The inverse isotope cannot be explained entirely in terms of the difference of steric factors (*i.e.*, the fact that an X–D bond is about 0.01 Å. shorter than the X–H bond due to the anharmonicity of the Morse curve potential). Nor does the effect find its explanation in any possible electronic differences of hydrogen or deuterium.[2,10]

Robertson has reported inverse secondary isotope effectsi n the solvolyses of propyl and propyl-γ-d_3 compounds (k_H/k_D of 0.92–0.95)[11] and of methyl and methyl-d_3 compounds (bromide 9.90, iodide 0.87, tosylate 0.96).[12] Elliot and Mason found 0.86 for the pyridine-catalyzed reactions of aniline and aniline-N,N-d_2 with benzoyl chloride in benzene solutions at 25°.[13]

[10] R. E. Weston, Jr., *Tetrahedron*, **6**, 31 (1959). The author correctly points out that interaction between electronic and nuclear motion is very small (Born–Oppenheimer theory) and that all the evidence for an electronic isotope effect is explicable on the basis of vibrational effects.

[11] K. T. Leffek, J. A. Llewellyn and R. E. Robertson, *Chem. and Ind.*, 588 (1960).

[12] J. A. Llewellyn, R. E. Robertson and J. M. W. Scott, *ibid.*, 732 (1959).

[13] J. J. Elliot and S. F. Mason, *ibid.*, 488 (1959).

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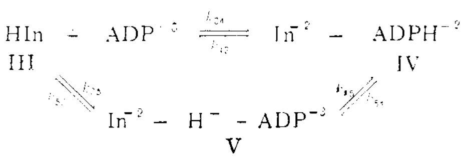
RECEIVED SEPTEMBER 7, 1960

KINETIC STUDIES OF ADP REACTIONS WITH THE TEMPERATURE JUMP METHOD

Sir:

A previous communication[1] described kinetic studies of the reactions of adenosine 5′-triphosphate (ATP) with Ca^{++}, Mg^{++} and a proton donor-acceptor system (phenol red). The purpose of this note is to present the results of a similar study with adenosine 5′-diphosphate (ADP). The temperature jump method[2,3] was used to obtain the relaxation spectrum of three systems: ADP and proton donor-acceptors (chlor phenol red = CPR, and phenol red = PR); ADP, Mg^{++} and a proton donor-acceptor (CPR); and ADP, Ca^{++} and a proton donor-acceptor (CPR). The method of analysis of the spectrum of relaxation times obtained is similar to that described previously[1] so that only the results and significant differences will be presented here.

As with ATP, a short relaxation time (10–70 μsec.) was found identically for all three systems (under equivalent conditions), while in the case of the system containing Mg^{++} (with CPR) a longer relaxation time (100–250 μsec.) was also present. The short relaxation time could be identified with the mechanism



where In represents the *p*H indicator used as the proton donor-acceptor (either CPR or PR) and all other symbols have their obvious meaning. By studying the concentration dependence of the relaxation time, all rate constants except k_{45} and k_{54} could be obtained. The results obtained for both ADP and ATP are summarized in Table I.

TABLE I

The temperature was 13° (±2°) for experimental reasons,[1] and the ionic strength 0.1 (KNO_3). The experimental error is about ±30%, from the evaluation of the relatively complicated form of the relaxation times (which are measured with a precision of about ±10%). For both ATP and ADP k_{54} can be estimated to the order of magnitude of 10^{11} M^{-1} sec.$^{-1}$. The equilibration of this step always was complete within the time of measurement. The longer relaxation time found in the presence of Mg^{++} can be explained by the mechanism

$$\begin{tikzcd}
\text{I}\ \mathrm{MeADP}^- + \mathrm{HIn}^- \arrow[r, shift left, "k_{12}"] \arrow[d, shift right, "k_{13}"'] & \mathrm{MeADPH} + \mathrm{In}^{-1}\ \text{II} \arrow[l, shift left, "k_{21}"] \arrow[d, shift right, "k_{24}"'] \\
\underset{\text{III}}{\mathrm{Me}^{+2} + \mathrm{ADP}^{-3} + \mathrm{HIn}^-} \arrow[r, shift left, "k_{34}"] \arrow[u, shift right, "k_{31}"'] & \underset{\text{IV}}{\mathrm{Me}^{+2} + \mathrm{ADPH}^{-2} + \mathrm{In}^{-2}} \arrow[l, shift left, "k_{43}"] \arrow[u, shift right, "k_{42}"']
\end{tikzcd}$$

Reactants	k_{34} × 10^{-1} M^{-1} sec.$^{-1}$	k_{43} × 10^{-1} M^{-1} sec.$^{-1}$	k_{35} × 10^{-4} M^{-1} sec.$^{-1}$	k_{53} × 10^{-11} M^{-1} sec.$^{-1}$	k_{43}/k_{34}[a]
ADP + PR	3.4	7	0.06	3.0	21
ADP + CPR	20	1	1.9	2.3	0.53
ATP + PR	4.8	7	0.06	3.0	15
ATP + CPR	20	0.8	1.9	2.3	0.39

[a] Determined from the equilibrium constants of ADP, ATP^{-4} and the indicators (measured by *p*H and spectrophotometric titration).

