Contribution from the Department of Chemistry,
The University of Akron, Akron, Ohio 44325

Inhibition of Phosphoryl-Transferring Enzymes by Aluminum-ATP

Norbert C. Furumo† and Ronald E. Viola*

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The effect of trivalent metal ions has been examined for a group of enzymes that catalyze phosphoryl-transfer reactions utilizing ATP as the phosphoryl donor. Hexokinase and glycerokinase were found to be extremely sensitive to inhibition by aluminum-ATP, with inhibition constants in the low micromolar range at pH 7. In contrast, for all of the other kinases examined, aluminum was either a relatively weak inhibitor (K_i = 150-750 μM) or a noninhibitor. The specificity of aluminum-ATP inhibition has been correlated with the structural isomer specificity of the enzymes that have been examined. Kinases that show a preference for the Λ β,γ-bidentate isomer of the metal-ATP complex are strongly inhibited by aluminum. The lanthanide complexes of ADP and ATP were also found to be strong inhibitors of several phosphoryl-transferring enzymes that were examined. Inhibition constants ranged from 10^{-4} M for europium-ATP with creatine kinase to 10^{-9} M for leutetium-ADP with creatine kinase.

Introduction

Aluminum has been proposed to be a causative agent in a wide range of diseases. The uptake, excretion, and distribution of aluminum in tissues and other general characteristics of aluminum metabolism have been examined.[2] Numerous studies have established a fairly strong cause and effect relationship for aluminum in dialysis encephalopathy[3,4] and in certain bone disorders.[5,6] The possible role of aluminum has also been examined in disorders such as amyotrophic lateral sclerosis, Parkinson's disease,[7] and Alzheimer's disease.[8,9] While evidence has been accumulating that suggests that aluminum may be involved in the etiology of these disorders,[10,11] there certainly has not been universal acceptance of these hypotheses. There has been a vast literature in recent years reporting the possible involvement of aluminum in these disorders. There have, however, been relatively few reports that have examined the possible effects of aluminum at the molecular level. Studies at this level have been impeded by the effects of pH and temperature on the complex solution equilibria of aluminum, making it difficult to determine which of a range of possible species is responsible for the observed effects.[12]

Previous work has shown that aluminum ion is a potent inhibitor of the Mg-ATP-requiring enzyme hexokinase from yeast and mammalian brain.[13-16] Womack and Colowick first related the biphasic kinetic behavior of hexokinase to the presence of contaminating aluminum in commercial preparations of ATP. We have previously shown[15] that the Al-ATP complex is a time-dependent, competitive inhibitor vs Mg-ATP for the nucleotide binding site of yeast hexokinase.

One of the questions to be addressed by this study is whether the strong inhibition of hexokinase by aluminum-ATP that has previously been observed is representative of the class of enzymes that utilize ATP as a phosphoryl donor. Here we present the results of inhibition studies of a wide range of phosphoryl-transferring enzymes by aluminum and lanthanide metal-ATP and -ADP complexes.

Experimental Section

Materials. All enzymes and cofactors were purchased from Sigma Chemical Co. and used without further purification. Low metal-ATP was purchased from Calbiochem and, if necessary, was further purified by the method of Schloss et al.[17] Stock solution of Al^{3+} were prepared from $AlCl_3 \cdot 6H_2O$, Puratronic from Alfa Products.

Enzyme Assays. All assays were performed at pH 7.0 and 30 °C by monitoring the change in absorbance at 340 nm of the pyruvate kinase-lactate dehydrogenase-coupled reaction with a Perkin-Elmer Lambda-1 spectrophotometer. Cuvette temperature was regulated to ±1 °C by a circulating water bath. The assay mixture contained 50 mM NaPIPES[1] (pH 7.0), 1.0 mM magnesium acetate, 0.3 mM NADH, 0.4 mM phosphoenolpyruvate, 6-20 units of LDH, 14-50 units of PK, and approximately 10 K_m of the substrate other than ATP. The concentrations of PK and LDH in the assay were in sufficient excess to ensure that the coupling reaction was not rate limiting. The reaction volume was 3.0 mL. Inhibition studies were carried out at levels of ATP above and below the K_m of ATP for that particular enzyme and at aluminum concentrations ranging from 0.1 to 800 μM, depending on the sensitivity of the enzyme. The reaction was initiated by the addition of 0.1-0.5 units of the enzyme under investigation.

