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A ^{31}P-NMR study of the mechanism of and salt effect on Mg^{2+}-ATP exchange

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The study of exchange between free and metal-bound ligands by NMR methods is discussed with reference to differentiation between unimolecular dissociation of the metal-bound complex and bimolecular exchange of metal ion between two ligands. This is applied to exchange of ATP^{4-} between the free and Mg^{2+}-bound states and problems of interpretation in the presence of a strong kinetic salt effect are discussed. Contrary to a previous report, exchange is inferred to occur mainly via unimolecular dissociation of $MgATP^{2-}$ over a range of temperatures, concentrations, and pH values, which include those expected in vivo. For the model system Mg^{2+}-tripolyphosphate, an activation energy of 52 ± 5 kJ · mol^{-1}, inferred to be that for dissociation of $MgTPPH^{2-}$, is found for the exchange process.

Introduction

ATP plays an essential energy-transfer role in many metabolic processes, often with magnesium ion Mg^{2+} as an essential cofactor. Understanding the mechanisms of these processes requires knowledge of the component equilibria; thus the formation and dissociation of the Mg^{2+}-ATP complex at physiological pH is of considerable interest.

In an earlier paper [1] on binding of Mg^{2+} to ATP and other triphosphates, we reported ^{31}P-NMR spectra that showed fast-exchange averaging between free and Mg^{2+}-bound triphosphate, but with some residual broadening of the P_β triplet (the P_β chemical shift difference between the two sites is greater than that for P_α or P_γ). Higher field spectrometers increase the chemical shift separation and hence the exchange broadening. Vasavada et al. [2] have analysed such spectra from 5 to 50 °C at 121 MHz (7.0 T) for a Mg^{2+}/ATP molar ratio of 0.5 : 1, and obtained an activation energy. They reported the bandshape to be independent of concentration, and inferred first order dissociation of the metal-bound complex. This is contrary to a report by Misawa et al. [3], who observed strong concentration-dependence of the P_β (and also P_γ) bandshape at 145.7 MHz (8.5 T), leading to separate P_β peaks for ATP and MgATP (i.e., approaching the slow-exchange condition) at a combination of low concentration and temperature. They concluded that exchange was 'in many cases' dominated by a bimolecular process between free ATP and the MgATP complex. Sontheimer et al. [4] have since extended activation energy measurements to still higher field (8.5 and 11.7 T), but did not investigate the exchange mechanism.

Observation of a strong kinetic salt effect, in such a sense as to simulate the spectral changes ascribed to the bimolecular mechanism in dilution studies, prompted us to study the system further. Interpretation of such studies is complicated also by concentration-dependence of the chemical shifts. We have therefore sought an independent measure of the dominant exchange mechanism, through NMR linewidth changes near the fast-exchange limit (at low magnetic field) on titration with Mg^{2+}. Finally, we have extended activation energy measurement to the model system Mg^{2+}-TPP.

Materials and Methods

Experimental

ATP and TPP were obtained as the sodium salt (highest purity grade) from Sigma. The resolution of the ^{31}P-NMR spectra was equal to that previously obtainable after passing ATP samples through a Chelex column and the samples were considered to be free from detec-

Abbreviations: ATP, adenosine 5′-triphosphate; TPP, tripolyphosphate.

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table traces of paramagnetic ions. Stock solutions of 50 mM were adjusted to pH 7.4 or 8.4 and either buffered with Tris-HCl, or left unbuffered. Magnesium chloride was obtained as AnalaR grade from B.D.H., and the concentration of stock solution (1 M) was checked by atomic absorption. NMR samples contained 5% D_2O as a lock signal. The concentration of triphosphate in such samples was limited (see Figure legends) both to minimize association through ring-stacking and to avoid precipitation at low temperatures on addition of Mg^{2+}. For both ATP and TPP, the Mg^{2+} complex is less soluble than the free triphosphate (i.e., sodium salt) particularly so in the case of TPP.

Temperature variation and dilution studies on ^{31}P-NMR bandshape were carried out at 145.8 MHz using a Brüker WM360 spectrometer, and chemical shift measurements on individual species were obtained using a Jeol GSX-270 spectrometer at 109.38 MHz. The effect of varying Mg^{2+}/ATP molar ratio (R) near the fast-exchange limit was studied at 32.2 MHz using a Brüker WP80 spectrometer. For this purpose, stock 1 M $MgCl_2$ solution was added incrementally to the triphosphate (sodium salt) sample using a 25 μl microsyringe. Use of a relatively high concentration of $MgCl_2$ ensured that dilution of the NMR sample was minimized (upto 10% during the titration from $R = 0$ to 1). The chemical shift of the averaged P_β resonance shows a displacement with change of composition, and the reversal of the direction of this displacement at $R = 1$ [1] provided a check on the molar ratio at this point. NaCl and KCl were also obtained from BDH and salt effects were studied by microsyringe addition of 4 M solutions.

