Metal Ion Effects in Isotopic Hydrogen Exchange in Biologically Important Heterocycles

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ABSTRACT

The binding of metal ions to heteroatomic centers of biomolecules has been utilized as a probe of metal ion effects in living systems. This article focuses on the effect of N-coordination by transition metals, especially Pt(II), Co(III), Cr(III), on isotopic C(2)−H or C(8)−H exchange of imidazoles, thiazoles, and purines. The usual reactivity trend, protonated ≫ metalated ≫ neutral substrate, is excepted for Cr(III)/1-methylimidazole, where Cr(III) activates stronger than H^+. An interplay of factors is considered, including metal-to-ligand back-bonding, electronic structure of metal ions, and differences in crystal field stabilization energy.

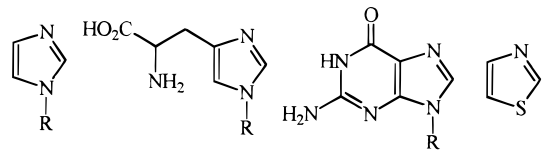
Introduction

Metal ions play vital roles in important biochemical processes involving biomolecules such as proteins, enzymes, nucleic acids, etc.; these molecules generally contain the imidazole moiety and similar heterocyclic residues as part of their basic structure.[1] The roles played by metal ions could include binding interactions at the active sites of biological substrates or catalysts, stabilization of conformational structures of biomolecules, and metal ion-mediated electron transfer processes, among others. Metallopharmaceuticals now form an integral part of modern medicine, being actively deployed for diagnostic purposes and in cancer chemotherapy.[1−3] The toxicity of various heavy metals has received considerable attention.[1]

A number of reactions of biological importance are limited or facilitated by proton or metal ion activation. Metalloenzymes have metal ions as part of their structure.[4−8] In living systems, metal ions can function either as electrophilic catalysts or as sources of OH^- at neutral pH. Thus, an understanding of the roles of metal ions in living systems as well as the magnitude and direction of their effects continues to attact the active attention of both chemists and biologists.

The binding of metal ions to heteroatomic centers of biomolecules has been exploited as a probe of metal ion effects in living systems. The C−H acidity in the heterocyclic fragments of biologically active molecules, which renders the protons susceptible to isotopic exchange, is a useful probe for metal ion effects in biomolecules and for characterizing kinetic and thermodynamic aspects of enzyme-catalyzed biological processes.[9] Studies of isotopic hydrogen exchange in imidazoles,[10−12] histidines,[12,13] thiazoles,[12,14] purine derivatives,[12,15] etc. have therefore been pursued. This article is a contextual account of our studies of metal ion effects in isotopic hydrogen exchange in a variety of biomolecules, aimed at evaluating the role and importance of metal ions in biological systems. Observed generalizations and peculiarities provide an insight into some of the factors that determine the direction and magnitude of metal ion effects. The relative importance of, and the kinetic and thermodynamic rationale for, proton and metal ion activation in these processes are considered. A holistic approach to the interpretation of metal ion effects in biological systems is emphasized since the overall effect of a metal ion results from a complex interplay of a number of factors.

Data for isotopic hydrogen exchange in selected biomolecules and their models, including those for which metal ion effects have been reported, are assembled in Table 1. Structures **1**−**4** present a sample of heterocyclic moieties in important biomolecules whose isotopic exchange reactions are mediated by metal ions and generally form the focus of the present review.



1(a) R = H (b) R = CH_3 2(a) R = H (b) R = CH_3 3(a) R = H (b) R = Ribosyl 4

Erwin Buncel, Professor Emeritus of Chemistry at Queen?s University, is a physical organic chemist with strong interests in bioorganic and bioinorganic chemistry (for a biography summarizing his earlier research activities, see *Acc. Chem. Res.* **1990**, *23*, 226; **1979**, *12*, 42). Recent research in Buncel's group in these areas has focused on metal ion catalysis in nucleophilic displacements at carbon, phosphorus, and sulfur centers, and metal ion−biomolecule interactions. In 1985 Buncel was the recipient of the SYNTAX Award in Physical Organic Chemistry, and in 1999 he received the R. U. Lemieux Award in Organic Chemistry, both from the Canadian Society for Chemistry. Buncel was an editor for the *Canadian Journal of Chemistry* (1981−1993) and since 1995 he has been an editor for the *Journal of Labelled Compounds and Radiopharmaceuticals.* Since becoming Professor Emeritus in 1996, Buncel has continued an active research program with both graduate students and postdoctoral fellows.