Assays in the reverse direction (ADP → ATP) employed the hexokinase-glucose 6-phosphate dehydrogenase couple. The reaction mix contained 50 mM PIPES (pH 7.0), 2.0 mM glucose, 0.4 mM NADP, 1.0-5.0 mM magnesium acetate, 2 units of HK, 5 units of glucose 6-phosphate dehydrogenase, and 10 times the K_m of the other substrate. For the examination of the hexokinase-catalyzed reaction, a coupled reaction consisting of peroxidase (10 units), glucose oxidase (10 units), glucose 6-phosphate (20 mM), and *o*-dianisidine (0.3 mM) at pH 7.0 was followed at 450 nm.

Metal-nucleotide dissociation constants were determined, for the trivalent metal ions of interest, by the procedure of Morrison and Cleland.[19] Trivalent metal-ATP dissociation constants were determined by using either hexokinase or creatine kinase, while the ADP dissociation constants were assessed by using the glycerokinase-catalyzed reaction. The levels of total metal ions were maintained to ensure a negligible concentration of free adenine nucleotide. The ratio of divalent (Mg^{2+}) to trivalent (Al^{3+} or lanthanide) metal ion was adjusted to allow Mg^{2+} to compete with the trivalent metal ion for binding to ADP or ATP. The concentration of trivalent metal ion was always considerably lower than that of Mg^{2+} and would therefore not deplete the nominal concentration of Mg^{2+}-nucleotide. Under these conditions the levels of the inhibitor (M^{3+}-nucleotide) will vary as a function of the Mg^{2+} concentration. This causes concave reciprocal plots ($1/v$ vs $1/[Mg\text{-}ATP]$), with the degree of curvature related to the relative K_d values for Mg^{2+}-nucleotide and M^{3+}-nucleotide.

Data Analysis. All data were fitted by the computer programs of Cleland,[18] translated to BASIC and adapted for use on a microcomputer,

(1) Abbreviations: HK, hexokinase; GK, glycerokinase; CPK, creatine kinase; MK, myokinase; PFK, phosphofructokinase; PK, pyruvate kinase; LDH, lactate dehydrogenase, PIPES, piperazine-*N*,*N*'-bis(2-ethanesulfonic acid).

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† Current address: Department of Biochemistry, University of California, Berkeley, CA 94720.

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Table I. Dissociation Constants of Trivalent Metal-ATP and -ADP Complexes

metal ion	K_d, μM	conditions[a] pH	conditions[a] enzyme assay
	ATP		
Mg	52[b]	7.0	HK
Gd	0.087 ± 0.019[a]	8.0	HK
Eu	0.16 ± 0.04[a]	8.0	HK
Nd	0.48 ± 0.38	7.0	CPK
Al	0.73 ± 0.22[b]	7.0	HK
Lu	2.51 ± 0.02	7.0	CPK
	ADP		
La	0.71 ± 0.39	7.0	GK
Eu	1.2 ± 0.6	7.0	GK
Lu	7.9 ± 4.1	7.0	GK

[a] From Morrison and Cleland.[19] [b] From Viola et al.[15]

which assume equal variance for the velocities of the fitted parameter. Reciprocal plots, with the fixed substrate saturating, were fit to eq 1,

$$v = \frac{VA}{K + A} \tag{1}$$

$$v = \frac{VA}{K_a(1 + I/K_{is}) + A} \tag{2}$$

where v is the experimentally determined velocity, V is the maximum velocity, A is the variable substrate concentration, and K is the Michaelis constant. Competitive inhibition data were fit to eq 2, where K_{is} is the slope inhibition constant.

