_a = 2.2 × 10^3 M^{-1}, ΔG^* = 12.8 kcal mol^{-1}, k_{off} = 2.5 × 10^3 s^{-1}), $ADPH^{2-}$ (K_a = 13 M^{-1}, ΔG^* = 12.1 kcal mol^{-1}, k_{off} = 7.7 × 10^3 s^{-1}), ATP^{4-} (K_a = 3.0 × 10^3 M^{-1}, ΔG^* = 12.4 kcal mol^{-1}, k_{off} = 5 × 10^3 s^{-1}), and $ATPH^{3-}$ (K_a = 6 M^{-1}, ΔG^* = 12.5 kcal mol^{-1}, k_{off} = 4.2 × 10^3 M^{-1}) have been determined by a total line shape analysis of ^{25}Mg NMR spectra. The results were compared with data previously determined for $tRNA^{Phe}$ (yeast) (Reid, S. S.; Cowan, J. A. *Biochemistry* **1990**, *29*, 6025-6032). Estimates of the on-rate for magnesium binding (k_{on} = K_a/k_{off}) were made; k_{on} (s^{-1}) = 4.3 × 10^4, 2.5 × 10^4, 1.4 × 10^4, 6.2 × 10^4, 5.5 × 10^6, 8.5 × 10^4, 1.5 × 10^7, 5.1 × 10^4, and 5.5 × 10^4, (tRNA), respectively. Outer-sphere association constants were also estimated; K_{os} (M^{-1}) = 0.43, 0.25, 0.14, 0.62, 55, 0.85, 150, 0.51, and 220 (tRNA), respectively. The exchange regime for Mg^{2+} binding shows no trend with K_a but appears to correlate with the number of inner- and outer-sphere binding contacts to $Mg(H_2O)_n^{2+}$. Magnesium binding to $ADPH^{2-}$ and $ATPH^{3-}$ occurs preferentially at the terminal phosphate dianion, and the α- or β-phosphate, respectively, is protonated. Possible relevance to the role of Mg^{2+} in enzymatic catalysis is discussed briefly. Magnesium binding to a terminal phosphate apparently leads to facile protonation of an inner phosphate that results in a reactive pyrophosphate-type center.

Introduction

Alkali-metal and alkaline-earth-metal ions (Na^+, K^+, Mg^{2+}, and Ca^{2+}) are the most abundant metal ions in biology; however, their biochemistry has received less attention than that of transition metals because the latter are more readily studied by common spectroscopic and electrochemical methods. Magnesium is a frequent cofactor for RNA and DNA processing enzymes, ribozymes, and an essential component of the ribosome.[1-8] It is important, therefore, to understand the manner in which magnesium binds to its "biological ligands" and activates them structurally and catalytically.[9] In aqueous solution, Mg^{2+} will typically form the hexahydrated $Mg(H_2O)_6^{2+}$ species; however, when it is bound to a protein or RNA, the inner and outer coordination environment is less well characterized. Magnesium readily forms complexes with biological substrates and may serve to either define a particular conformation or catalytically activate chemical functionality toward reaction.[10-16] The role of the metal

† Abbreviations: deoxyribonucleic acid (DNA); phenylalanine transfer ribonucleic acid ($tRNA^{Phe}$); adenosine monophosphate (AMP^{2-}); adenosine diphosphate (ADP^{3-}); adenosine triphosphate (ATP^{4-}); single strand (ss); double strand (ds); variable temperature (VT); room temperature (RT).

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