Calculation of exchange rates

This is simplest for exchange of non-spin-coupled nuclei between two sites (A, B) with different chemical shifts [5]. The NMR bandshape can then be given in terms of (i) the molar fraction (p_A, p_B) at each site; (ii) the half-height linewidths ($1/\pi T_2$ in Hz) at each site in absence of exchange; (iii) the chemical shift separation $\Delta\nu$ (in Hz; or $\Delta\omega$ in angular frequency units, rad $\cdot$ s^{-1}); and (iv) the mean lifetimes (τ_A, τ_B) of nuclei in sites A and B. At equilibrium,

$$p_A\tau_A^{-1} = p_B\tau_B^{-1}$$

so the exchange rate can be expressed in terms of a single variable τ^{-1}, defined as $(\tau_A^{-1} + \tau_B^{-1})$. For the common case of equally populated sites, it follows that $2\tau = \tau_A = \tau_B$; this has been a source of ambiguity where the subscripts A, B, have been omitted (see below). The relationship of τ values to rate constants depends on the mechanism of the exchange process. At rates up to the coalescence point, both τ^{-1} and $\Delta\nu$ may reasonably be found by fitting calculated to experimental spectra. Above the coalescence point $\Delta\nu$ is usually taken to be the difference between the chemical shifts of the separate components A and B, but with some degree of uncertainty if these shifts are concentration- (or mixing-) dependent.

The two-site analysis can be applied to many cases of ligand exchange between free and metal-bound states, and is here applied to the P_β resonance of free and Mg^{2+}-bound triphosphate. The presence of spin coupling ($J = 15 - 20$ Hz) of P_β to P_α and P_γ nuclei must however be considered. $J_{\alpha\beta}$ and $J_{\beta\gamma}$ are equal (by symmetry) in the case of TPP, and nearly so in ATP, so that the P_β resonance of the free and Mg^{2+}-bound states separately is a first-order 1 : 2 : 1 triplet rather than a singlet [1,2,4]. Integrity of the P_α–O–P_β and P_β–O–P_γ bonding, and hence the spin coupling, is maintained during exchange. The latter may therefore be treated as three superimposed two-site exchanges, with one-to-one transfer of nuclear magnetization between corresponding components of the free and metal-bound triplets [2]. If $J_{\alpha\beta}$ and $J_{\beta\gamma}$ have the same sign, then the corresponding pairs of components will be the low-field, central and high-field lines of each triplet (this applies to the present sysems). Vasavada et al. [2] have calculated P_β bandshapes for Mg^{2+}-ATP exchange at 121 MHz both with and without the triplet structure, and show that in the region of maximum exchange broadening ($0.1 < \tau^{-1}/\Delta\omega < 10$) the bandshapes are not significantly different. This is no doubt because the coupling constants are a small fraction ($< 10\%$) of the chemical shift separation at this frequency. Misawa et al. [3] and Sontheimer et al. [4] have both used the two-site model, and we have done the same for 145.8 MHz spectra of our systems.

We have also used bandwidth at half-height as an alternative to complete bandshape analysis for exchange between two equally-populated sites [6]. If $\Delta\nu$ is known, this is a useful method of obtaining $\tau_A^{-1} = \tau_B^{-1}$ ($= k$ in the notation of [6] p. 78) from spectra in the region of maximum exchange broadening.

Comparison of mechanisms for metal-ligand exchange

Two mechanisms are considered:

(a) Unimolecular dissociation of the metal-ligand complex (MA)

$$MA \underset{k_2}{\overset{k_1}{\rightleftharpoons}} M + A$$

The recombination process will be bimolecular, with a second order rate constant k_2. For each species (MA and A) the mean lifetime (τ_{MA}, τ_A respectively) is equal to the concentration divided by the turnover rate, so that

$$\tau_{MA} = \frac{[MA]}{k_1[MA]}; \quad \tau_{MA}^{-1} = k_1$$

$$\tau_A = \frac{[A]}{k_2[M][A]}; \quad \tau_A^{-1} = k_2[M]$$

The mean lifetime variable τ, used in the NMR analysis, is then

$$\tau^{-1} = \tau_{MA}^{-1} + \tau_A^{-1} = k_1\left(1 + \frac{[MA]}{[A]}\right)$$

At a given temperature, τ will therefore depend only on the ratio of the concentrations of metal-bound to free ligand and is independent of the total ligand concentration. This ratio will remain effectively constant on dilution of a sample if the formation constant of the complex MA is reasonably high (as in the cases considered here).