The dissociation constants of lanthanide-ATP and -ADP complexes were determined by the kinetic method of Morrison and Cleland[19] by fitting data to eq 3 with a computer program transcribed from Fortran to BASIC.

$$\log y = \frac{\log (V(b + 2c/K + (b^2 - 4ac)^{1/2})}{(aK + b + c/K)/2} \tag{3}$$

$$a = (1 + I/K_i)(1 + (S + I)/K_i) + K_d(R/K_i)$$

$$b = S(1 + (S + I)/K_i) - K_dR(1 - (S + I)/K_i)$$

$$c = -K_dR(S + I)$$

$K = K_m$ for Mg-ATP (Mg-ADP)

S = concentration of Mg-ATP (Mg-ADP)

I = concentration of Ln-ATP (Ln-ADP)

K_d = estimated dissociation constant of Ln-ATP (Ln-ADP)

R = [free Mg]$/K_d$ for Mg-ATP (Mg-ADP)

Results

Trivalent Metal Ion Dissociation Constants. Before the effect of trivalent metal ions on enzyme-catalyzed phosphoryl-transfer reactions can be assessed, it is necessary to establish the affinity of these metal ions for the adenine nucleotide substrates under the assay conditions. Metal-nucleotide dissociation constants are reported in Table I for several lanthanide ions binding to ATP and ADP. Also included in Table I are dissociation constants for M^{3+}-ATP complexes that had been previously determined by the kinetic technique of Morrison and Cleland.[19] The affinity of ATP for these trivalent metal ions ranges from a factor of 20 to multihundred-fold greater than that for divalent magnesium ion.

Aluminum Inhibition of Kinases. The effect of added aluminum on the kinetics of phosphoryl-transfer was examined for a wide range of kinases. The inhibition experiments were designed, on the basis of the dissociation constants of the aluminum-nucleotide complexes determined above, so that all of the aluminum is present as the nucleotide complex. This is to ensure that inhibition is due to the aluminum-nucleotide complex and not to the interaction of free metal ion with the protein. The linear competitive inhibition observed by Al-ATP for the enzymes listed in Table II can be divided into three groups on the basis of the degree of inhibition. Group 1 consists of hexokinase and glycerokinase, enzymes that are strongly inhibited by Al-ATP, with K_i values in the micromolar to submicromolar range. Group 2 consists of enzymes that are moderately inhibited by Al-ATP, with K_i values in the range of several hundred micromolar. Group 3 consists of either enzymes that are very weakly inhibited, with K_i values in the millimolar range, or enzymes that are not inhibited by Al-ATP.

Table II. Inhibition of Kinases by Aluminum-ATP

enzyme	K_i(Al-ATP), μm	K_m(Mg-ATP), mM	isomer[a]
	Group 1		
hexokinase (yeast)	0.32 ± 0.06[b]	0.13 ± 0.02	Λ
glycerokinase (bacterial)	1.00 ± 0.15	0.023 ± 0.002	Λ
	Group 2		
creatine kinase (muscle)	[c]	0.096 ± 0.029	[d]
myokinase (muscle)	160 ± 33	0.010 ± 0.004	Δ
acetate kinase (bacterial)	176 ± 89	0.018 ± 0.006	
galactokinase (yeast)	524 ± 117	0.024 ± 0.003	
glucokinase (bacterial)	555 ± 120	0.027 ± 0.015	
arginine kinase (lobster)	730 ± 197	0.093 ± 0.031	[d]
	Group 3		
gluconate kinase	>750		
phosphofructokinase	>750	0.021 ± 0.011	Δ
phosphoribulokinase	>750	0.239 ± 0.055	
choline kinase	>750	0.215 ± 0.032	
3-phosphoglycerate kinase	>750	0.261 ± 0.083	
guanylate kinase	>750	0.147 ± 0.015	
nucleotide monophosphokinase	>750		
nucleotide diphosphokinase	>750	0.119 ± 0.001	

[a] Geometry of the bidentate metal-ATP complex.[20] [b] From Viola et al.[15] [c] Aluminum-ATP is a slow substrate for this enzyme.[32] [d] These enzymes have been shown to utilize a tridentate metal-ATP complex as the nucleotide substrate.