Omoshile Clement is a Senior Scientist at Molecular Simulations Inc. in San Diego, CA. Omoshile was born in Nigeria and received his B.Sc. degree in chemistry from the University of Ibadan. He then began graduate work at Queen's University under Erwin Buncel and earned the M.Sc. and Ph.D. degrees in bioinorganic chemistry. Following postdoctoral work at the Battelle Pacific Northwest National Laboratory from 1996 to 1998, Omoshile joined MSI as an applications scientist. He continues active research interests in various aspects of experimental and computational chemistry.

Ikenna Onyido graduated from the University of Ibadan, Nigeria, with a B.Sc. First Class Honours (1974) and Ph.D. (1979). After a year of postdoctoral work with Per Ahlberg at the University of Uppsala, Sweden, he returned to Ibadan to take up a lectureship position in chemistry. In 1989, he was appointed Professor and Head of Department of Chemistry in the new University of Agriculture, Makurdi, where he also served as the foundation Dean of Science and, later, became Deputy Vice-Chancellor for four years. Since 1983, he has maintained an active collaboration with Erwin Buncel with whom he shares common research interests in different aspects of chemistry.

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Table 1. Rate Data for H/D and H/T Exchange at C(2)/C(8) of Some Representative Biomolecules[a,b]

substrate (ref.)[c]	exchange reaction (T/°C)	k^{H^+} (M^{-1} s^{-1})[d]	k^{M^+} (M^{-1} s^{-1})[e]	k (M^{-1} s^{-1})
1a (19a)	H/T (85)	6.0×10^3		
5a (25a)	H/T (85)		1.24	
1b (10)	H/T (85)	1.0×10^4		
5b (25a)	H/T (60)		0.15	
6 (26,27)	H/T (35)		6.0×10^3[f]	
9[g] (30,31)	H/D (60)		2.6×10^{-2}[h]	
12 (30,31)	H/D (60)		1.7×10^{-2}	
BzImH[i] (10,12)	H/T (85)	6.2×10^4	~0.21, Ag(I)	
4 (17,29)	H/D (60)	4.2×10^6[j]		9.3×10^{-4}
13[k] (29)	H/D (60)		9.1×10^2[l]	
2a (12)	H/T (85)	6.1×10^3		49
2b (13,15c)	H/T (85)	1.0×10^4	$\leq 1 \times 10^{-1}$, $CH_3Hg(II)$	1.0×10^2[m]
3b (12,15)	H/D (30)	3.0×10^5	1.5×10^2, Pt(II)[n]	0.32
	H/T (85)	3.1×10^6		6.05
16 (12)	H/T (85)		2.7×10^5[o]	
MeGuo[i](12)	H/T (85)	5.3×10^6	1.4×10^4, Cu(II)	2.5×10^{-3}
InoH[i](15c)	H/D (61)	1.3×10^8	1.4×10^3, Pt(II)	16.8
(12)	H/T (85)	1.6×10^7		
MeIno[I] (12)	H/T (85)	1.8×10^7	2.0×10^4, Cu(II)	1.9×10^{-2}
			4×10^2, Ag(I)	
AdeH[i] (12)	H/T (85)	3.0×10^4	4.0×10^2, Ag(I)	
5′-AMP[i] (12)	H/T (85)	8.6×10^6	8.2×10^3, Cu(II)	7.3×10^2
3′-AMP[i] (12)	H/T (85)	1.7×10^5	1.3×10^3, Ag(I)	3.2×10^2