(b) Bimolecular exchange between metal-bound and free ligand

$$MA + A^* \overset{k_3}{\rightleftharpoons} MA^* + A$$

Here

$$\tau_{MA} = \frac{[MA]}{k_3[MA][A]}; \quad \tau_{MA}^{-1} = k_3[A]$$

and

$$\tau_A = \frac{[A]}{k_3[MA][A]}; \quad \tau_A^{-1} = k_3[MA]$$

so that

$$\tau^{-1} = k_3\{[MA] + [A]\}$$

i.e., the mean life-time variable τ is inversely proportional to the total concentration of free and bound ligand.

In most cases, the two mechanisms may thus be differentiated easily by the concentration-dependence of the spectrum (e..g, by successive dilution of a single sample), maintaining a constant molar ratio of the exchanging species. In the case of unimolecular dissociation (a) the bandshape will be unaffected if $\Delta\nu$ is independent of concentration. For bimolecular exchange (b), the bandshape will alter progressively towards the slow-exchange condition on dilution.

Dilution studies of the present system are not entirely straightforward (see below). A different approach to the dominant mechanism was also used, based on the half-height width $1/\pi T_2$ of individual peaks near the fast exchange limit. For exchange between two uncoupled sites (A, B) it was shown by Meiboom et al. [7] that

$$T_2^{-1} = p_A T_{2A}^{-1} + p_B T_{2B}^{-1} + p_A^2 p_B^2 \Delta\omega^2(\tau_A + \tau_B) \qquad (1)$$

The last term in this expression ($= \chi$, say) corresponds to the exchange broadening. If B is identified with a metal-bound complex MA, and A with the free ligand, then since molar fraction $p_B = 1 - p_A$, we have

$$\chi = p_A^2(1 - p_A)^2\Delta\omega^2(\tau_A + \tau_{MA})$$

Mechanism (a).

For unimolecular dissociation of MA,

$$\tau_{MA} = k_1^{-1}; \quad \tau_A = \frac{[A]}{k_2[M][A]} = \frac{[A]}{k_1[MA]} = k_1^{-1}\frac{p_A}{1 - p_A}$$

so that

$$\tau_A + \tau_{MA} = k_1^{-1}(1 - p_A)^{-1}$$

Hence

$$\chi = p_A^2(1 - p_A)\Delta\omega^2 k_1^{-1}$$

This confirms that there is no broadening at $p_A = 0$ or 1. Taking the derivative of χ with respect to mol fraction,

$$\frac{d\chi}{dp_A} = (2p_A - 3p_A^2)\Delta\omega^2 k_1^{-1}$$

The turning point at $p_A = 2/3$ corresponds to maximum χ, since the second differential is negative. The exchange broadening is therefore asymmetric with respect to composition and is greatest at molar ratio $[MA]/[A] = 1:2$, (i.e., when $R = 1/3$). The asymmetry associated with this mechanism has been noted by Sudmeier et al. [8], and also by Vasavada et al. [2] in a series of computer-simulated spectra.

Mechanism (b).

For bimolecular exchange,

$$\chi = p_A^2(1 - p_A)^2\Delta\omega^2 k_3^{-1}\left(\frac{1}{[MA]} + \frac{1}{[A]}\right)$$

Substituting

$$[MA] = (1 - p_A)[MA + A]; \quad [A] = p_A[MA + A]$$

Then

$$\chi = p_A^2(1 - p_A)^2\Delta\omega^2 k_3^{-1}\left[\frac{1}{p_A(1 - p_A)[MA + A]}\right]$$

or

$$\chi = \frac{\Delta\omega^2 k_3^{-1}}{[MA + A]}p_A(1 - p_A)$$

In this case, the exchange broadening is concentration-dependent, being inversely proportional to the total concentration [MA + A] of free and bound ligand. Additionally, the variation of broadening with molar ratio is:

$$\frac{d\chi}{dp_A} = \frac{\Delta\omega^2 k_3^{-1}}{[MA + A]}(1 - 2p_A)$$

Exchange broadening is now symmetrical with respect to composition, with a maximum at mol fraction $p_A = p_{MA} = \frac{1}{2}$.