Also listed in Table II are the Michaelis constants for Mg-ATP that were determined for each enzyme. When the geometric requirement of a particular enzyme system for the metal-ATP complex has been established, that information is listed. The geometric requirement refers to the screw sense isomer formed by the binding of metal ion to the nucleotide phosphates. If the resulting complex forms a right-handed helix about an axis through the metal ion perpendicular to the chelate ring, then the complex has been designated Δ, while the left-handed screw sense isomer is called Λ.[21] It appears that enzymes that prefer the Λ bidentate isomer of M^{2+}-ATP, hexokinase and glycerokinase, are strongly inhibited by aluminum, while those enzymes that have a preference for the Δ isomer are only weakly inhibited. To test this hypothesis, glutamine synthetase was examined for its sensitivity to aluminum ion inhibition. The enzyme purified from *Escherichia coli* was also strongly inhibited by aluminum, with a K_i value of about 2 μM. In contrast, glutamine synthetase from sheep brain was very insensitive to aluminum ion inhibition.

Lanthanide Inhibition of Kinases. To further assess the role of aluminum in the inhibition of phosphoryl-transferring enzymes, the effects of other trivalent metal ions have been examined for selected kinases. Previous work has shown that yeast hexokinase is inhibited by trivalent lanthanide ions, with the degree of inhibition dependent on the ionic radius of the metal ion.[15] The present study has been expanded to examine the inhibitory effects of lanthanides with small (lutetium, ytterbium), intermediate (europium, neodymium), and large (lanthanum) atomic radii on the phosphoryl-transferring enzymes that were most strongly inhibited by aluminum. All of the lanthanide-ATP complexes examined were very good inhibitors of creatine kinase, myokinase, and glycerokinase (Table III), with K_i values in the micromolar

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Table III. Inhibition Constants for Trivalent Metal–ATP Complexes

metal	ionic radius, Å[a]	K_i for M^{3+}–ATP, μM HK[b]	CPK	MK	GK
Al	0.535	0.32 ± 0.06	240 ± 81	160 ± 33	1.00 ± 0.15
Lu	0.861	0.84 ± 0.36	0.96 ± 0.13	1.1 ± 0.3	4.0 ± 1.4
Yb	0.868	0.84 ± 0.22	17.1 ± 3.7	nd	nd
Eu	0.947	36 ± 4	100 ± 24	35 ± 16	3.4 ± 0.8
Nd	0.983	105 ± 4	51 ± 15	43 ± 23	nd
La	1.032	nd	nd	nd	3.8 ± 0.4

[a] From Shannon.[22] Ionic radius of Mg^{2+} is 0.72 Å. [b] From Viola et al.[15]

Table IV. Inhibition Constants for Trivalent Metal–ADP Complexes

metal	K_i for M^{3+}–ADP, μM CPK	PK	GK	HK
Al		349 ± 107	28 ± 4	7.8 ± 2.4
Lu	0.012 ± 0.004	9.4 ± 2.5	1.4 ± 0.4	0.77 ± 0.29
Eu	0.11 ± 0.03	20 ± 4	20 ± 2	
La	0.34 ± 0.08	20 ± 4	39 ± 5	

range. The lutetium–ATP complex was generally the strongest inhibitor, with a K_i value near 1 μM for each of the enzyme systems examined. Lutetium–ADP was also a potent inhibitor of the reverse reaction catalyzed by phosphoryl-transferring enzymes, with K_i values ranging from 9 μM for pyruvate kinase down to 12 nM for creatine kinase (Table IV).

Discussion

Rather than being the rule, the potent inhibition of hexokinase and glycerokinase by Al–ATP that was previously observed[15] appears to be the exception when the sensitivity of kinases to aluminum inhibition is examined. The results of this study indicate that aluminum is either a noninhibitor or a fairly weak inhibitor of most of the phosphoryl-transferring enzymes tested. Surprisingly, Al–ATP was observed to be a slow substrate for creatine kinase.[31] There are several general conclusions that can be drawn concerning the interactions of aluminum–ATP with this group of enzymes (Table II): There is a general trend in which the enzymes that have a high affinity for the Mg–ATP substrate, as measured by the Michaelis constant,[23] are inhibited by Al–ATP. However, there are two notable exceptions to this general observation. Hexokinase has a relatively high K_m for Mg–ATP, in line with those of enzymes in group 3, yet this enzyme is strongly inhibited by Al–ATP. On the other hand, PFK has a lower K_m for Mg–ATP, similar to those of the group 1 and 2 enzymes, but it is not inhibited by Al–ATP.

