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RELAXATION SPECTRA OF COPPER(II)-ATP COMPLEXES AT LOW pH

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SUMMARY

1. Acidic solutions (pH 1–5) of copper(II)-adenosine triphosphate yield ultrasonic attenuation spectra consisting of three maxima. From the concentration and pH dependence of these peaks, and by comparison with the spectra of low pH ATP, $CuCl_2$ and $Cu_3(PO_4)_2$ solutions, we have assigned the spectrum as follows. The overall reaction which fits the ultrasonic attenuation data best is

$$Cu^{2+} + H_2ATP^{2-} \rightleftharpoons CuHATP^{-} + H^{+}$$

The shortest relaxation time (approx. $2 \cdot 10^{-9}$ s) is ascribed to penetration of the inner copper(II) hydration shell by the ligand. The next slower process (approx. $1 \cdot 10^{-8}$ s) is then due to chelate ring closure of the species formed in the faster step. The lowest frequency peak (< 2 MHz) has been taken to be either a characteristic of aqueous copper(II) ion, as already observed in other systems (*e.g.* $CuSO_4$), or to be due to formation of another (minority) complex species such as CuH_2ATP. In either case, the presence of this peak does not interfere with the kinetic analysis of the faster processes.

2. On the assumption that formation of copper(II) complexes follows the established pattern of other divalent transition metal ions, we obtain at 25 °C and variable ionic strength (0.1–0.8 M), $8.8 \cdot 10^{8}$ s^{-1} for the water elimination rate constant, and $6.3 \cdot 10^{7}$ s^{-1} for the sterically controlled ring closure. The ion-pair formation constant consistent with the data is 16 M^{-1}. The absolute value of the standard volume change, $|\Delta V^{\circ}|$ has been estimated to be 42 ml·mole^{-1} for the above overall reaction.

Although the experimental error associated with the determination of the relaxation maxima is on the order of 10–20 %, the values of the rate constants, ion-pair formation constant and standard volume changes are reliable to only a factor of approximately two, due to uncertainties in solution composition and approximations made in solving the rate equations.

INTRODUCTION

This study extends the investigation of the kinetics of association of adenosine triphosphate with divalent cations. The temperature-jump method has been used to determine the complexation rate constants for ATP with Mg^{2+} and Ca^{2+} (refs 1–3), and for ATP with Ni^{2+}, Co^{2+} and Mn^{2+} (ref. 4). The latter ions have also been examined at high nucleotide concentration by NMR[5]. Enzymatic reactions incorporating ATP usually involve specific divalent cations. However, the metal binding site and the conformation of the metal-ATP complex have not been fully established[6–9*], so the specificity is not well understood. The kinetic study by ultrasonic attenuation of copper(II) binding, when compared with other metal ions, aids in the development of a detailed model of ATP activity.

METHODS AND MATERIALS

An apparatus incorporating a cell with a relatively small liquid sample (approx. 80 ml) was used for making the ultrasonic attenuation measurements. The basic design is an optical bench arrangement which remains in perfect alignment throughout the experiment, with easily changed transducers of different fundamental frequencies. A detailed analysis of the electronic components of this pulse apparatus and of the acoustical wave path is available elsewhere[10,11]. The standard procedure for carrying out kinetic determinations by this technique has been followed in this study[12].

Crystalline disodium ATP (Nutritional Biochemicals Corp., Cleveland) was dissolved in distilled water (Belmont Springs), neutralized with NaOH (Fisher), and then passed through a Dowex 50W-X4 (Baker) column. A Beckman DU Spectrophotometer was used to determine the ATP concentration by the absorption at 260 nm[13]. The purified, acidified ATP solution was used to dissolve the appropriate quantity of reagent grade $Cu(NO_3)_2$ (B and A). Reagent grade HCl or HNO_3 (Fisher) were used in conjunction with NaOH to adjust the pH. Within experimental error, no difference in attenuation was detected when HCl replaced HNO_3.