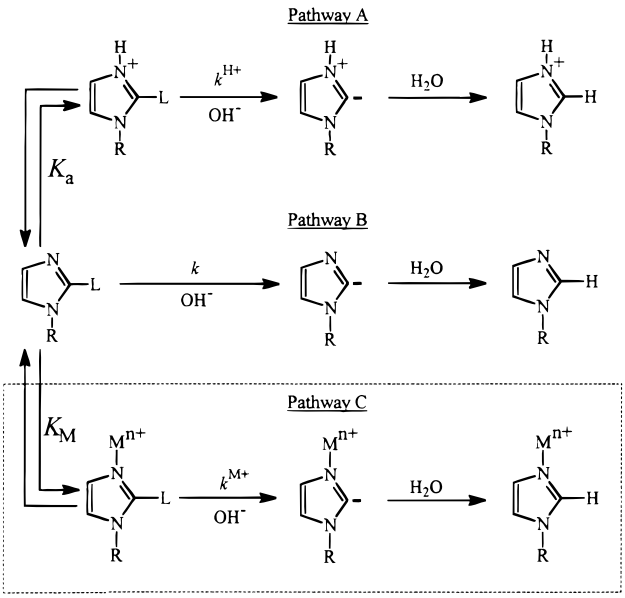
[a] The rate constants in this table are defined in Scheme 1. [b] Exchange at C(2) for imidazoles/histidines/thiazoles and at C(8) for purine derivatives. [c] Original data can be found in the references. [d] For H/D exchange, reactions were carried out in D_2O solutions, with pD = pH + 0.4. [e] Metal ions following k^{M^+} values were studied by addition of metal salts to solutions of the ligand (see text). [f] Exchange at C(4)/C(5) was also observed with $k^{M^+} = 7.1 \times 10^2$ M^{-1} s^{-1}. [g] Similar results were obtained for complex **10** and **11** (refs 30 and 31). [h] Exchange occurred at C(5) with $k^{M^+} = 1.8 \times 10^{-5}$ M^{-1} s^{-1}; in **11**, exchange was also observed at C(4) with $k^{M^+} = 1.0 \times 10^{-4}$ M^{-1} s^{-1} (see refs 30 and 31). [i] Abbreviations: MeGuO = 1-methylguanosine; InoH = inosine; MeIno = 1-methylinosine; AdeH = adenosine; 5′-AMP = 5′-adenosine monophosphate; 3′-AMP = 3′-adenosine monophosphate. [j] Calculated from data at pD 2.80 and T = 60.7 °C (see ref 17). [k] Similar results were obtained for complexes **14** and **15**. [l] Exchange was observed at C(5) with k^{M^+} = 3.69, 4.22, and 4.83 M^{-1} s^{-1} for **13**, **14**, and **15**, respectively; C(4)−H exchange also occurred in **15** with $k^{M^+} = 2.54$ M^{-1} s^{-1}. [m] This value of k contains a contribution from the exchange reaction of its zwitterionic form (see text). [n] Data for the effect of Pt(II) obtained at 61 °C (ref 15c). [o] This value pertains to N−T/N−H exchange rather than reaction at C(8) (see ref 31).

Isotopic Exchange in Uncomplexed Substrates: Mechanism, Rate Equations, and Ring Proton Reactivities

The generalized mechanism[12−16] for C(2)−H exchange in imidazoles and related substrates as well as C(8)−H in purines in aqueous buffer solutions is given in Scheme 1, which includes the various forms of the substrate present in solution.

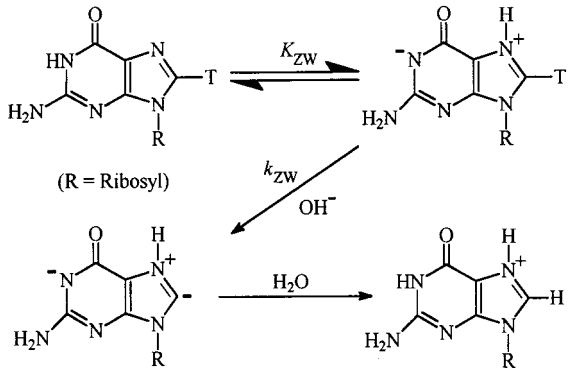
Rate-limiting attack by OH^- on the protonated substrate (pathway A) and the neutral substrate (pathway B) gives rise to intermediates which are rapidly reprotonated by solvent to yield the exchanged product. Pathway A is vastly superior to pathway B, k^{H^+}/k (the so-called *proton activating factor*) ≈ 10^7−10^{10}, due to resonance stabilization of the ylide intermediate in the former route. Pathway B in substrates with zwitterionic forms, e.g., histidines[12,13] and several purine derivatives,[12,15a,16b] incorporates exchange via the tautomeric form whose reactivity compares to that of the N(3)/N(7) protonated form in pathway A (Scheme 2). Consequently, the relevant second-order rate constant, k, is a composite quantity ($k = k_o + K_{zw}k_{zw}$); its magnitude is augmented by the additional term $K_{zw}k_{zw}$.[12,13a,15b,25a]

Scheme 1[a]



[a] L = H, D, or T; R = H, alkyl, or ribosyl.

Scheme 2



The kinetic expression which accommodates pathways A and B is given by eq 1, where SH_2^+ and SH refer to protonated and neutral forms of the substrate. Under first-

$$\text{rate} = k^{H^+}[SH_2^+][OH^-] + k[SH][OH^-] \qquad (1)$$

order conditions, eq 2 obtains, k_{obs} being the pseudo-first-order rate constant, while K_a and K_w represent the ionization constant for N(3)/N(7) protonation and the ionic product of water, respectively. Equation 2 can be

$$k_{obs} = \frac{k^{H^+}K_w + kK_a[OH^-]}{K_a + [H^+]} \qquad (2)$$

simplified for different pH regions to reflect the relative values of $[H^+]$, $[OH^-]$, and K_a.[10]

