A Kinetic Investigation of the Interaction of Adenosine-5'-triphosphate with Divalent Metal Ions*

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Rate constants for the formation and dissociation of Ni(II) and Co(II) complexes of ATP have been determined utilizing the temperature-jump method; lower bounds of the rate constants for the corresponding Mn(II) complex have also been determined. The values of the formation rate constant at 25° in 0.1 M KNO₃ for Ni(II), Co(II), and Mn(II) are 4.1×10^6, 9.2×10^7, and $>10^9$ M^{-1} sec^{-1}, respectively, while those for the dissociation rate constant are 38, 2.0×10^3, and $>10^4$ sec^{-1}. Experimental values of the formation rate constant for these ions plus those for Ca(II) and Mg(II) are in reasonable agreement with calculated values based on a previously proposed mechanism involving water dissociation from the inner hydration shell of the metal ion as the rate-determining step in complex formation (M. Eigen, *Z. Elektrochem.* **64**, 115 [1960]). The implications of these results for the understanding of metal ion specificity in biological systems are discussed.

The role of metal ions in biological systems has been investigated by many workers in recent years (see Vallee, 1955; Malmström and Rosenberg, 1959; Williams, 1959). In particular, enzymatic reactions involving ATP generally require the presence of some divalent cation (see Bock, 1959). The metal-ion requirement is usually quite specific, although the reason for this specificity is not at all well understood. Numerous thermodynamic investigations of the interaction of metal ions with various ligands have been carried out (see Bjerrum et al., 1957), particularly utilizing ATP as a ligand (see Taqui Khan and Martell, 1962). The results unfortunately do not shed much light on the nature of metal-ion specificity. For example, Ca^{2+} and Mg^{2+} generally form complexes of comparable thermodynamic stability; a similar situation is found for Ni^{2+} and Co^{2+} ions. Yet Ca^{2+} and Ni^{2+} are generally very poor metal-ion activators, while Mg^{2+} and Co^{2+} are often good activators. The possibility exists that the structure of the ATP–metal ion complexes is markedly different for various metals; however, current experimental evidence suggests this is not the case—at least as far as the predominant structures in solution are concerned (Cohn and Hughes, 1960, 1962).

An alternative approach to the problem of metal-ion specificity is an investigation of the kinetic properties of the various ions. In recent years relaxation techniques have been used to study the kinetics of metal-complex formation (Eigen, 1960; Eigen and Tamm, 1962a,b; Hammes and Steinfeld, 1962). From this work, a general mechanism for complex formation has emerged. The interactions of Ca^{2+} and Mg^{2+} with ATP and ADP have already been reported (Diebler et al., 1960; Eigen and Hammes, 1960, 1961). This study is concerned with the kinetics of the interaction of ATP with Ni^{2+}, Co^{2+}, and Mn^{2+}, thus essentially completing the list of divalent metal ions of particular interest as metal-ion activators.

EXPERIMENTAL

Stock solutions of Ni^{2+}, Co^{2+}, and Mn^{2+} were prepared from reagent grade nitrate salts (Mallinckrodt), while stock solutions of ATP were made up from the disodium salt of adenosine-5'-triphophate (Pabst).

The ATP stock solution was prepared fresh every few days and refrigerated. All solutions were prepared with distilled deionized water and brought to an ionic strength of 0.1 M with reagent grade KNO₃ (Baker). The pH of the solutions to be studied was adjusted by adding NaOH dropwise. The final pH of the solution was measured with a Radiometer pH meter. Kinetic studies were carried out with the temperature-jump apparatus as previously described (Czerlinski and Eigen, 1959; Hammes and Steinfeld, 1962; Hammes and Fasella, 1962). To detect pH changes related to the metal-ligand reaction, all solutions were made 2.0×10^{-5} M in chlorphenol red, a pH indicator. In order to assign the observed relaxation effects to the metal-ligand reaction, blank solutions of indicator-metal and indicator-ligand were also studied. All reactions were investigated over as wide a concentration range as possible at two different pH values. For Ni^{2+} the range of ATP and metal concentration employed was from 4.16×10^{-5} to 4.82×10^{-4} M and from 2.89×10^{-5} to 3.12×10^{-4} M, respectively, while for the Co^{2+} study the concentration values of ATP and metal varied from 3.22×10^{-5} to 3.39×10^{-4} M and from 1.97×10^{-5} to 1.65×10^{-4} M, respectively. Similar concentration ranges were probed using Mn^{2+} as the divalent metal ion. The pH range studied for both metals was from 6.00 to 6.55.