All measurements were made at 25 ± 1 °C. Aqueous ATP blanks were studied at pH 1.7 and 5.0 indicating high-frequency absorption at the upper pH limit only. The $CuCl_2$ blank was essentially identical to that of pure water in agreement with results already reported[14]. A $Cu_3(PO_4)_2$ solution was also measured and showed the characteristic higher frequency relaxations of divalent aqueous complexation such as $CuSO_4$[15–17].

RESULTS AND TREATMENT OF DATA

It is known from potentiometric titration data[18], that the copper–ATP system becomes unstable with respect to precipitation around pH 5, but that further increases in pH lead to solubilization. These results have been interpreted as indicating the presence of mixed hydroxo-, ATP complexes at high pH.

A recent determination of metal–ATP binding[19], which eliminated the presence of disturbing alkali metal ions, produced results in accord with the predominance of two complexes, $CuATP^{2-}$ and $CuHATP^{-}$, at low pH. It was found, after preliminary analysis of our data, that the latter study was most consistent with our results. Therefore the stability constants of Perrin and Sharma[19] have been used to describe the compositions of these solutions, even though their determination was at 30 °C. The error due to the use of 30 °C rather than 25 °C stability constants is small compared to the unreliability of constants determined in the presence of alkali metal ions[19]. The equilibrium constants and concentrations used in this study are given in Table I.

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[*] These papers, reporting on NMR studies, are representative of the many studies on metal–ATP binding using ESR, optical and other techniques. They may be consulted for further references.

TABLE I

EQUILIBRIUM CONSTANTS AND SAMPLE SOLUTION CONCENTRATIONS

Equilibrium constants are at 30 °C with ionic strength of 0.1 M (ref. 11). All concentrations are in molar units. The subscript $[\,]_o$ indicates total stoichiometric amounts initially present.

$$K_{a1} = \frac{[H^+]\,[HATP^{3-}]}{[H_2ATP^{2-}]} = 10^{-3.83} \qquad K_{a2} = \frac{[H^+]\,[ATP^{4-}]}{[HATP^{3-}]} = 10^{-6.81} \qquad K_{CuATP} = \frac{[CuATP^{2-}]}{[Cu^{2+}]\,[ATP^{4-}]} = 10^{6.83}$$

$$K_{CuHATP} = \frac{[CuHATP^{-}]}{[Cu^{2+}]\,[HATP^{3-}]} = 10^{3.97}$$

$[ATP]_o$	$[Cu^{2+}]_o$	pH	$[H^+] \times 10^3$	$[Cu^{2+}] \times 10^3$	$[CuATP^{2-}] \times 10^3$	$[CuHATP^{-}] \times 10^3$	$[H_2ATP^{2-}] \times 10^3$	$[HATP^{3-}] \times 10^3$	$[ATP^{4-}] \times 10^9$
0.17	0.17	1.03	93	79	0.11	91	78	0.12	0.21
0.17	0.085	1.08	83	29	0.075	56	114	0.20	0.38
0.10	0.10	1.30	50	45	0.12	55	44	0.13	0.41
0.03	0.09	2.65	2.2	61	1.4	27	0.72	0.048	3.2
0.03	0.03	2.90	1.5	5.2	1.7	23	4.7	0.46	47
0.08	0.08	2.05	9.0	20	0.74	59	19.5	0.32	5.5

The range of ATP and metal concentration was chosen so as to locate the relaxation times within the present capabilities of the equipment. The practical concentration range was, consequently, 0.065–0.17 M for ATP and 0.016–1.7 M for copper. The pH was, of course, kept low, ensuring reproducible results. In particular, a region around pH 2 was chosen, where free ATP is mainly in the form H_2ATP^{2-} and $HATP^{3-}$.