In calculating the relaxation time, the presence of the indicator must be taken into account and the complete expression given previously must be used to evaluate the data. By varying the concentration and *p*H (5.7–6.1) and utilizing the equilibrium constants determined by Martell and Schwarzenbach[4] (which refer to the conditions of our experiments, *cf.* also ref. 5), k_{13}, k_{31}, k_{24} and k_{42} could be obtained. These results together with data obtained previously for ATP are summarized in Table II. The precision of the rate constants is about ± 35% for the ADP reactions.

Since only one relaxation time is observed when Ca^{++} is substituted for Mg^{++}, the relaxation time characteristic of the Ca^{++}–ADP reaction must be shorter than 5 μsec. and a lower bound can be obtained for k_{31} and k_{13} (see ref. 1 for details of this procedure). These minimum values (which do not differ greatly from the upper limits for these rate constants) also are presented in Table II.

TABLE II

The temperature was 26° (+2°) and the ionic strength 0.1 *M* (KNO_3).

Reactants	k_{13} × 10^{-3} sec.$^{-1}$	k_{31} × 10^{-7} sec.$^{-1}$	k_{24} × M^{-1} sec.$^{-1}$	k_{42} × 10^{-6} M^{-1} sec.$^{-1}$	k_{31}/k_{13}[4] M^{-1}	k_{42}/k_{24}[4] M^{-1}
Mg^{++} + ADP	2.5	0.3	3	1	$10^{3.1}$	$10^{1.0}$
Mg^{++} + ATP	1.2	1.2	3	3	$10^{4.0}$	$10^{2.0}$
Ca^{++} + ADP	>400	> 25	..	..	$10^{2.8}$	..
Ca^{++} + ATP	>250	>100	..	..	$10^{3.6}$	$10^{1.8}$

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[1] H. Diebeler, M. Eigen and G. G. Hammes, *Z. Naturforsch.*, in press.

[2] G. Czerlinski and M. Eigen, *Z. Elektrochem.*, **63**, 652 (1959).

[3] H. Diebeler and M. Eigen, in preparation.

[4] A. E. Martell and G. Schwarzenbach, *Helv. Chim. Acta*, **39**, 653 (1956).

[5] R. A. Alberty and R. M. Smith, *J. Am. Chem. Soc.*, **78**, 2376 (1956); *cf.* also L. B. Nanninga, *J. Phys, Chem.*, **61**, 1144 (1957),

In discussing these results let us first consider the proton transfer rate constants found in Table I (k_{34} and k_{43}). It is evident that the faster rate constant for a given system is identical for ATP and ADP within experimental error. At first glance, this appears to be somewhat unexpected since the charges on ATP, ADP and the indicators are such that proton transfers involving ADP should be faster than those for ATP. However, the ionic strength effect partially cancels out the difference in charge between ATP and ADP.[6]

It now appears that proton transfer reactions, although fast,[7,8] are not always diffusion controlled even if the equilibrium favors the transfer in that direction. From these studies the difference between the donor and acceptor pK's (log k_{43}/k_{34}) seems to be important.

As expected, the metal ions react faster with ATP than ADP. The magnitude of the differences found is just about what would be estimated from elementary electrostatic considerations. The ionic strength effect of KNO_3 would not be of great importance here since the doubly positive Mg^{++} or Ca^{++} would undoubtedly dislodge any weakly bound potassium ion upon reaching the vicinity of the ATP molecule. Since the rate controlling step of complete complex formation is the substitution of a water molecule from the complex initially formed by the metal and ligand,[8] the charge of the partners after formation of the initial complex should be decisive.

From these studies of ATP and ADP, it is clear that although the equilibrium constants for Ca^{++} and Mg^{++} complex formation (k_{31}/k_{13} and k_{42}/k_{24}) do not differ greatly, the kinetics of the two ions differ by at least two orders of magnitude. This is a general property of the two metal ions[8] and may be of significance in explaining why the two metals differ greatly as enzymatic activators (*cf.* also ref. 1). This problem will be discussed in more detail in a forthcoming publication.[9]

We are indebted to the National Science Foundation for granting a postdoctoral Fellowship to G.G.H. during the tenure of which this work was done.