A better criterion for determining whether Al–ATP will be a potent inhibitor can be obtained by observing the preference of these enzymes for the screw-sense isomer of the metal–ATP substrate. Hexokinase and glycerokinase are the only enzymes listed in Table II that have been definitively shown, by an examination of the binding preference for inert metal–nucleotide complexes, to prefer the Λ bidentate screw-sense isomer.[21] These enzymes are both strongly inhibited by Al–ATP (Table II). Glutamine synthetase from *E. coli*, which utilizes Mg–ATP to activate the γ-carboxyl group of glutamate, has been shown to bind both the Δ and Λ β,γ-bidentate isomers of Cr–ATP, but only the Λ isomer causes irreversible inactivation of the enzyme.[24] An examination of the effect of aluminum on glutamine synthetase from *E. coli* showed the enzyme to be strongly inhibited by Al–ATP, with a K_i value in the low micromolar range. Glutamine synthetases isolated from mammalian brain have considerable structural differences from the *E. coli* enzyme.[25] In agreement with an earlier observation,[26] the sheep brain form of this enzyme was found not to be inhibited by aluminum ions. These results suggest a significant difference between the metal–nucleotide isomer specificity of *E. coli* and sheep brain enzymes that can be probed by Al–ATP.

These results lend support to the hypothesis that it is the Λ bidentate isomer of Al–ATP that is the inhibitory form of this trivalent metal–ATP complex. NMR studies have shown that the complex of Al^{3+} with ATP exists in solution primarily as a β,γ-bidentate chelate at low pH.[27] In the neutral-pH range evidence has been presented to support base stacking, and possibly even Al-bridging between nucleotides, with the β,γ-bidentate chelation at the phosphates preserved. The nucleotide binding sites on kinases are not expected to be able to accommodate binding of these stacked complexes. Selective binding of the monomeric bidentate form of the complex will, however, shift the equilibrium in solution and allow enzyme inhibition by Al–ATP at neutral pH.

The metal–nucleotide specificity of several phosphoryl-transferring enzymes has also been examined by using thiophosphate analogues of ADP and ATP. This approach has exploited the change in coordination specificity between Mg^{2+}, which has a preference for oxygen coordination, and Cd^{2+}, which prefers sulfur coordination.[28] On the basis of a decrease in isomer specificity for ATPβS in the presence of Cd^{2+}, Romaniuk and Eckstein[29] proposed a Λ bidentate isomer specificity for acetate kinase from *E. coli*. However, the generally accepted test for isomer specificity, reversal of specificity with a change in metal ion, was not observed. Acetate kinase was observed to be only weakly inhibited by Al–ATP (Table II), suggesting the need for additional studies to definitively assign isomer specificity for this enzyme. Mammalian glucokinase was determined to utilize the Λ bidentate isomer of the metal nucleotide substrate, on the basis of a reversal of the stereospecificity for ATPβS.[30] The substrate specificity of bacterial glucokinases has not been established; however, glucokinase from *Bacillus stearothermophilus* was only weakly inhibited by Al–ATP.

Other trivalent metal ions were also found to be inhibitors of this group of phosphoryl-transferring enzymes. In fact, in contrast to the selectivity observed by aluminum–nucleotides, the lanthanide nucleotides are potent inhibitors of all of the enzymes that have been examined. The enzyme hexokinase has been previously been observed to show a well-correlated size dependence on lanthanide ion inhibition,[15] with trivalent ions below an ionic radius of 0.9 Å showing strong inhibition of yeast hexokinase. For the other kinases that were examined in this paper, the relationship between the ionic radius of the lanthanide and the magnitude of the inhibition is less well correlated, but the same general trend was observed with creatine kinase and myokinase. Lutetium, the smallest lanthanide ion examined with an ionic radius of 0.86 Å, is a potent inhibitor of both enzymes, with K_i values 150- to 250-fold tighter than that of aluminum. Europium and neodymium, by contrast, with ionic radii of 0.95–0.98 Å, are only a factor of 2–4 more potent than aluminum. However, glycerokinase shows no ionic radius dependence on inhibition. All of the lanthanides examined were strong inhibitors of this enzyme system, with K_i values comparable to that of aluminum. There are clearly some differences in the manner in which certain groups of kinases interact with their metal–nucleotide substrates that remain to be elucidated.