Composition (i.e., molar ratio)-dependence of linewidth near (but not at) the fast-exchange limit could therefore be used to identify the dominant mechanism. The ratios derived above apply also to the total observed line width if the non-exchange linewidths associated with each site are the same. This is often nearly so, especially if such linewidths are dominated by field inhomogeneity. However, if they are significantly different then the mol ratio of maximum broadening would be displaced by an amount that can be calculated from Eqn. 1.

Results

Dilution studies

P_β spectra ranging from fast to slow exchange could be obtained both for the Mg^{2+}-ATP and the Mg^{2+}TPP systems at 145.8 MHz by appropriate choice of conditions. At a given temperature the exchange rate for the Mg^{2+}-TPP system was considerably slower than that for Mg^{2+}-ATP, in accord with our earlier observation [1]. At the same concentration, the Mg^{2+}-ATP system required a temperature 15 C° lower than that of Mg^{2+}-TPP to bring about similar exchange rates. The effect of dilution (into buffer solution; at pH 7.4) on samples initially 40 mM in total triphosphate and close to 1 : 2 in Mg^{2+}/triphosphate ratio, was therefore studied at 288 K in the case of TPP and 273 K for ATP. Both cases showed marked progressive changes in band shape on dilution, from a single broad peak at 40 mM to well-resolved peaks at 10 mM. The ATP system was studied also at pH 8.4, where acid dissociation is essentially complete, and showed a similar progressive change from fast to slow exchange on dilution (Fig. 1a). This is contrary to the invariance of bandshape reported by Vasavada et al. [2] and appeared to support quantitatively the bimolecular exchange mechanism (b) favoured by Misawa et al. [3].

Further experiments were carried out on the Mg^{2+}-ATP system to investigate possible kinetic salt effects upon the exchange rate. At both pH 7.4 and 8.4 it was found that the spectrum could be converted progressively from slow to fast exchange by incremental addition of 4 M NaCl solution. One such experiment gave almost identical bandshape changes on additions of KCl and of NaCl, so that we did not observe a specific cation effect under these conditions. The earlier dilution experiments were associated with a progressive reduction in ionic strength, which is unusually pronounced in the present system because of the high ionic charges associated with the triphosphate species. Since the positive salt effect is in the correct sense to slow down the exchange on dilution, the possibility arises that changes in bandshape on dilution were due largely or wholly to a self-salt effect which could mask the true mechanism. Dilution of the Mg^{2+}-ATP system (at pH 8.4 and