In principle, all three C-bound protons of imidazole-type nuclei can undergo isotopic exchange. The order C(2)−H ≫ C(5)−H > C(4)−H has been observed for neutral imidazoles largely due to the effects of the heteroatomic flanking of C(2)[10,11] and the *adjacent lone pair* (ALP) phenomenon.[11b] Protonation/alkylation of N(3) (path A, Scheme 1) substantially enhances the rate of C(2)−H abstraction due to resonance stabilization of the ylide intermediate. Olofson and co-workers[17] report similar rates for C(2)−H and C(5)−H and a nonexchanging C(4)−H for thiazole in its neutral and protonated forms, although C(2)−H exchanges 10^9−10^{10} times faster in the protonated substrate than in the neutral form. N(3)-alkylated thiazole exchanges only at C(2); ring cleavage competes with C(5)−H exchange, and this is very much faster than C(4)−H exchange.[14a]

A variety of rate−pH profiles providing definitive information regarding the identity of the exchanging species have been discussed; as well, the presence of unreactive species is revealed.[10−12,18] These rate profiles demonstrate exchange from protonated,[10−12] neutral,[12,15a,18] and anionic substrate forms with the negative charge remotely located from the exchanging site;[12,18] zwitterionic forms of purines and histidines;[12,13] and adenosine 5′-monophosphate[12] with ionizable acidic and/or basic side chains. Bell-shaped profiles provide evidence for unreactive pools of substrate forms in which a negative charge is located adjacent to the exchange site.[12,18]

Isotopic Hydrogen Exchange in Metal Ion−Biomolecule Complexes

(i) Exchange from Metal Ion−Substrate Complexes Derived in Situ in Solution. The impetus for the study of metal ion effects in isotopic hydrogen exchange in biomolecules came from the expectation that metal coordination at N(3)/N(7) of imidazole- and purine-type substrates would mimic the effect of the proton and induce large rate enhancements of C(2)−H/C(8)−H exchange.[12,13b,15b,19−21] The historical antecedents of CH_3Hg-(II) complexation of inosine and guanosine nucleosides at N(7)[22] and enhanced lability of C(8)−H in purine nucleosides and nucleotides following heavy metal ion complexation in D_2O[23] provided a basis for this hypothesis.

The effect of metal ions on H/D or H/T exchange in heterocycles has been studied in two ways:[12] (a) by adding excess metal ion salts to aqueous solutions of the substrates, and studying the exchange in situ, and (b) by synthesizing, isolating, and characterizing the metal ion−biomolecule complex followed by the exchange study.

The appropriate pH region for study of the effect of added metal ions is predetermined in H/D or H/T exchange experiments at a pH at which the substrate is completely protonated.[12] This ensures that the exchange routes, upon addition of metal ions, simplify to pathways A and C in Scheme 1, for which eq 3 is the relevant kinetic expression,[12,15] assuming that both protonated and metalated substrate forms undergo H/D or H/T exchange. A

$$k_{obs}^{M^+} = \frac{k^{H^+}K_M'K_w + k^{M^+}K_a[M^{n^+}][OH^-]}{K_M'[H^+] + K_aK_M' + K_a[M^{n^+}]} \qquad (3)$$

model in which the metalated substrate form is unreactive, i.e., $k^{M^+} = 0$ (Scheme 1), is conceivable[21] but is not pursued further since this assigns an inhibitory role to the metal ion in the exchange process. The quantity $K_M' = 1/K_M$, where K_M is the stability constant of the metal ion−biomolecule complex.

A number of studies[12,13,15a,20] have revealed that metal ions depress C(2)−H exchange rates, relative to the effect of the proton, in imidazole and its derivatives (Table 1). For example, first-order rates for the detritiation of C(2)−T in [2-^{3}H]imidazole at 85 °C and pH 5.70 are retarded by added metal ions according to the order Cu(II) > Zn(II) ∼ Ni(II) ≫ CH_3Hg(II).[15a] Similar observations have been made in our laboratory with 1-methylimidazole,[20,21] thiazole,[20,21] benzothiazole,[20,21] and 1-methylhistidine.[13] Without exception, the observed rates for the detritiation of 1-methyl[2-^{3}H]imidazole were decreased by added Ag(I), Cu(II), Pb(II), Co(II), Cd(II), Zn(II), Ni(II), and CH_3Hg-(II).[20,21] Values of k^{H^+}/k^{M^+}, comparing proton and metal ion activation, ranged from 10 for Cu(II) to 150 for CH_3-Hg(II). The detritiation rates of [2-^{3}H]thiazole were decreased by Ag(I) and Cu(II); Ag(I) was found to inhibit the detritiation of [2-^{3}H]benzothiazole. Values of k^{H^+}/k^{M^+} were evaluated as 3×10^2 for Cu(II) and 5×10^5 for Ag(I) in the case of thiazole and 3×10^5 for Ag(I) with benzothiazole.[20,21] For 1-methyl[2-^{3}H]histidine, k^{H^+}/k^{M^+} in the presence of CH_3Hg(II) was estimated[13] as $\sim 10^3$. The bis(1-methylhistidine) complex of Pd(II) prepared in situ exchanges C(2)−H 2.5×10^5-fold slower than the protonated ligand.[15c]