For the enzyme systems that have been examined, the lanthanide complexes with ADP and ATP are more potent inhibitors than the corresponding aluminum complexes for HK, CPK, and MK. Because all of the lanthanides have the same charge as aluminum (+3), and are good inhibitors of all the enzymes tested, there must be some additional factors that are important in the ability of this group of metal ions to inhibit kinases. Differences

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between the lanthanides and aluminum that could offer explanations for their higher enzyme affinity include the possible involvement of the f electrons of the lanthanide ions in binding or the higher ligand exchange rates of the lanthanides (10^7–10^8 s^{-1} for water) as compared to that of aluminum (10 s^{-1} for water).[31] Higher exchange rates would permit the lanthanide complexes to undergo rapid coordination isomerization during binding to the enzyme active site and during the conformational changes that are required throughout a catalytic cycle.

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Contribution from the Laboratoire de Chimie des Organométalliques associé au CNRS (URA 415), Université de Rennes I, 35042 Rennes Cedex, France

Synthesis and Proton NMR Studies of the Electronic and Magnetic Properties of Low-Spin Ferric Isocyanide Tetraphenylporphyrin Complexes

G. Simonneaux,* F. Hindre, and M. Le Plouzennec

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The preparation and spectral properties of new low-spin ferric isocyanide complexes of tetraphenylporphyrin have been reported. The increase of the CN stretching frequency in IR spectra indicates a higher bond order in the complex than in the free ligand. The proton NMR spectrum of Fe(TPP)(*t*-BuNC)$_2$ClO$_4$ has been analyzed. The hyperfine shifts have been separated into their dipolar and contact contributions. The separated components reflect the very low magnetic anisotropy of the iron, and the unusual orientation of the unpaired spin density when the nitrogen axial ligands are exchanged for isocyanide ligands leads to complete reverse localization.

The relationship between metalloporphyrin stereochemistry and the biochemical functions of hemoproteins has been the subject of extensive investigations. Previous reports from this laboratory have been directed toward systematic examination of new probes of hemoglobins.[1-3] Studies of acyl isocyanide[4] and phosphine[5,6] binding to metalloporphyrins have been of particular interest in exploring the structure of the binding site in natural hemoproteins. We now want to describe the preparation of new low-spin ferric porphyrin isocyanide complexes and their studies by various spectroscopic methods (IR and ^{1}H NMR). A quantitative separation of the dipolar and contact contributions to the hyperfine shifts for Fe(TPP)(*t*-BuNC)$_2$ClO$_4$ shows that isocyanide ligand greatly decreases the magnetic anisotropy of the iron as compared with the effect of the cyanide ligand.[7]

Experimental Section

The following iron porphyrins[8] were prepared by literature methods: Fe(TPP)ClO$_4$,[9] Fe[T(*m*-Me)PP]ClO$_4$,[10] Fe[T(*p*-Me)PP]ClO$_4$,[10] and Fe[T(*o*-Me)PP]ClO$_4$.[10]

tert-Butyl isocyanide, methyl isocyanide, and *o*-dimethylphenyl isocyanide are commercially available (Fluka AG).

Caution! We have not observed detonation of iron porphyrin perchlorates under our conditions, but care is urged.

Fe(TPP)(*t*-BuNC)$_2$ClO$_4$·CH$_2$Cl$_2$. Addition of an excess of *tert*-butyl isocyanide (8 equiv) in toluene (5 mL) to 200 mg (0.26 mM) of Fe(TPP)ClO$_4$ in toluene (60 mL) at 25 °C under argon results in rapid formation of Fe(TPP)(*t*-BuNC)$_2$ClO$_4$. The solution was set aside overnight for crystallization. Fine crystals were collected by filtration and washed with toluene. Recrystallization was achieved by dissolving the product in a minimum amount of CH_2Cl_2 (10 mL) and adding hexane. The yield of CH_2Cl_2 solvate was 0.22 g (81%). Anal. Calcd for $C_{56}H_{48}O_4N_6Cl_3Fe$: C, 64.48; H, 4.74; N, 8.24. Found: C, 64.54; H, 4.75; N, 8.52. UV-vis (λ_{max}, nm (ϵ, mM^{-1} cm^{-1}); toluene): 417 (122), 506 (12), 573 (7.8).

