solutions should cause band narrowing of the resonance of the rapidly exchanging $^{43}Ca^{2+}$ ions bound to the protein and/or a decrease in NMR intensity of those slowly exchanging bound $^{43}Ca^{2+}$ ion resonances.[2] Since addition of excess Mg^{2+} to the Ca^{2+}–S100$_b$ and Ca^{2+}–S100$_{a0}$ solutions changes both ^{43}Ca NMR signals, the two environmentally different Ca^{2+}-binding sites of S100$_b$ and S100$_{a0}$ appear to be Mg^{2+}-binding sites. Similarly at least 10 Ca^{2+}-binding sites of S100$_a$ may be Mg^{2+}-binding sites since addition of excess Mg^{2+} to the Ca^{2+}–S100$_a$ solution caused ^{43}Ca NMR resonance narrowing. Our ^{43}Ca NMR findings on the Ca^{2+}- and Mg^{2+}-binding sites of the S100 proteins are in accordance with the previous suggestion that Ca^{2+}-binding sites of S100 proteins are Mg^{2+}-binding sites.[15,16]

Using ^{43}Ca NMR studies, we have presented direct evidence that (1) S100$_b$, S100$_{a0}$, and S100$_b$ proteins have at least 20, 7, and 10 Ca^{2+}-binding sites, respectively, and that (2) the Ca^{2+}-binding sites of S100 proteins are also Mg^{2+}-binding sites.

We would like to emphasize the utility of ^{43}Ca NMR in the study of Ca^{2+}-binding proteins using a conventional NMR spectrometer and probe.

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Exploitation of Crystalline Architecture and Solution Data in the Rational Preparation of Novel Mixed-Metal ATP Complexes

Sir:

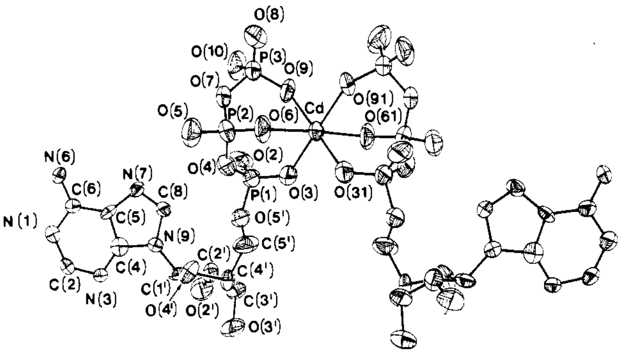

Figure 1. ORTEP drawing showing the $[Cd(HATP)_2]^{4-}$ ion of I with the labeling scheme of the atoms. The view is parallel to the *b* axis. Ellipsoids enclose 30% probability.

Metal ions and metalloenzymes are ubiquitous in nucleotide and nucleic acid biochemistry.[1-8] However, crystallization of relevant metal complexes is difficult since nucleotides have three types of moieties (charged phosphate; flexible puckered sugar; planar aromatic bases). Crystallization of nucleoside triphosphate complexes has been particularly difficult due to the high negative charge of species such as ATP^{4-}. Nevertheless, considerable information concerning metal binding modes and complex stability in solution has been gathered.[2,3,5] We now report that we have rationally exploited this solution information, together with the sparse crystallographic information on ATP complexes, to prepare the first X-ray quality mixed-metal ATP crystals. Examples include the first crystalline Cd complex and a new Zn complex.

Solutions of bis(2-pyridyl)amine (BPA), ATP, and Mg^{2+}, Ca^{2+}, or Mn^{2+} cations at ca. pH 4.5 formed single crystals suitable for X-ray analysis formulated as $[M(H_2O)_6][M'(HATP)_2]\cdot 2HBPA\cdot nH_2O$.[6,7] In previous studies, M = M′ and it appears essential that the $[M(H_2O)_6]^{2+}$ cations insulate the highly charged $[M'(HATP)_2]^{4-}$ anions from each other. For such a role, each cation forms four H-bonds to two anions for a total of eight H-bonds. The $HBPA^+$ moieties stack with the adenine, which is protonated at N(1). Those metal species that gave the best crystals (Mg^{2+}, Ca^{2+}, Mn^{2+}) have been characterized by solution studies as having a high preference for O over N and for having a >80% "open" coordination of ATP with little involvement of adenine directly or indirectly (via a bridging H_2O) with the metal.[2,3] Zn^{2+} gives crystals of poor quality, and Fe^{2+}, Ni^{2+}, Cu^{2+} and Cd^{2+} have not given similar crystalline materials. Compared to Mg^{2+}, Ca^{2+}, and Mn^{2+}, all these latter metal species either have a greater preference for N or have >30% "closed" coordination by ATP, i.e. involvement of the base either via indirect interaction