RESULTS

The reaction mechanism can be postulated as

$$M^{2+} + ATP^{4-} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} MATP^{2-} \qquad (1)$$

$$H^+ \updownarrow$$
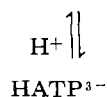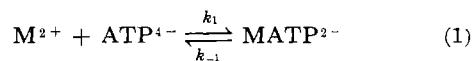
$$HATP^{3-}$$

In the pH range investigated, the concentration of $MHATP^-$ was negligible compared to that of the above-mentioned species. In addition, the indicator-proton equilibrium must be taken into account:

$$HIn \rightleftharpoons In^- + H^+ \qquad (2)$$

If the protolytic equilibria can be assumed to be adjusted rapidly compared to that of the metal-complex reaction (this is, in fact, proved by the "blank" experiments), the reciprocal of the longest relaxation time,
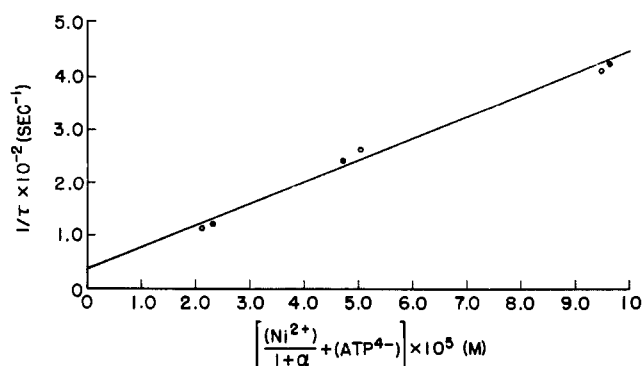
Fig. 1.—Plot of $1/\tau$ versus $(Ni^{2+})/(1 + \alpha) + (ATP^{4-})$ in 0.1 M KNO_3 at 25°. Open and solid circles represent experiments at pH 6.00 and 6.55, respectively.

Fig. 2.—Plot of $1/\tau$ versus $(Co^{2+})/(1 + \alpha) + (ATP^{4-})$ in 0.1 M KNO_3 at 25°. Open and solid circles represent experiments at pH 6.00 and 6.55, respectively.

$1/\tau$, can be written as (Hammes and Steinfeld, 1962):

$$1/\tau = k_1 \left[\frac{(M^{2+})}{1 + \alpha} + (ATP^{4-}) \right] + k_{-1} \qquad (3)$$

where $\alpha = \dfrac{(H^+)}{K_A + (ATP^{4-}) \left[\dfrac{K_I + (H^+)}{K_I + (H^+) + (In^-)} \right]}$

Here, K_A and K_I refer to the acid ionization constants of ATP and indicator which are 2.95×10^{-7} (Taqui Khan and Martell, 1962) and 7.58×10^{-7} M (unpublished experiments), respectively. All concentrations are the equilibrium values at 25°.