Fe(TPP)(CNR)$_2$ClO$_4$ (R = Me, *o*-(CH$_3$)$_2$C$_6$H$_3$) was prepared similarly. Other Fe(TPP)(CNR)$_2$ClO$_4$ complexes also gave correct analyses. The *tert*-butyl isocyanide derivatives of Fe[T(*m*-Me)PP]ClO$_4$, Fe[T(*p*-Me)PP]ClO$_4$, and Fe[T(*o*-Me)PP]ClO$_4$ were prepared as described above. The products were not recrystallized but were characterized by ^{1}H NMR, IR, and UV-visible spectroscopy. UV-vis (λ_{max}, nm; toluene): Fe(TPP)(CN-*o*-(Me)$_2$C$_6$H$_3$)$_2$ClO$_4$, 418, 505, 569; Fe(TPP)(CN-CH$_3$)$_2$ClO$_4$, 418, 507, 572; Fe[T(*m*-Me)PP](*t*-BuNC)$_2$ClO$_4$, 417, 508, 571; Fe[T(*p*-Me)PP](*t*-BuNC)$_2$ClO$_4$, 416, 508, 572.

The synthesis of Fe(TPP)(*t*-BuNC)$_2$ has been previously reported by Jameson and Ibers.[11] Other Fe(P)(CNR)$_2$ compounds were prepared similarly (P = T(*m*-Me)PP and T(*p*-Me)PP). UV-vis (λ_{max}, nm; toluene): Fe[T(*m*-Me)PP](*t*-BuNC)$_2$, 433, 538; Fe[T(*p*-Me)PP](*t*-BuNC)$_2$, 434, 537. Electronic spectra were measured with a Jobin Yvon Hitachi spectrophotometer as dichloromethane (or toluene) solutions with small

Table I. Isocyanide Stretching Frequencies of FeTPP Complexes[a]

L	free ligand	Fe^{III}TPP(L)$_2$ClO$_4$	Fe^{II}TPP(L)$_2$
t-BuNC	2130	2220	2129[b]
MeNC	2155	2250	2150
Me$_2$C$_6$H$_3$NC	2135	2195	2130

[a] Nujol; cm^{-1}. [b] From ref 11.

Table II. Observed Shifts and Separation of the Isotropic Shift into Contact and Dipolar Contributions in Fe(TPP)(*t*-BuNC)$_2$ClO$_4$ (ppm)

proton type	$\Delta H/H$[a]	$(\Delta H/H)_{iso}$[b]	$(\Delta H/H)_{dip}$	$(\Delta H/H)_{con}$
o-H	0.96	-7.06	0	-7.06
m-H	13.75	6.12		6.12
m-CH$_3$	1.29	-1.22		-1.22
p-H	3.21	-4.42		-4.42
p-CH$_3$	8.95	6.31		6.31
pyrr H	9.73	1.28		1.28
t-BuNC H	-1.87	-1.38		-1.38

[a] Chemical shift at 298 K with Me$_4$Si as internal reference. [b] Isotropic shift with diamagnetic Fe(TPP)(*t*-BuNC)$_2$ complex as reference.

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(8) Abbreviations used: P, any porphyrin; (TPP)H$_2$, tetraphenylporphyrin; [T(*o*-Me)PP]H$_2$, tetrakis(*o*-methylphenyl)porphyrin; [T(*m*-Me)PP]H$_2$, tetrakis(*m*-methylphenyl)porphyrin; T[(*p*-Me)PP]H$_2$, tetrakis(*p*-methylphenyl)porphyrin; 1-MeIm, 1-methylimidazole; Im, imidazole.
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