For n discrete relaxation processes, the ultrasonic attenuation is related to the relaxation times, τ_i, of the individual steps in the mechanism by the relation

$$\alpha\lambda_{\text{chem}} = \sum_{i=1}^{n} A_i \frac{\omega\tau_i}{1+\omega^2\tau_i^2} \qquad (1)$$

In the above, $\alpha\lambda$ is the attenuation coefficient per wavelength, λ (where $\alpha\lambda_{\text{chem}} = \alpha\lambda_{\text{exp}} - \alpha\lambda_{\text{solu}}$); A_i is an experimentally measurable constant; and $\omega = 2\pi f$ (rad·sec^{-1}), f = frequency (Hz). The absorption spectra for these solutions show two peaks in the 7–220 MHz range as shown in Fig. 1. Inspection of these data indicates that a multistep relaxation process is occurring. When $\alpha\lambda$ is a maximum, $\omega\tau_i \cong 1$ (ref. 12).

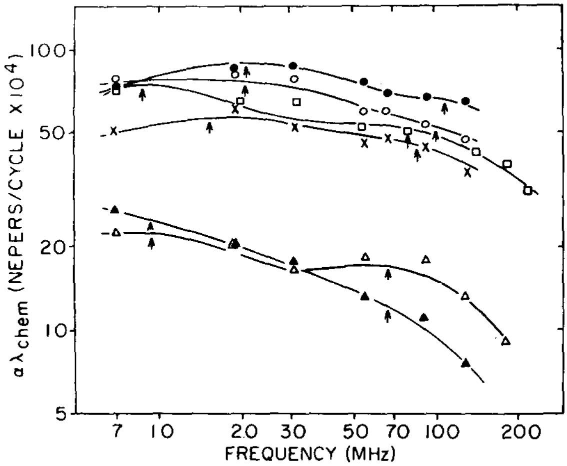


Fig. 1. Experimental absorption data for the sample solutions of Table I at 25 °C, molar concentrations. The curves are calculated from Eqn 1. Peak positions are tabulated in Table II, with the exception of <4 MHz peaks. The existence of these peaks has been indicated by the curve resolution program, but cannot be quantitatively evaluated. Arrows indicate the two maxima used in computing rate constants. Concentrations of individual species are given in Table I. Data: ●—●, $[ATP]_0$ = 0.17 M, $[Cu]_0$ = 0.17 M, pH 1.03; ○—○, $[ATP]_0$ = 0.17 M, $[Cu]_0$ = 0.085 M, pH 1.08; □—□, $[ATP]_0$ = 0.08 M, $[Cu]_0$ = 0.08 M, pH 2.05; ×—×, $[ATP]_0$ = 0.10 M, $[Cu]_0$ = 0.10 M, pH 1.30; △—△, $[ATP]_0$ = 0.03 M, $[Cu]_0$ = 0.03 M, pH 2.65; ▲—▲, $[ATP]_0$ = 0.03 M, $[Cu]_0$ = 0.03 M, pH 2.90.

A standard curve resolution program for the Wang 370 calculator was used to determine the curves with the form Eqn 1 which together sum to fit the observed values. The $(\alpha\lambda)_i^{\max}$ and $f_i^{\max} \cong 1/2\pi\tau_i$, i = 1,2 values are given in Table II.

A peak < 2MHz, the presence of which was indicated by the data (*vide* Fig. 1 caption), has been taken to be either a characteristic of aqueous copper(II) ion, as already observed in other systems (*e.g.* $CuSO_4$[14]), or to be due to formation of another (minority) complex species such as CuH_2ATP. Whatever the reason for its existence, the presence of this peak does not interfere with the analysis of the faster processes[12].