The effect of the added metal salts on the detritiation of a number of purine derivatives has been reported.[12] The detritiation rate of 1-methyl[8-^{3}H]inosine is strongly depressed by Cu(II) and Ag(I), $k^{H^+}/k^{M^+} = 8.2 \times 10^2$ and 4.5×10^4, respectively. Cu(II) strongly inhibits detritiation of 1-methyl[8-^{3}H]guanosine, with $k^{H^+}/k^{M^+} = 3.8 \times 10^2$. Pt(II) complexes of **16**−**19** were prepared in situ and were found to undergo C(8)−H/D exchange at rates by far slower than the free ligands under catalysis by H^+ ($k^{H^+}/k^{M^+} \approx 10^5$).[15b]

These studies, involving different substrates, reveal that the kinetic condition $k^{H^+} > k^{M^+}$ exists in Scheme 1. Data for metal ion effects on exchange in purines[12] show clearly that metalated biomolecules react faster than their neutral forms, k^{M^+}/k (Scheme 1), the so-called "metal activating factor" (maf) being of the order of 10^4−10^6.

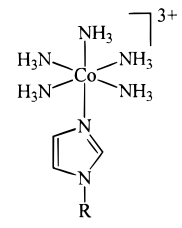
The approach employed in the studies discussed above is, however, fraught with a number of experimental problems. First, there is precipitation of metal ions at high pH.[12] Second, most of the metal ions involved form *kinetically labile* complexes in solution,[24] giving rise to concurrent multiple stoichiometric equilibria in solution. These invariably lead to unknown dissociation equilibria and complicated kinetics.[13,20]

(ii) Exchange from Presynthesized Metal Ion−Biomolecule Complexes. The difficulties pointed out above are avoided by the synthesis, isolation, and characterization of *substitution-inert*[24] complexes; exchange studies are then undertaken on the ligand portion of the isolated complexes under optimized experimental conditions, ensuring the identity of the reacting species. Typical transition metal ions studied by this method include Co(III),[19,25] Cr(III),[26−28] and Pt(II).[29−31] Pt(II)−biomolecule complexes are strategic in bioinorganic chemistry for their chemotherapeutic potential in cancer treatment;[1,3] we have recently reported[30−33] a number of new platinum complexes of biomolecules.

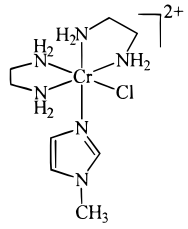
The exchange mechanism of these stable metal ion−biomolecule complexes simplifies to the boxed-in portion of Scheme 1, i.e., pathway C, to which the rate expression of eq 4 applies.

$$k_{obs}^{M^+} = k^{M^+}[OH^-] \qquad (4)$$

(a) Co(III)−Biomolecule Complexes. Qualitative evidence for inhibition by Co(III) in H/D exchange in **1a** and **1b** was provided by Rowan and co-workers.[19b] A detailed kinetic study of the detritiation of **5a** and **5b**, following treatment with HTO under standard conditions, was undertaken in our laboratory.[25a] Exchange was observed at C(2) only, to yield values of $k^{H^+}/k^{M^+} \geq 8 \times 10^2$ and $\geq 10^4$ for **5a** and **5b**, respectively. A value of $k^{M^+}/k \approx 10^3$ was calculated for both complexes, thus quantifying Co(III) activation relative to the reaction of the neutral substrate. A curve-fitting procedure suggested exchange at C(2) in N(1)-deprotonated **5a** with a rate 1/1000 that of the N(1)-protonated form;[25a] this could not be confirmed in a subsequent NMR study of H/D exchange in **5a**.[25b] The lack of measurable reactivity of N(1)-deprotonated **5a** is in accord with anticipated unfavorable electrostatic interactions in such a process. However, exchange from anionic species occurred in purines and related substrates in which the negative charge is remotely located from the exchange site.[12,15a]



5(a) R = H
(b) R = CH_3



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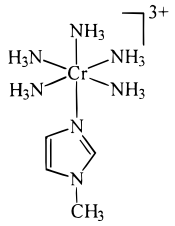

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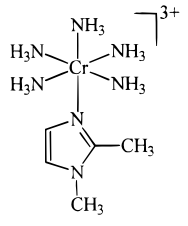