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[6] R. M. Smith and R. A. Alberty, *J. Phys. Chem.*, **60**, 180 (1956).

[7] A. Weller, *Z. Elektrochem.*, **64**, 55 (1960).

[8] M. Eigen, *ibid.*, **64**, 115 (1960).

[9] M. Eigen and G. G. Hammes, in preparation.

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RECEIVED SEPTEMBER 26, 1960

THE INFLUENCE OF STERIC FACTORS IN FAST PROTOLYTIC REACTIONS AS STUDIED WITH HF, H_2S AND SUBSTITUTED PHENOLS

Sir:

The development of relaxation spectrometry has led to the study of a wide variety of diffusion controlled protolytic reactions. The rate constants associated with these very rapid recombinations which are of the order of 10^{10} to 10^{11} M^{-1} sec.$^{-1}$ can be represented by a formula derived by Debye[1]

$$k_R = \frac{4\pi N_A\,(z_+ z_- e_0^2)(u_+ + u_-)}{\epsilon[\exp(z_+ z_- e_0^2/\epsilon a k T) - 1]} \qquad (1)$$

where N_A is Avogadro's number, $z_\pm$ the ionic valence, e_0 the elementary charge, $u_\pm$ the ionic mobilities ($u_i = D_i/kT$; D_i = diffusion coefficient of ion i) ϵ the dielectric constant, "a" an effective reaction distance, k Boltzmann's constant, and T the absolute temperature. At ionic concentrations less than $\sim 10^{-3}$ M the applicability of this equation is limited only by the fact that the dielectric constant depends upon the effective reaction distance a, and that steric factors may restrict the total number of possible approaches for successful encounters. It has been shown previously[2] that for most of these reactions the effective reaction distance is large enough (two to three hydrogen bonds, *i.e.*, approximately 7.5 Å.) to permit the use of the macroscopic dielectric constant. This investigation has been undertaken to demonstrate the influence of steric factors on the rate of diffusion controlled reactions. HF and H_2S have been chosen because of the simple structure of the anions.

The experiments were performed by using the dissociation field method.[3] The principle of this method is the perturbation of a system at chemical equilibrium by rapidly changing the electric field density (up to 10^5 kv./cm.). The measured increase in conductance under the application of high fields (and Wien effect) is a (known) function of the impulse duration due to relaxation. From the dispersion of the amplitude of the conductivity change in sinusoidal pulses the relaxation time necessary for the reattainment of equilibrium can be determined and related to the rate constants of the chemical reactions of the system.[4] Detailed descriptions of the 2nd Wien effect[5,6] and experimental procedures[3,4] may be found elsewhere.

For these investigations the H_2S was prepared from reagent grade FeS and HCl.[7] Similarly, reagent grade HF was used, and measurements were carried out in a glass vessel lined with pure paraffin wax. All measurements were done at 25° and at essentially zero ionic strength.

The reactions studied are

$$H^+ + HS^- \underset{k_D}{\overset{k_R}{\rightleftharpoons}} H_2S \qquad (I)$$

$$H^+ + F^- \underset{k_D}{\overset{k_R}{\rightleftharpoons}} HF \qquad (II)$$

where k_R has the same meaning as in equation (1), and k_D is the dissociation rate constant. The relaxation time is given by[3]

$$\tau = [k_D + k_R(C_+ + C_-)]^{-1} \qquad (2)$$

Since the equilibrium constants are known,[8,9] both rate constants may be determined. The rate constants obtained are:

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[1] P. Debye, *Trans. Electrochem. Soc.*, **82**, 265 (1942).

[2] M. Eigen and L. De Maeyer, *Proc. Roy. Soc.*, **A247**, 505 (1958).

[3] M. Eigen and J. Schoen, *Z. Elektrochem.*, **59**, 483 (1955).

[4] (a) M. Eigen, *ibid.*, **64**, 115 (1960); (b) L. De Maeyer, *ibid.*, **64** 65 (1960).

[5] M. Wien and J. Schiele, *Z. Physik*, **32**, 545 (1931).

[6] L. Onsager, *J. Chem. Phys.*, **2**, 599 (1934).

[7] "Handbuch der Praeparativen Anorganischen Chemie," Georg Bauer, Ferdinand Enke Verlag, Stuttgart, 1960, p. 313.

[8] Lange's "Handbook of Chemistry," Ninth Edition, p. 1198.

[9] H. H. Broene and T. De Vries, *J. Am. Chem. Soc.*, **69**, 1644 (1947).