TABLE II

RELAXATION SPECTRA FOR COPPER(II)–ATP SOLUTIONS

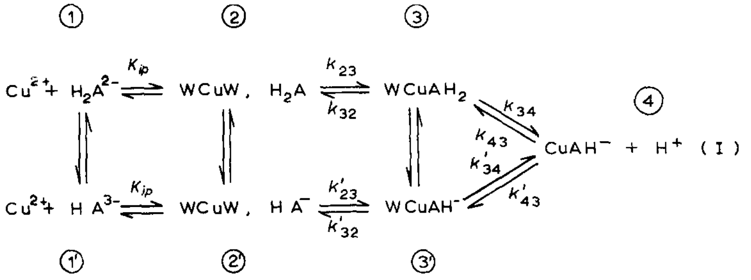
All frequencies are in MHz. All absorption amplitudes are × 10^4 nepers/cycle; concentrations in molar units.

$[ATP]_0$	$[Cu^{2+}]_0$	*pH*	*Calcd* f_1^{max}	*Obsd* f_1^{max}	$(\alpha\lambda)_1^{max}$	*Obsd* f_2^{max}	$(\alpha\lambda)_2^{max}$
0.17	0.17	1.03	100	120	50	20	50
0.17	0.085	1.08	96	100	35	20	50
0.10	0.10	1.30	83	85	35	14	35
0.03	0.09	2.65	70	70	approx. 14	10	approx. 10
0.03	0.03	2.90	*	70	approx. 7	10	approx. 15
0.08	0.08	2.05	60	80	40	9	50

* See Results and Treatment of Data.

To determine rate constants, the procedure adopted in analyzing the data follows Eigen and Tamm's[20,21] work with sulfates of many transition metals. In essence, this procedure starts by assuming a single-step association process and then fits the experimental data to a suitable relaxation mechanism. If an unsatisfactory fit results, a two-step mechanism is assumed, and an equation is rederived consistent with the new assumption. In this way Eigen and co-workers obtained very satisfactory results with 2- or 3-step mechanisms.

The ATP ligand, however, presents more complications than sulfate since it undergoes 4 stepwise protolytic dissociations[18,19]. In addition, the copper(II) –ATP system titration shows at least four separate chelates[18]. Below a pH of 5.5 the simplest aquated forms predominate over the various hydroxo and polymeric species. A complete, multi-step acidic reaction scheme is then as follows, neglecting H_4ATP, H_3ATP^- and ATP^{4-}, and in which the symbol A replaces ATP.



States 2 and 2′ represent ion pairs, in which the two water molecules of the inner coordination sphere that are eventually displaced by the incoming ligand are represented by the symbol W.

It is clear from the multi-peak nature of the absorption spectrum, that the minimum number of steps required to explain the data is two. However, a 2-step mechanism of ion-pair formation followed by inner-sphere substitution is inconsistent with the results for similar systems[20,21]. That is, the observed upper frequency maximum, for example, is too low to correspond to diffusion controlled formation of an ion-pair. Furthermore, on the basis of the control experiments, it is also clear that protolytic steps are too rapid to account for the observed maxima. As a working hypothesis, we assumed that the two peaks corresponded to (a) elimination of the first water molecule (b) slower chelate ring closure. Mechanism I is still not amenable to analysis, since only one ($1 \rightleftharpoons 1'$) of the three vertical equilibrium quotients is known. Therefore, in addition to assigning the spectrum, some further assumptions had to be made.

Consider the five solutions of lowest pH. A simplifying assumption is that pathway $1' \rightleftharpoons 2' \rightleftharpoons 3' \rightleftharpoons 4$ may be neglected for these solutions, since at these pH values $[H_2A^{2-}] > [HA^{3-}]$. If the following conditions hold the rate equations can be solved for this modified mechanism. These conditions are

(i) Step $1 \rightleftharpoons 2$ is always in equilibrium

(ii) $\tau_1 \ll \tau_2$

Under these conditions, the application of the standard methods for obtaining relaxation times yields[12]

$$\begin{aligned} \frac{1}{\tau_1} &\approx k_{32} + k_{23}\left[\frac{K_{ip}([Cu^{2+}] + [H_2A^{2-}])}{1 + K_{ip}([Cu^{2+}] + [H_2A^{2-}])}\right] \\ \frac{1}{\tau_2} &\approx k_{34} + k_{43}([H^+] + [CuHA^-]) \end{aligned} \tag{2}$$

The equation for the longer relaxation time, τ_2, should be modified by the equilibration of the faster process, $2 \rightleftharpoons 3$. However, from an *a posteriori* calculation of the factor arising from the more rapid step, we know that it is always around unity for these four systems, hence it has been omitted in writing Eqn 2.