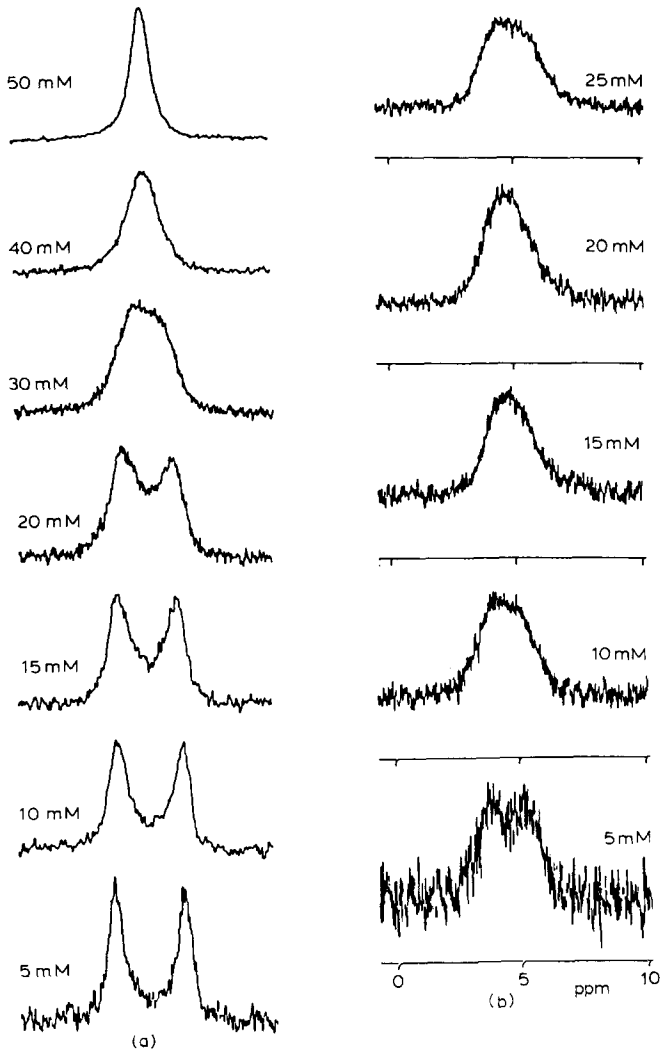

Fig. 1. Dilution experiments on Mg^{2+}-ATP system (1 : 2 molar ratio Mg^{2+}/ATP) at pH 8.4 (equimolar in Tris buffer); P_β-NMR resonance at 145.8 MHz; 273 ± 1 K. (a) Dilution with 50 mM buffer solution only; (b) dilution at constant ionic strength with 25 mM buffer solution containing 187 mM NaCl (the temperature is 1–2° higher for this run). Total ATP concentrations are shown for each spectrum.

therefore in known ionization state, i.e., equimolar in $MgATP^{2-}$ and ATP^{4-} with Na^+ as counter-ion) was therefore carried out with buffer solution containing sufficient NaCl to maintain constant ionic strength. The latter was calculated on the assumption of a full multiple-charge contribution from the triphosphate species present *. In duplicated experiments, the bandshape was now only slightly altered on five-fold dilution (an example is shown in Fig. 1b). This is in marked contrast to Fig. 1a and in broad agreement with mechanism (a). The changes that do occur might reflect either a small contribution from mechanism (b) or changes in chemical shift separation (see below).

* We are indebted to our colleague Dr. J.G. Stamper for discussion of this point.

The dilution experiment of Misawa et al. [3] on samples with $R \approx 0.5$ and an initial total ATP concentration of 10 mM, were carried out in the presence of 100 mM KCl. We have repeated this study under similar conditions (pH 7.4; no buffer) but with dilution into 175 mM KCl to maintain constant ionic strength, and making allowance for a small increase in $\Delta\nu$ on dilution. Our values for τ_{MA}^{-1}, extrapolated to zero concentration of ATP, are $1.1 \cdot 10^3$ s^{-1} (25 °C); $0.5 \cdot 10^3$ s^{-1} (10 °C); $0.1 \cdot 10^3$ s^{-1} (−1 °C). These are identified with k_1 of the dissociative mechanism (a) and indicate an activation energy for this of 33 ± 5 kJ · mol^{-1}. This is consistent with values in lit. [2,4] for total exchange, and agrees well with that of Vasavada et al. in absence of buffer [2]. Our results indicate slight increase of τ_{MA}^{-1} with concentration but at a lower rate than Misawa et al. [3]. The ratio * of slope to intercept suggests that the relative contribution of mechanism (b) is at most no more than 25% of that found previously [3], making it a minor contributor in these conditions. Closer assessment would require more precise $\Delta\nu$ values for the mixed ATP + MgATP system than we presently have.

Molar ratio studies

In view of the complications encountered in dilution studies, confirmatory evidence was sought from dependence of the P_β bandwidth on Mg^{2+}/ATP ratio near the fast exchange limit. Higher temperatures and a lower operational frequency (32.2 MHz) were used for this purpose. Under these conditions, P_β resonances from free and Mg^{2+}-bound ATP merge into one resolved triplet (due to spin-coupling to P_α and P_γ [1]), with slight exchange broadening. The latter was measured as a function of Mg^{2+}/ATP ratio (from $R = 0$ to > 1) on incremental addition of $MgCl_2$ to a solution of ATP (sodium salt; Tris-buffered or unbuffered at pH 7.4). The ratio of P_α to P_β peak heights (sum of doublet and triplet components respectively) was measured in addition to P_β line width. Since the P_α doublet shows no exchange broadening under these conditions, this gives an alternative indication of P_β broadening. Results for two experiments are shown in Fig. 2. All cases showed an asymmetry of broadening with respect to molar ratio (this was noted in our earlier paper [1] for both Mg^{2+}-ATP and Mg^{2+}-TPP systems), with a maximum at $R = 0.3$ to 0.4. This provides further support for the predominance of mechanism (a) (see above). It should be noted that the kinetic salt effect cannot be responsible for the observed asymmetry. There will be some reduction in ionic strength (smaller than that in the dilution experiments) on addition of $MgCl_2$, due to partial neutralization of the multiple charge on the triphosphate ion. However, the expected reduction in exchange rate will here increase the exchange broadening and should therefore displace the observed maximum slightly to the right, i.e., to higher value of the molar ratio R.