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(9) Crystal data for I: $C_{40}H_{76}N_{16}O_{41}P_6CaCd$, mol wt 1775.4, orthorhombic, space group $C222_1$, $a = 22.846$ (3) Å, $b = 10.252$ (2) Å, $c = 31.914$ (6) Å, $V = 7475$ (1) Å^3, $Z = 4$, $d_{calcd} = 1.58$ g/cm^3, μ(Mo Kα) = 5.79 cm^{-1}. Crystal data for II: $C_{40}H_{82}N_{16}O_{44}P_6Mg_{0.72}Zn_{1.28}$, mol wt 1778.2, orthorhombic, space group $C222_1$, $a = 22.666$ (3) Å, $b = 10.131$ (2) Å, $c = 30.893$ (6) Å, $V = 7094$ (1) Å^3, $Z = 4$, $d_{calcd} = 1.66$ g/cm^3, μ(Mo Kα) = 6.25 cm^{-1}. Data collection and structure solution for I and II: Data were collected on a Syntex P2$_1$ diffractometer with crystals measuring about 0.15 × 0.15 × 0.10 mm. A total of 3632 (I) and 2612 (II) independent reflections were measured in the range $3° < 2\theta < 50°$ (I) and $3° < 2\theta < 45°$ (II) at 20 ± 1 °C. The data were corrected for *Lp* effects but not for absorption because of the small absorption coefficients and crystal sizes. Only the 2394 (I) and 2206 (II) observed reflections with $F > 3\sigma(F)$ were used subsequently. The cell parameters and the distribution of reflection intensities indicated that the structures of I and II are essentially isomorphous with reported ternary complexes.[6,7,10] However, the Patterson synthesis was still carried out for I and II. The positions of the Cd^{2+} and Zn^{2+} ions were determined and the structures were completed by a series of three-dimensional difference-Fourier maps. All the metals and the ATP non-hydrogen atoms were refined anisotropically for I and II. The N(1) hydrogen atom, located for II from the Fourier-difference analysis, was included in the refinement. Twelve blocked full-matrix least-squares cycles (SHELXTL program system 1985, implemented on a Data General Eclipse S/140 minicomputer) decreased the $R = (\sum(|F_o| - |F_c|)/\sum|F_o|)$ index to 10.15% (I) and 9.03% (II). The $R_w = ([\sum w(|F_o| - |F_c|)^2/\sum wF_o^2]^{1/2})$ index converged to 10.89% (I) and 9.89% (II). The weights were of the form $w = a/(\sigma^2(F) + bF^2)$. Scattering factors were from SHELXTL.

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through a bridging H_2O or indeed directly by a base N to form a "macrochelate".[2] We reasoned that crystals could be grown when M′ = a metal ion with an intermediate O/N preference and where solution studies suggest an open coordination mode (>50%) (Co, Zn, Cd), if the $[M(H_2O)_6]^{2+}$ site needed for crystallization consists of Mg^{2+} or Ca^{2+}. Our rationale includes the following: (a) metals with a N preference would preferably coordinate to N rather than occupy the $[M(H_2O)_6]^{2+}$ site; (b) an open configuration suggested by solution data was that found in the crystals;[6,7] (c) the d-outer-shell metal ions have a greater preference for phosphate coordination over H_2O coordination; (d) Mg^{2+} and particularly Ca^{2+} appear to have a distorted $[M(H_2O)_6]^{2+}$ coordination sphere possibly especially suited for H bonding.[6]

By the use of two metal salts, crystals could be obtained that contained the two metal species, as analyzed by atomic absorption. For example, concentration of an aqueous ca. pH 4.5 solution of $Cd(NO_3)_2$, $Ca(NO_3)_2$, Na_2H_2ATP, and BPA in the molar ratio 1:1:2:2 afforded crystals of I. X-ray and elemental analyses of these crystals agreed with the formulation $[Ca(H_2O)_6][Cd(HATP)_2]\cdot 2HBPA\cdot 9H_2O$. The site occupation factor for Cd was determined by constraining its isotropic thermal parameter *U* to the reasonable value of 0.05 Å^2 and assuming that Ca fully occupied the $[M(H_2O)_6]^{2+}$ site. For this formulation, the analytical data calculated for I (2.26% Ca, 6.33% Cd) were in very good agreement with the values found (2.07% Ca, 6.10% Cd). The $[Cd(HATP)_2]^{4-}$ unit is depicted in Figure 1.

In an analogous experiment using $ZnSO_4$ and $MgSO_4$, crystals II were obtained and analyzed as above. However, the $[M(H_2O)_6]^{2+}$ site gave two peaks on the same C_2 axis with a total of 15 electrons. If we assume that there is a statistical disorder with Zn occupying the minor site, II can be formulated as $[Mg(H_2O)_6]_{0.72}[Zn(H_2O)_6]_{0.28}[Zn(HATP)_2]\cdot 2HBPA\cdot 12H_2O$ based on the X-ray data. This formulation (Anal. Calcd: Mg, 0.98; Zn, 4.70) is in excellent agreement with the analysis (Found: Mg, 1.02; Zn, 4.70). Although a consistent picture emerges for both I and II, we cannot rule out some deviation from these site occupations.