Condition (ii) implies that k_{23}, $k_{32} \gg k_{34}$, k_{43}. From the magnitude of the stability constant, and the monotonic variation of the highest absorption maxima with concentration, we have made the further assumptions $k_{23} \gtrsim k_{32}$ and $k_{34} \gtrsim k_{43}([H^+] + [CuHA^-])$. The differences among the middle peaks are not reflected in concentration variation, and may, in fact, be due to coupling with the observed low (< 2 MHz) frequency peaks. A trial and error procedure was adopted to find the best set of constants—k_{23}, k_{34}, K_{ip}—which would fit the data. A satisfactory fit was obtained with the constants shown in Table III. (*Cf.* observed *vs* calculated frequency maxima, Table II.) This procedure did not work for the highest (2.90) pH solution.

Allowing the unprimed pathway to be non-contributory in analogy to the preceding did not produce agreement between observed and calculated results for the solution of pH 2.90. From this fact we may conclude that, at these ionic strengths, $K_{ip} \lesssim K'_{ip}$, so that the apparent reactivity of $HATP^{3-}$ towards Cu^{2+} is similar to that of H_2ATP^{2-} towards this ion. There was thus no choice but to evaluate the complete mechanism. The straightforward, although lengthy, process yielded expressions of τ_1, and τ_2 based on the additional assumption, justified by the ATP blanks,

(iii) All vertical steps are always in equilibrium.

The new equations contain the equilibrium quotients $K_{11'}, K_{22'}, K_{33'}$. Now $K_{11'}$ is known (Table I); as a working hypothesis we have assumed that the loss of a proton from the ligand in States 2 and 3 is similar to that from 1. Consequently, the following conditions and assumptions were made, on this basis, and from concentrations in Table I.

$$\text{(a) } K_{33'} \approx K_{22'} \approx K_{11'} = 1.5 \cdot 10^{-4}$$

$$\text{(b) } [H^+] \sim [Cu^{2+}]$$

$$\text{(c) } \frac{[HA^{3-}]}{K_{11'}} = \frac{[H_2A^{2-}]}{[H^+]} \approx 1$$

$$\text{(d) } K'_{ip} \simeq 3\,K_{ip}$$

Using these assumptions and the observed relaxation times, we calculated values of k_{23} and k_{34} (the primed terms do not contribute to the final expression, being unfavorably weighted by equilibration factors), in excellent agreement with the values found for the other four solutions, and given in Table III.

Using the equations developed by Eigen and De Maeyer (*vide* p. 937, ref. 12), which apply to the three solutions in which $[Cu^{2+}]_0 \cong [ATP]_0$, we calculate $|\Delta V°|$ approx. $|42|$ ml·mole^{-1} for the overall reaction. Any analysis of the significance of this number is negated, in this case, by the activity coefficient dependence of apparent partial molar volumes of several reaction components (*cf.* Fig. 13, ref. 14).

TABLE III

KINETIC PARAMETERS FOR COPPER(II)–ATP INTERACTION

Temperature 25 °C, variable ionic strength of 0.1–0.8 M.