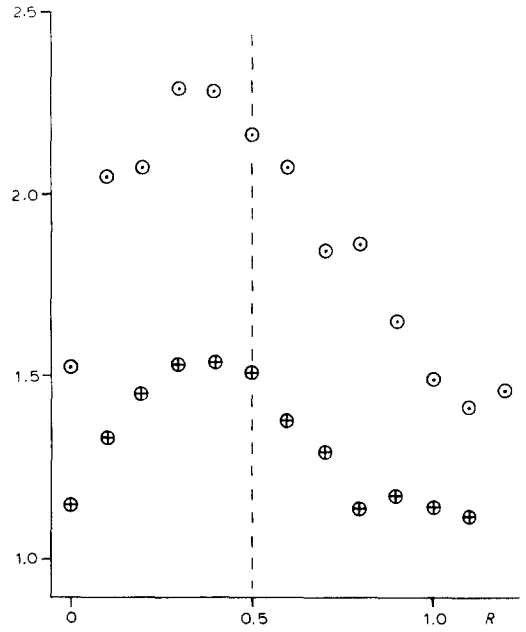

Fig. 2. Exchange broadening of P_β-NMR-resonance of ATP at 32.2 MHz, near the fast-exchange limit, as a function of added $MgCl_2$ solution (x-axis = molar ratio R, of Mg^{2+} to total ATP). Series ⊙: 40 mM ATP, pH 7.4 (Tris buffered), 288 K, y-axis = ratio of P_α/P_β peak heights. Series ⊕; 25 mM ATP, pH 7.4 (unbuffered), 311 K, y-axis = average of P_β line width and P_α/P_β peak height measurements (see text).

Temperature variation for the system Mg^{2+}-TTP

The P_β resonance for this model system at 145.8 MHz is shown in Fig. 3 over the temperature range 273–298 K. The sample was 40 mM in total TPP, approx. 0.5 : 1 in Mg^{2+}/TPP molar ratio and Tris-buffered at pH 7.4 as before. Matched calculated spectra are also shown in Fig. 3. The Arrhenius plot (using τ^{-1} values) is linear within experimental error (Fig. 4) giving an activation energy for exchange of $E_a = 52 \pm 5$ kJ · mol^{-1}.

Discussion

The present study demonstrates the difficulties of kinetic analysis in the presence of a strong salt effect. This poses a particular problem over dilution studies when the reactants are ions carrying a high electrical charge. Whilst we have attempted to compensate for this by maintaining constant ionic strength, it is by no means certain that, at the reactant concentrations needed for the NMR study, such salt effects can be represented by a single ionic strength factor. The further evidence

* Comparison of our τ_{MA}^{-1} values with the previous [3] spectra and reported τ_{MA}^{-1} values suggested that the latter may in fact be $\tau^{-1} = 2\tau_{MA}^{-1}$.