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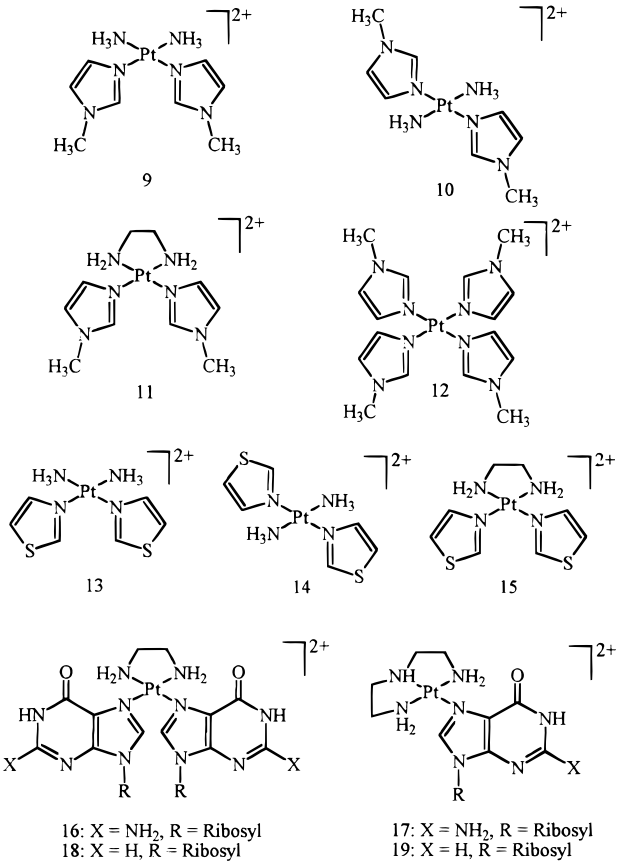
(b) Cr(III)−Biomolecule Complexes. A clear departure from the behavior of Co(III) was found in the detritiation of the Cr(III)−imidazole complexes **6**−**8** in aqueous buffer, pH range 3.8−6.5, at 35 °C following treatment with HTO.[26,27] Two parallel exchange ("fast" and "slow") processes were observed for these complexes. The "slow" process in all cases is C(4,5)−H exchange, hitherto reported in the free ligand only under extreme temperature and pH conditions.[10,11] The "fast" process is assigned to C(2)−T exchange in **6**, competing N−T and C(2)−T exchange in **7**, and N−T exchange in **8**.[26,27] Quantitation of the effect of Cr(III) coordination on H/T exchange was fully achieved in **6**, giving k^{H^+}/k^{M^+} and k^{M^+}/k values of 0.05 and $\sim 2 \times 10^7$, respectively.[26] The significant finding, reported for the first time,[26,28] is the 20-fold superior catalytic effect of Cr(III) over H^+ in C(2)−H exchange in imidazoles, in contrast to the inhibitory roles relative to H^+ catalysis by Co(III)[19,25] and other metal ions noted above. Comparing the effectiveness of Cr(III) and Co(III) in catalyzing C(2)−H exchange in imidazoles, it is noted that a 3×10^5-fold difference exists between the two transition metal ions, favoring the former.

(c) Pt(II)−Biomolecule Complexes. Isotopic hydrogen exchange studies in isolated complexes of Pt(II) with 1-methylimidazole[30,31] (**9**−**12**), thiazole[28,29] (**13**−**15**), and guanosine[12] (**16**) have been reported. Pt(II) forms d^8 square planar complexes, in contrast to the d^3 and d^6 octahedral *substitution-inert* complexes of Cr(III) and Co(III), respectively, described above. H/D exchange in **9**−**12** determined in D_2O/NaOD solutions[30,31] using ^1H NMR spectroscopy revealed that Pt(II) coordination at N(3) enhances C(2)−H exchange by a factor of ca. 10^2 relative to the neutral substrate; values of k^{M^+}/k and k^{H^+}/k^{M^+} of $\sim 8 \times 10^4$ and 5×10^2, respectively, were calculated. Exploiting the diagnostic utility of $^1H-^{195}Pt$ coupling unambiguously assigned ^1H chemical shifts of C(2)−H, C(4)−H, and C(5)−H;[30] measurable exchange at both C(4) and C(5) of **11** established the reactivity order C(2)−H ≫ C(5)−H > C(4)−H. The 5.9-fold faster exchange of C(5)−H relative to C(4)−H in **11** contrasts with expectations based on inductive, through-bond electron withdrawal by N(3)-coordinated Pt(II). A similar reactivity order in H/D exchange in the free ligand has been attributed to the ALP effect.[11b] This order of reactivity in Pt(II)−imidazole complexes invokes[30] through-bond propagation of the partial positive charge (δ^+) placed at N(3) by Pt(II) coordination to the more stable N(1) position, where the inductive/hyperconjugative effect of the CH_3 substituent is called into play.