$k_{23} = 8.8 \cdot 10^8\ s^{-1}$

$k_{34} = 6.3 \cdot 10^7\ s^{-1}$

$K_{ip} = 16\ M^{-1}$

$K'_{ip} = 50\ M^{-1}$

$K_{33'} \approx K_{22'} \approx K_{11'} = 1.5 \cdot 10^{-4}$

$|\Delta V°| \approx |42|$ ml·mole^{-1}

DISCUSSION

It is worth pointing out that to interpret the results of four out of the five solutions studied, no drastic assumptions were necessary. The loss in the accuracy of the kinetic parameters determined in the analysis stems from the assumption of complete separability of relaxation times and, more importantly, the use of equilibrium quotients determined at 30 °C and ionic strength 0.1 M. On the other hand, the main features of the mechanism emerge unobscured by these assumptions; whereas the greater preponderance of the protonated complex, CuHATP, necessary for even a rough fit of the data, is not available from any other set of equilibrium constants than the ones we have used. In view of this fact, the use of the approximate equations for the relaxation times, for which $\tau_2 \cong 7\,\tau_1$, instead of the exact solution seems justified. Nevertheless, some apparently arbitrary assumptions were required to achieve a fit of the highest pH data. A brief consideration of some relevant structural data for ATP complexes will indicate that these hypotheses are not unreasonable.

That is, briefly, that protolytic reactions between states 2 and 2′, and 3 and 3′ should be similar to that of free ATP itself. The protolytic dissociation in question arises from the adenine $-NH_3^+$ group[18]. In the ion-pair this group should, on electrostatic grounds, be oriented away and fairly far (on the average about 7 Å) from the metal ion, so as to be essentially free. Penetration into the metal ion's inner coordination sphere would not alter this picture, appreciably, as the ATP molecule is relatively large in comparison with the metal ion, and would continue to exhibit many internal rotations and vibrations quite freely. The other assumptions are based on the calculated concentrations (*vide* Table I), and are justified to the accuracy given by the choice of constants. Implicit in this discussion is that the metal binding is mainly to the phosphate moiety. This, and other, structural aspects of the complex that we may infer from kinetic data will be considered as we discuss the parameters determined in this study.

Ion-pair formation constants have been directly measured[22], calculated from theory[23], and obtained indirectly from kinetic studies[24,25]. Using the latter approach, Hammes and Levison found a value of 400 M^{-1} for the ion-pair formation between ATP^{4-} and Ni^{2+}, Co^{2+}, Mn^{2+} at 25 °C and ionic strength 0.1 M. Since we are dealing with ATP species of reduced charge a lower value would be anticipated. Moreover, for highly charged ions, the effect of increasing ionic strength is significant and would further reduce the magnitude of the ion-pairing constant compared with the previous study. From the Davies equation for activity coefficients[22], and a theoretical model[23], we calculate that, at ionic strengths in the range of this study, $K'_{ip} \approx 3K_{ip}$. Thus the values $K_{ip} = 16$ and $K'_{ip} = 50$ M^{-1}, are self-consistent, and in agreement with previous results for similar systems[4].

Unlike the ion-pairing constant, the first-order substitution rate constant should be independent of such factors as charge and molecular structure, provided the mechanism is dissociative[26]. That is, when elimination of water is rate controlling, the mechanism is SN1 in the older terminology[27]. For most divalent transition metal ions, this water dissociation constant has been well-established through a comparison of NMR-line broadening and relaxation measurements. For Cu^{2+} aq., however, the NMR results do not yield the necessary kinetic information[28]. The latest available studies place the subsitution rate constant in the range $2.0 \cdot 10^8$ s^{-1} (20 °C) for copper sulfate[15], to $2.0 \cdot 10^9$ s^{-1} (25 °C) for copper glycinate[29]. The value of approx. $9 \cdot 10^8$ s^{-1} found in this work is therefore essentially in agreement with the values reported in the literature, indicating that initial ATP penetration into the copper ion's inner hydration sheath follows the same mechanism as has been observed for other ligands. The existence of the last step in Mechanism I is, so far, unique for ATP, although a similar process has been observed for other combinations of metal ion and ligand[30–32].