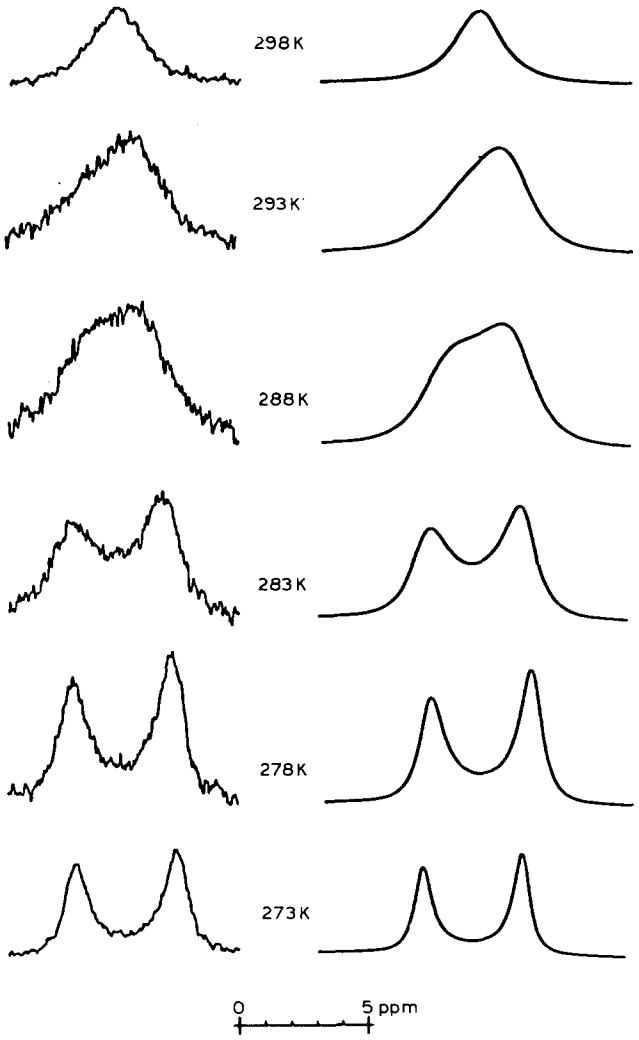

Fig. 3. Temperature dependence of P_β-NMR resonance at 145.8 MHz of Mg^{2+}-TPP system (0.48:0.52 molar ratio Mg^{2+}/TPP); 40 mM total TPP/Tris-buffered at pH 7.4. Experimental spectra are shown on the left; Corresponding computer-fitted spectra are shown on the right.

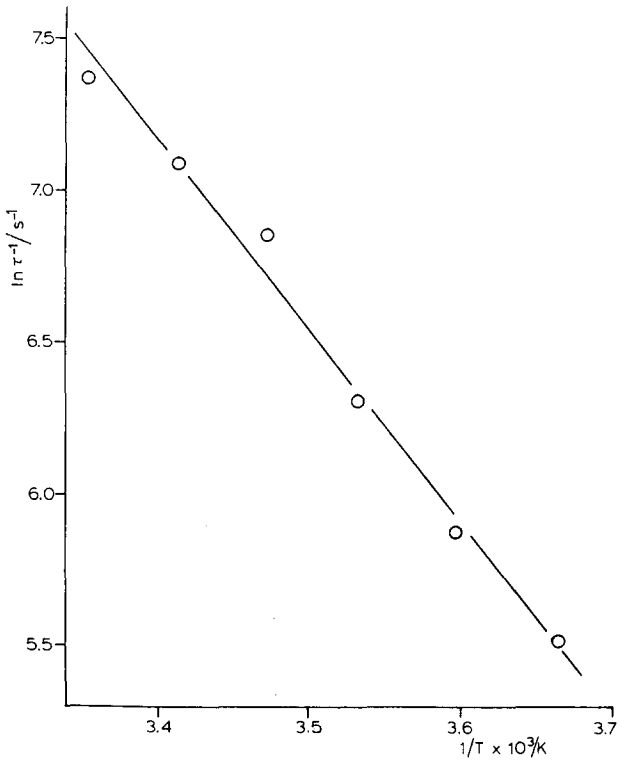

Fig. 4. Arrhenius plot for exchange in the Mg^{2+}-TPP system. The parameters are those of the computer-fitted spectra of Fig. 3.

provided by titration with Mg^{2+} near the fast exchange limit is then valuable. Confirmation of the dissociative mechanism (a) is also given by the agreement between τ_{MA}^{-1} and the rate constant for dissociation of $MgATP^{2-}$ (1200 s^{-1} at 25°C) found by the T-jump method [3,9,10].

The activation energy for Mg^{2+}-ATP exchange has been reported to depend on buffering conditions by Vasavada et al. [2] who found somewhat lower values than that of Sontheimer et al. [4] which was 41 kJ · mol^{-1} in Tris buffer (pH 8.4). This is probably the most accurate determination, since it used well-resolved spectra with the overlapping temperature ranges of two high-field spectrometers. Our comparable value of 52 ± 5 kJ · mol^{-1} for Mg^{2+}-TPP exchange is also in Tris buffer and in the same state of ionization and suggests similar binding of Mg^{2+} through the triphosphate chains. The pK_a of $ATPH^{3-}$ at 293–303 K has been variously reported between 6.5 and 7.0 with a preferred value close to the latter [9]. The predominant species at pH 7.4 and 8.4 is therefore ATP^{4-} with $ATPH^{3-}$ as a minor component at the lower pH. (The pK_a of $MgHATP^-$ is much lower than these pH values, being quoted e.g., as 5.26 in Ref. 11, so we infer that the exchange studied here is via the unprotonated species $MgATP^{2-}$ and ATP^{4-}.) For TPP, our pH of 7.4 is intermediate between the widely-spaced pK_a values for the last two acid dissociations (quoted e.g., as 6.57 and 7.93 at 298 K [12]). The predominant species is therefore $TPPH^{4-}$, in which the terminal H substitutes for the ribose linkage in ATP [12] *.