Equation (3) predicts that a plot of $1/\tau$ versus $(M^{2+})/(1 + \alpha) + (ATP^{4-})$ should be a straight line with a slope equal to k_1 and an intercept equal to k_{-1}. Such plots are shown for the data obtained with the Co^{2+} and Ni^{2+} complexes in Figures 1 and 2. A good linear fit was obtained consistent with independently measured equilibrium constants (Taqui Khan and Martell, 1962). The rate constants obtained are presented in Table I and have probable error limits of about ±25%. The reaction involving Mn^{2+} and ATP^{4-} was probably too fast to be measured. (There was some evidence of a relaxation time, but interference of the indicator-proton reaction made a quantitative determination impossible.) However, an alternative possibility is that the enthalpy of complex formation may be too small for the appropriate relaxation effect to be detected. Assuming the former to be true, lower bounds for the rate constants can be obtained and are included in Table I. This calculation assumes a resolution time of the temperature-jump apparatus of about 20 μsec.

DISCUSSION

Kinetic studies of metal complex formation with ATP have now been carried out with five different metals, Mg^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+}, and Ca^{2+}. All the pertinent rate constants are assembled in Table I. A number of kinetic investigations of complex formation involving these metals and various ligands indicate that the mechanism of complex formation involves formation of an ion pair followed by the rate-determining dissociation of one or more water molecules from the inner hydration shell of the metal ion (Eigen, 1960; Eigen and Tamm, 1962a,b). The rate constant characterizing the dissociation of a water molecule from the completely aquated metal ion is essentially independent of the particular ligand. This general mechanism can be depicted as (Hammes and Steinfeld, 1962):
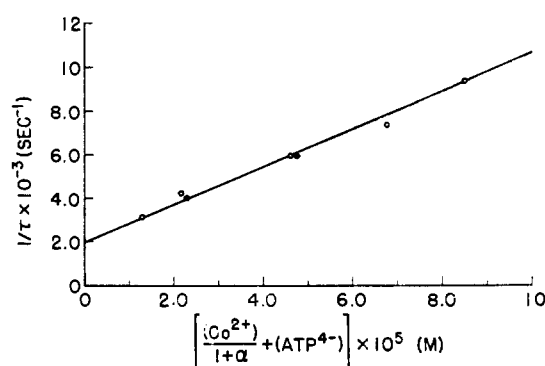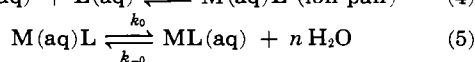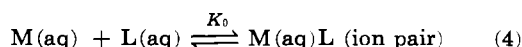
$$M(aq) + L(aq) \xrightleftharpoons{K_0} M(aq)L \text{ (ion pair)} \qquad (4)$$

$$M(aq)L \xrightleftharpoons[k_{-0}]{k_0} ML(aq) + n\, H_2O \qquad (5)$$

Assuming the rate of equilibration of the initial ion-pair formation is fast compared to the rate-determining step of water dissociation, it can be easily shown that $k_1 = K_0 k_0$ and $k_{-1} = k_{-0}$ (Hammes and Steinfeld, 1962). Here K_0 is the equilibrium constant characterizing the ion-pair formation, and k_0 is the rate constant characterizing the rate of dissociation of the first water molecule from the metal ion's inner hydration shell; k_{-0} is the rate of breaking the metal-ligand bond if n is equal to one, while if n is greater than one, k_{-0} is only a rough measure of this quantity.

The ion-pair constant, K_0, for the appropriate metal-ligand interaction can be approximately calculated (see, for example, Steinfeld and Hammes, 1962, and references therein) and is dependent among other things on the charge of the ions, distance of closest approach of the ions, ionic strength, and dielectric constant of the medium. However, because such calculations involving highly charged ions at high ionic strengths are not reliable, an alternate method of obtaining K_0 was used. For many ligands the experimentally determined k_0 for Ni^{2+} is independent of the nature of the ligand (Eigen, 1960; Hammes and Steinfeld, 1962; Hammes and Morrell, 1964). In the case of Ni^{2+}, because of the crystal-field stabilization energy involved (Pearson, 1959; Basolo and Pearson, 1958), it is quite reasonable to assume that the unimolecular dissociation constant, k_0, is completely independent of the type of ligand involved. This is apparently not always true for Co^{2+} ions (Hammes and Morrell, 1964). Accordingly, a value of 10^4 sec^{-1} for k_0 was chosen, which, when used with the k_1 determined for Ni^{2+} in this paper, gives a value of 4×10^2 M^{-1} for K_0. Now k_1 can be calculated for reactions involving other metal ions from the relationship $k_1 = 4 \times 10^2 k_0$, where k_0 has been determined independently in other investigations (Eigen and Tamm, 1962a,b). This assumes K_0 is roughly the same for all of the metal ions involved, which is not an unreasonable assumption. A comparison of experimental and calculated k_1 values is given in Table I.