Pt(II) activation of the thiazole ring toward C−H exchange in complexes **13**−**15** provided values of k^{H^+}/k^{M^+} and k^{M^+}/k of 4.5×10^3 and $\sim 10^6$, respectively, for H/D exchange at C(2). C(4)−H and C(5)−H exchange was also observed in **15**, with exchange at C(5) occurring ca. 2-fold faster than that at C(4) due to stabilization of the α-carbanion at C(5) by the adjacent S atom;[34] this effect is absent in the intermediate formed on proton abstraction at C(4). The observation of C(4)−H exchange in **15**



represents the first reported data for exchange at this site in a thiazole moiety.

Jones and Taylor[15b] measured H/T exchange in the isolated complex **16**; the value of $k^{M^+} = 2.7 \times 10^5$ M^{-1} s^{-1} obtained for exchange at C(8) is only 2.2-fold smaller than the rate constant obtained for CH_3^+ activation in 7-methyl-[8-^{3}H]guanosine.[12] A subsequent H/D study[15c] of in situ-generated **16** afforded k^{M^+} values which are $10^{4.3}$ times smaller than the value reported by Jones and co-workers,[15b] after correcting for temperature, solvent, and substrate isotope effects. The two studies[15b,c] can be reconciled on the basis that the rates reported by Jones and co-workers apply to N−T/N−H exchange of the ethylenediamine moiety of the complex rather than exchange at C(8), to which the value $k^{M^+} = 1.46 \times 10^{-2}$ M^{-1} s^{-1} was assigned;[31] hence, for **16** and similar complexes, the condition $k^{H^+} > k^{M^+}$ also holds.

Proton versus Metal Ion Activation

It is clear from Table 1 that metal ion coordination enhances the exchange reactions relative to the neutral substrates. In general, the protonated substrate exchanges faster than the metalated form, i.e., $k^{H^+} > k^{M^+}$, the only apparent exception being the case of Cr(III), which is 20 times more effective than H^+ in catalyzing H/T exchange in 1-methylimidazole.[26,27] The superiority of pathway A over pathway B in Scheme 1 has been discussed extensively[10−16,18−21] and is attributed to (i) substantial acidification of C(2)−H/C(8)−H by N(3)/N(7) protonation or methylation, and (ii) the resonance stabilization of the ylide intermediate generated in pathway A on proton abstraction. Metalation of the substrate also acidifies C(2)−H/C(8)−H relative to the neutral form. In addition, the carbenoid intermediate generated in pathway C is stabilized by the inductive/field effect of the metal through M^{n+}−N(3)/N(7) σ bond polarization,[25] establishing the order $k^{M^+} > k$ (Scheme 1).

The generally observed order of $k^{H^+} \gg k^{M^+}$ in H/D and H/T exchange in these systems has been ascribed[10,13,25] to the much higher fractional positive charge (δ^+) located on N(3)/N(7) by H^+ or CH_3^+ relative to metal ions, on the basis of the simple electrostatic model proposed by Norris, Buncel, and Taylor.[35] Data in Table 1 already reveal a large diversity in the properties of the mediating metal ions. The relative reactivities of coordinated metal ions therefore conceivably result from a complex interaction of several factors rather than simply the magnitude of the fractional charge on N(3)/N(7). The order of metal ion catalytic effectiveness of Cr(III) > H^+(CH_3+) ≫ CH_3Hg(II) > Co(III) ≥ Pt(II) which is evident from the data in Table 1 for C(2)−H exchange in 1-methylimidazole[10,13,25,27,30] can now form a basis for examining the factors that determine metal ion reactivities.

Cr(III) Versus Co(III) Activation: A Framework for Understanding Metal Ion Effects in Biological Systems

The highlight of the study of metal ion effects in C(2)−H exchange in 1-methylimidazole, with particular reference to Cr(III)[26,27] and Co(III),[25] is the emergence of the reactivity order Cr(III) > H^+ ≫ Co(III), which extrapolates to the reactivity ratio of 20:1:6 × 10^{-5} for Cr(III), H^+, and Co(III), respectively. This significant difference in the catalytic effects of Cr(III) and Co(III) in C(2)−H exchange in 1-methylimidazole is reminiscent of activation/inactivation at enzyme active sites consequent upon changes in metal ion identity, at constant ionic size and electrical charge. We summarize below a number of factors that could account for the disparity in the catalytic effects of these two metal ions.