For metal ions following a dissociative substitution mechanism, the loss of the first water molecule is rate determining. Thus, when the incoming ligand is multidentate, normally, the elimination of some or all of the remaining bound water molecules is a faster process[27]. If, however, the closure of the metal-chelate ring is hindered, the vacated site in the metal ion's inner coordination sheath may be repossessed by a solvent molecule, thereby marking a change in the rate limiting step. This process, first inferred indirectly from overall complexation kinetics has been termed sterically controlled substitution[31,32]. The presence of this effect has been confirmed directly, when, as in the present case, the individual steps may be observed separately[33].

This effect is more important for very labile ions. For, otherwise, the elimination of water molecules is always too slow to compete with ring closure. As one of the most labile (towards substitution) metal ions the effect, for copper(II), has already been observed with β-alanine[30] and L-carnosine[34]. The value determined here of $6.3 \cdot 10^7$ s^{-1} for this process cannot be directly compared with those of the other systems, as a direct determination was not possible in the other cases.

It is interesting to compare this result with other metal ion–ATP reactions. Since the value of k_{34} should be mainly determined by ligand properties, we would expect it to be approximately the same for reactions with other metal ions. For Ni^{2+} and Co^{2+}, k_{23} is on the order of 10^4 and $5 \cdot 10^5$ s^{-1}, which are small enough, so that normal substitution could be observed[4]. The reaction with manganese was too rapid to permit accurate evaluation of the rate constant by temperature-jump. A kinetic study by NMR resulted in an estimated value of approx. $3 \cdot 10^8$ $M^{-1} \cdot s^{-1}$ for the $Mn^{2+} + ATP^{4-}$ reaction at 25 °C and 0.1 M ionic strength[5]. For Mn^{2+} aq. k_{23} should be on the order of $5 \cdot 10^6$ to 10^7 s^{-1} (ref. 27). It is therefore probable that, although the fully limiting sterically controlled substitution mechanism may not have been operative for manganese(II), it is very likely that the two processes of ring closure and repossession of the vacant site by water are competitive, decreasing the observed bimolecular rate constant by the factor $k_{34}/(k_{34} + k_{32})$.

The last questions remaining to be answered are structural in nature. What produces sterically controlled ring closure? Is the presence of this effect supported by structural data? In answer to the first question, it appears that there is ring strain, resulting from a mismatch between the distance from binding site to binding site on the metal ion and the distance between dentate groups on the ligand[35].

Few structure determinations are available for comparable copper complexes, and none for the ATP complex. From NMR determinations[6–9], the copper is placed between β- and γ-phosphorous oxygens; *i.e.* on positions furthest from the ribose. The distance between the centers of two oxygen binding sites (which in solution, of course, is not fixed) is on the order of 3.1 Å, based on space-filling atomic models*. This distance exceeds the center to center distance along an edge of the square coplanar copper(II) coordination structure, which is usually around 2.8 Å[36]. Therefore, chelate ring closure could be strained due to the necessity for bringing the two binding groups into closer proximity.

It is interesting to consider whether the sterically controlled ring closure may entail binding between copper and a nitrogen of the ATP adenine moiety. Considerable controversy has arisen over the structure of ATP metal complexes in solution[6–9]. Clearly, a kinetic study such as this one does not furnish direct evidence for choosing any one of a number of possible structures. It has been shown that ATP binding with a d° ion (Mg^{2+}) occurs exclusively with the phosphate function[37,38]. For transition metal ions, the data is ambiguous in this regard; but, although not strongly favored in ATP[5], metal–nitrogen binding occurs in some nucleotides[7,8]. Of the divalent first-row transition metal ions, copper forms the strongest amine complexes; indeed, in general it forms the most stable complexes of the series[39]. If we assume that the initial attachment is to the phosphate moiety, then chelation of an adenine nitrogen to the copper(II) atom would, on steric grounds, be as slow, or slower, than phosphate

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* Averaged over all possible configurations of the Corey–Pauling–Koltun model.

ring closure. Therefore, those relaxation times associated with k_{34} may represent, in addition to rate-limiting ring strain in the formation of a second phosphate metal bond, a strained metal–adenine bond formation.

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