From solution studies, Mg^{2+} forms slightly more stable adducts than Ca^{2+} with triphosphate chains.[2] In direct competition experiments where crystals were grown in the presence of equimolar Mg^{2+} and Ca^{2+}, the Ca molar content exceeded the Mg content. Since it appears that $[Ca(H_2O)_6]^{2+}$ has a greater preference for the $[M(H_2O)_6]^{2+}$ site than does $[Mg(H_2O)_6]^{2+}$, we grew Mg/Zn crystals with the same amounts of all components used to grow crystals II, except for $MgSO_4$, which was doubled. The resulting material gave the following analytical data: 1.34% Mg, 3.76% Zn. These data agree with those expected for $[Mg(H_2O)_6][Zn(HATP)_2]\cdot 2HBPA\cdot 12H_2O$ (1.38% Mg, 3.70% Zn). If 10 equiv of $MgSO_4$ are present initially, the resulting crystals have analyses consistent with a ca. 40:60 Mg/Zn occupancy of the $[M'(HATP)_2]^{4-}$ site. This result is consistent with solution studies[2,3] which demonstrate a ca. 6-fold greater stability for triphosphate complexes with Zn^{2+} over Mg^{2+}.

Although Fe^{2+} and Fe^{3+} ATP complexes have not received detailed study, ATP is implicated in Fe biochemistry.[8] We have been able to obtain, to our knowledge, the first ATP crystals containing Fe. Using methods described above with a 2-fold amount of $MgSO_4$, we obtained crystals that can be formulated as $[Mg(H_2O)_6][Fe(HATP)_2]\cdot 2HBPA\cdot 12H_2O$ (Anal. Calcd: Mg, 1.38; Fe, 3.18. Found: Mg, 1.38; Fe, 3.29). These crystals oxidize slowly in air but are as yet too small for X-ray analysis.

The stability constants that guided our experiments are for 1:1 complexes and do not necessarily define the binding mode of the triphosphate chain. The parallels between our results and solution properties are most easily understood if the triphosphate chain is bound directly to the metal as a tridentate ligand. We believe that the small dependence of the stability constants on the M^{2+} species, which have prompted suggestions of outer-sphere phosphate complexes,[11] could, instead, reflect the remarkably similar conformation of the folded α, β, γ-coordinated triphosphate chain found in all tridentate ATP structures.[6,7,10,12] This folding is characterized by a P-P-P angle of ca. 90° in all previous structures and in I (86 (1)°) and II (85 (1)°). In all the structures, M-O_γ is shorter than M-O_β and M-O_α.

In I, a relatively large ion, Cd, is bound to the triphosphate (M-O α, β, and γ distances are 2.27 (1), 2.28 (1), and 2.23 (1) Å for Cd and 2.14 (1), 2.084 (8), and 2.040 (9) Å for Zn, respectively). The major consequence appears to be in some of the sugar torsion angles not in the triphosphate conformation. For example, II has fairly typical values[6,7] for the C(3′)-C(4′)-C(5′)-O(5′) and C(4′)-C(5′)-O(5′)-P(1) torsion angles of 58 (1) and 166.9 (9)°, respectively. However, in I these angles are 47 (2) and 175 (1)°, respectively.

MgATP is arguably the most important coordination complex in nature. Our knowledge of its structure relies on indirect methods. It is reassuring that the fundamental understanding of metal binding to ATP in solution[2,3] provided by these methods appears to explain our findings. Furthermore, our studies suggest that such solution information can be exploited to direct metal centers to particular sites even in crystals of labile metals containing complex organic species.

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Supplementary Material Available: Tables of positional and thermal parameters, bond distances, and bond angles (15 pages); a table of observed and calculated structure factors (7 pages). Ordering information is given on any current masthead page.

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Inclusion Tuning of Nonlinear Optical Materials: Sorbates in the KTP Structure

Sir:

Potassium titanyl phosphate, $KTiOPO_4$ (KTP)[1,2] is a relatively new nonlinear optical material that has recently attracted considerable attention because of its high nonlinear optical coefficient, high optical damage threshold and low phase matching temperature sensitivity.[3] These properties make KTP the premier material used for second harmonic generation (SHG) of the 1.06 μm YAG laser. In addition, recent studies have shown that large single crystals may make excellent wave guides and electrooptic modulators,[4] which can be used effectively in fiber optic telecommunications. Large, high-quality, single crystals of KTP can

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