The enthalpy of dissociation (ΔH) of the $MgATP^{2-}$ complex in aqueous solution is relevant. A value close to 18.8 kJ · mol^{-1} (4.50 kcal) has been found both indirectly [13] and by calorimetry [14]. This is lower than the range of activation energy values (E_a approx. 30–40 kJ · mol^{-1}) found for the Mg^{2+}-ATP exchange process [2,4], including that (approx. 33 kJ · mol^{-1}) found here. Since these values are associated mainly or wholly with unimolecular dissociation (for which $E_a = \Delta H^{\ddagger} + RT$ in solution) then the discrepancy between ΔH and E_a implies also a low but finite activation energy (approx. 10–20 kJ · mol^{-1}) for the reverse exothermic process, i.e., recombination of Mg^{2+} and ATP^{4-} under the given experimental conditions.

* This was quoted inadvertently as TPP^{5-} in our earlier paper [1].

The importance of exchange in the Mg^{2+}-ATP system in vivo is exemplified by a recent study by Apte et al. [15]. Our conclusion that the dissociation of $MgATP^{2-}$ is essentially unimolecular in the pH and concentration range studied will certainly apply also to the lower concentrations encountered in vivo. This is of relevance to the detailed study of the mechanisms of all MgATP-dependent enzymes. Of further importance is the observation of a strong salt effect on the kinetics of the equilibrium, which has at least two implications. Firstly, in NMR studies of biological systems involving Mg^{2+} and ATP^{4-} it is clear that the ATP^{4-} concentration and the total ionic strength will determine whether or not separate signals from free ATP^{4-} and its Mg^{2+} salt are observed. If this is not generally appreciated then false interpretations of the data may arise. Secondly, and probably more importantly, it seems unlikely that unimolecular dissociation and bimolecular ionic combination will be equally affected by the salt effect, i.e., the equilibrium will be dependent on the ionic strength of the solution. This effect would be masked in our experiments in which the concentration of Mg^{2+} and ATP^{4-} was high enough to ensure effectively complete binding. However, at lower, more physiological concentrations this effect could be considerable and result in erroneous interpretations of results.

References

1 Bishop, E.O., Kimber, S.J., Orchard, D. and Smith, B.E. (1981) Biochim. Biophys. Acta 635, 63–72.
2 Vasavada, K.V., Ray, B.D. and Nageswara Rao, B.D. (1984) J. Inorg. Biochem. 21, 323–335.
3 Misawa, K., Lee, T.M. and Ogawa, S. (1982) Biochim. Biophys. Acta 718, 227–229.
4 Sontheimer, G.M., Kuhn, W. and Kalbitzer, H.R. (1986) Biochem. Biophys. Res. Commun. 134, 1379–1386.
5 Gutowsky, H.S. and Holm, C.H. (1957) J. Chem. Phys. 25, 1228–1234.
6 Sandstrom, J. (1982) Dynamic NMR Spectroscopy, Academic, New York.
7 Meiboom, S., Luz, Z. and Gill, D. (1957) J. Chem. Phys. 27, 1411–1412.
8 Sudmeier, J.L., Evelhoch, J.L. and Jonsson, N.B.-H. (1980) J. Mag. Res. 40, 377–390.
9 O'Sullivan, W.J. and Smithers, G.W. (1979) Methods Enzymol. 63, 294–336.
10 Diebler, H., Eigen, M. and Hammes, G.G. (1960) Zeit. Naturforsch. 15B, 554–560.
11 Phillips, R.C. (1966) Chem. Rev. 66, 501–527.
12 Frey, C., Stuehr, J. and Banyasz, J. (1972) J. Am. Chem. Soc. 94, 9198–9204.
13 Sari, J.C., Ragot, M. and Belaich, J.P. (1973) Biochim. Biophys. Acta 305, 1–10.
14 Belaich, J.P. and Sari, J.C. (1969) Proc. Natl. Acad. Sci. USA 64, 763–770.
15 Apte, D.V., Koutalos, Y., McFarlane, D.K. and Ebrey, T.G. (1989) Biophys. J. 56, 447–452.