TABLE I
RATE CONSTANTS OF METAL ION-ATP COMPLEXES[a]

Metal	Experimental k_1 (M^{-1} sec^{-1})	Experimental k_{-1} (sec^{-1})	Calculated k_1 (M^{-1} sec^{-1})	k_0 (sec^{-1})[b]
Ni^{2+}	4.1×10^6	0.38×10^2		10^4
Mg^{2+c}	1.2×10^7	1.2×10^3	4×10^7	10^5
Co^{2+}	9.2×10^7	2.0×10^3	8×10^7	2×10^5
Mn^{2+}	$>10^9$	$>10^4$	2×10^9	4×10^6
Ca^{2+c}	$>10^9$	$>10^5$	8×10^9	2×10^7

[a] 25°, 0.1 M KNO_3. [b] Eigen and Tamm (1962a,b). [c] Diebler et al. (1960).

The experimental and calculated values are in reasonable agreement considering the approximations involved in the calculations and the experimental uncertainties, and give added support to the previously proposed general mechanism for complex formation. No significant effect of the ligand on k_0 can be discerned from these data, unlike the cases of pyrophosphate and tripolyphosphate as ligands (Hammes and Morrell, 1964).

Although not many enzymatic systems have been studied in sufficient detail to describe the overall mechanism in terms of elementary steps, steady-state measurements have nevertheless allowed determination of lower bounds of individual rate constants. Maximum velocities rarely exceed 10^3 sec^{-1} (see Eigen and Hammes, 1963, for a discussion of this point). Hence, if the rate-determining process in many enzymatic processes involving ATP were the rate of dissociation of an enzyme-substrate-metal complex, it would appear on the basis of the magnitude of the metal-complex-dissociation-rate constants that Ca^{2+}, Co^{2+}, Mn^{2+}, and Mg^{2+} would be more catalytically important than Ni^{2+}. Since the actual role of the metal ion in the catalytic process may be involved in bond breaking, the efficiency of the metal ion involved may be related to its acidity, that is, to its ability to dissociate a proton from its innermost hydration shell. Because of the fact that the reverse protolytic reaction is diffusion controlled (Eigen, 1962) and therefore roughly the same for all divalent metal ions, the catalytic efficiency of the metal ions would then parallel their acid-ionization constants. The order of increasing acidity for the metals considered here is $Ca^{2+} < Mg^{2+} < Ni^{2+} < Co^{2+}$ (Basolo and Pearson, 1958), while the acid strength of Mn^{2+} is probably about the same as Mg^{2+}. On this basis, Ca^{2+} would seem to be least effective. This suggests that the optimal activating metals from the standpoint of rapid dissociation of complexes and high acidity are Mg^{2+}, Mn^{2+}, and Co^{2+}. In point of fact, many enzymes are activated quite efficiently by Mg^{2+}, Mn^{2+}, and Co^{2+}, whereas Ni^{2+} and Ca^{2+} are often ineffective. On the other hand, the exceptionally high magnitude of Ca^{2+}-complex-dissociation-rate constants may explain the increased efficiency of Ca^{2+} in the hydrolysis of ATP catalyzed by myosin ATPase (Eigen and Hammes, 1963). Of course the above discussion is an oversimplification, but it may provide some insight into the mechanism of enzymatic processes. Quite clearly both the equilibrium and kinetic properties of metal ions should be considered in assessing the role of metal ions in biological systems.

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