(i) Magnitude of Fractional Positive Charge at N(3) of Substrate. For the electrophiles CH_3^+ (a good model for H^+), CH_3Hg(II), and $Co(NH_3)_5^{3+}$, Norris et al.[35] have shown that the magnitude of the fractional positive charge at N(3) of imidazole, which correlates with C(2)−H acidity, follows the order CH_3^+(H^+) > CH_3Hg(II) ∼ Co(III). NMR chemical shift differences ($\Delta\delta$) in electrophile-coordinated imidazoles and the pK_a values for the deprotonation of aquo and mixed aquo/ammine[36] metal complexes lead to the order of electrophile−N(3) σ bond polarization H^+ > Cr(III) > Co(III) ∼ CH_3Hg(II). Evidently, other factors must be considered in order to explain the significant difference in the catalytic effects of Cr(III) and Co(III), even though their acidifying effects on heterocycles differ only marginally, favoring Cr(III).

(ii) Electronic Structure of Metal Ions and Differences in Crystal Field Stabilization Energy (CFSE). According to crystal field theory (CFT) of bonding, the d^3 and d^6

structures for Cr(III) and Co(III), respectively, generate different energetic requirements and consequences in their complexes with ligands, including reactivity differences.[24,37] Cations with large crystal field stabilization energies (CFSE) have correspondingly high crystal field activation energies (CFAE), defined by eq 5 for substitution and dissociative processes.[37] In the dissociative process

$$\text{CFAE} = \text{CFSE(transition state)} - \text{CFSE (initial state)} \quad (5)$$

of H/D exchange in Cr(III) and Co(III) ammine complexes, the d^6 system suffers greater loss in CFSE relative to the d^3 system due to repulsive interactions between the amido p-electrons and nonbonding d-electrons in the transition state.[37] CFSE in the low-spin d^6 system of Co(III) is double that of the d^3 configuration of Cr(III), 2.4Δ versus 1.2Δ. This qualitative picture can conveniently be applied to the H/D and H/T exchange reactions of Cr(III) and Co(III) imidazole complexes; the rate-limiting transition state for H/T exchange is shown as eq 6, X depicting the ligand fragment from which tritium is abstracted. Charge devel-

$$\text{MX}-\text{T} + \text{OH}^- \rightarrow [\text{MX}^{\delta-}\cdots\text{T}\cdots\text{OH}^{\delta-}]^{\ddagger} \rightarrow \text{MX}^- + \text{T}-\text{OH} \quad (6)$$

opment at the ligand fragment carbon site introduces repulsive interactions between the incipient carbanionic site p-electrons and the nonbonding d-electrons of the metal ion, an effect which will be more important for Co(III) than Cr(III). Conceivably, the Co(III)/Cr(III)−imidazole system will show by far greater sensitivity to this phenomenon than the corresponding ammine system since proton transfers from carbon centers are energetically more demanding than transfers from electronegative atoms such as N.[38]

(iii) Metal-to-Ligand π Back-Bonding. Cr(III) and Co(III) have different capacities for π-bonding.[24,37] The d^3 configuration of Cr(III) is mainly σ-withdrawing and shows little tendency to π back-bonding,[29,37] whereas the low-spin d^6 structure of Co(III) predisposes it to $\sigma + \pi$-(covalent) interactions so that it can engage in π-bonding with electron acceptor ligands,[27,29,37] such as imidazole-type compounds. This effect predicts a lower intrinsic acidity of the ring hydrogens as well as an enhanced barrier for OH^- attack on the ring due to accentuated ring electron density for Co(III)-bound imidazoles relative to their Cr(III) counterparts.

If, in the imidazole system, factors i−iii above act in favor of Cr(III), as is apparently the case, the large difference in the catalytic abilities of Cr(III) and Co(III) toward proton exchange in imidazoles can then be adequately accounted for.

Moreover, the confluence of factors i−iii above in promoting the efficacy of Cr(III) catalysis apparently also leads to the catalytic order Cr(III) > H^+ for C(2)−H isotopic exchange in the imidazole system.

Concluding Remarks

Isotopic hydrogen exchange in molecules of biological significance is catalyzed by metal ions. Quantitative estimates of the catalytic effects of metal ions in this process show metalated biomolecules reacting 10^1-10^7 times faster than their neutral forms. Larger rate enhancements, up to 10^{10}-fold over the neutral substrates, are generally recorded by H^+ or CH_3^+ coordination at N(3)/N(7) of these biomolecules. The conventional order of reactivity, protonated ≫ metalated ≫ neutral form of substrates, is excepted in the case of Cr(III) catalysis, which mediates C(2)−T exchange in 1-methylimidazole 20 and 3×10^5 times faster than H^+ and Co(III), respectively. In addition to the magnitude of the fractional charge located at N(3)/N(7) of the biomolecule through metal−N(3)/N(7) σ bond polarization, we believe that other important factors such as the electronic configuration of the metal ion, differences in CFSE and CFAE between one metal ion and another, and π back-bonding effects are critical to an understanding of the magnitude and direction of metal ion reactivities in these systems. This realization consequently emphasizes a holistic approach in considering and interpreting the biochemistry of metal ions.

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