Peters, R. A. (1954), *Endeavour 13*, 147.
Rétey, J., Lüthy, J., and Arigoni, D. (1970), *Nature (London)*, *226*, 519.
Rose, I. A. (1958), *J. Amer. Chem. Soc. 80*, 5835.
Rose, I. A. (1970a), *J. Biol. Chem. 245*, 6052.
Rose, I. A. (1970b), *in* The Enzymes, Boyer, P. D., Ed., Vol. II, 3rd ed, New York, N. Y., Academic Press.
Rose, I. A., and Rieder, S. V. (1958), *J. Biol. Chem. 231*, 315.
Rosso, R. G., and Adams, E. J. (1967), *J. Biol. Chem. 242*, 5524.
Sprecher, M., Berger, R., and Sprinson, D. B. (1964), *J. Biol. Chem. 239*, 268.
Srere, P. A. (1967), *Biochem. Biophys. Res. Commun. 26*, 609.
Swain, C. G., Stivers, E. C., Reuwer, J. F., Jr., and Schaad, L. J. (1958), *J. Amer. Chem. Soc. 80*, 5885.

Studies on the Allosteric Modification of Nucleoside Diphosphatase Activity by Magnesium Nucleoside Triphosphates and Inosine Diphosphate*

Vern L. Schramm† and John F. Morrison

ABSTRACT: A kinetic study has been made of the effects of various nucleotide species as modifiers of the reaction catalyzed by nucleoside diphosphatase when magnesium–inosine diphosphate is used as the substrate. The results showed that the magnesium complexes of both nucleoside and deoxynucleoside triphosphates, as well as free inosine diphosphate, are capable of activating the enzyme at lower concentrations of substrate. The experimental data have been analyzed in terms of the mechanism previously proposed for the reaction (*Biochemistry 8*, 3821 (1969)) and values obtained for the various kinetic constants. These indicate that the structure of the modifier determines the strength of its binding to free enzyme and that the resulting enzyme–modifier complexes have different abilities to combine with the substrate. Certain nucleotide species were also able to significantly lower the maximum velocity of the reaction.

Nucleoside diphosphatase (EC 3.6.1.6) catalyzes the hydrolysis of a wide range of nucleoside diphosphates and hence the reaction can be written in general form as

$$\text{nucleoside diphosphate} \xrightarrow{M^{2+}} \text{nucleoside monophosphate} + P_i$$

where M^{2+} represents an essential divalent metal ion which may be Mg^{2+}, Mn^{2+}, or Ca^{2+}. Characteristic features of the enzyme freshly prepared from rat liver are that it does not exhibit Michaelis–Menten kinetics and can be activated by $MgATP^{2-}$ which functions as an allosteric modifier (Yamazaki and Hayaishi, 1965, 1968; Schramm and Morrison, 1968). Detailed kinetic investigations of the reaction using $MgIDP^-$ as the substrate in the presence and absence of $MgATP^{2-}$ (Schramm and Morrison, 1969) have led to the proposal that the reaction proceeds *via* a rapid equilibrium, random mechanism that allows for (a) the interdependent reaction of two molecules of substrate at two identical catalytic sites, (b) differences in the rate of product formation from enzyme forms containing one or two molecules of substrate, and (c) combination of a modifier at a distinct site on the enzyme which can affect both the binding of substrate and rate of product formation.

Since various magnesium nucleoside and deoxynucleoside triphosphate complexes, as well as IDP^{3-}, are capable of activating the enzyme, it was of interest to determine the effect of these compounds relative to that of $MgATP^{2-}$. The results of such experiments have been found to be in accord with the mechanism previously proposed and quantitative analysis of the data in terms of the initial rate equation for the mechanism has shown the different nucleotide species exhibit varying abilities to combine with the enzyme and to affect the maximum velocity of the reaction.

Materials and Methods

All nucleoside and deoxynucleoside triphosphates were products of P-L Biochemicals. The deoxynucleoside triphosphates were not further purified as they were purchased just prior to use and did not contain significant amounts of the corresponding deoxynucleoside diphosphates. On the other hand, ITP, UTP, GTP, and CTP, which had been stored as the crystalline sodium salts at $-10°$ for about 2 years, contained 6–10% of the corresponding diphosphates. ITP was not purified since allowance could be made for the presence of IDP when calculating the total amount of IDP required to give particular concentrations of $MgIDP^-$ which was used as the substrate. The removal of CDP from solutions of CTP was also unnecessary as CDP is a poor substrate (Schramm and Morrison, 1968) and the amounts introduced with CTP would have no significant effect on the reaction velocity. Because $MgUDP^-$ and $MgGDP^-$ are good substrates for nucleoside diphosphatase, it was essential to purify the samples of UTP

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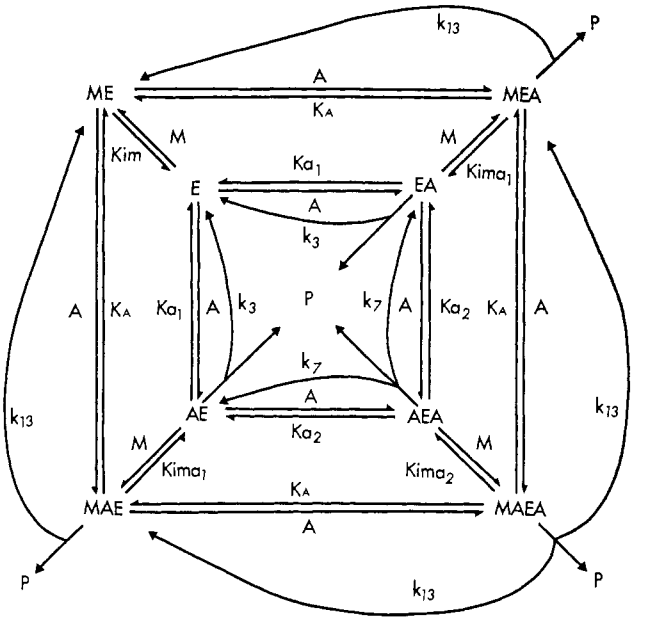
and GTP and this was done by a modification of the method of Hurlbert (1957). The nucleoside triphosphate (200 mg) was adsorbed onto a column (6 × 1.5 cm) of Dowex 1 (200–400 mesh, formate form) and eluted by the use of linear gradients of 0.4–1.0 or 0.2–0.8 N ammonium formate in 4 N formic acid. The total volume of the eluting solution was 200 ml. After elution from the column, the nucleoside triphosphates were adsorbed onto a pad of acid-washed Norit A in a small Büchner funnel, washed with water, and eluted with a mixture of ethanol–water (1:1, v/v). The eluent was made 85% with respect to ethanol and the nucleotide precipitated by the addition of a 50% molar excess of 3% (w/v) $BaBr_2$ solution. The precipitate was washed with absolute ethanol, anhydrous ether, and dried in a vacuum desiccator. A solution of the sodium salt was obtained by mixing a slurry of the barium nucleoside triphosphate into the top of a column (1.8 × 9 cm) of Zeo Carb 225 (Na^+ form) which was then washed with water. The purified preparations of UTP and GTP contained approximately 1% of the corresponding nucleoside diphosphate as determined by use of a coupled assay system involving pyruvate kinase and lactate dehydrogenase (Morrison and James, 1965). The other reagents and the preparation of nucleoside diphosphatase have been described previously (Schramm and Morrison, 1968).

Determination of Reaction Velocities. Enzymic activity was determined at 30° in the presence of 0.1 M triethanolamine-HCl buffer containing 0.01 mM EDTA (pH 8.5). The total volume of reaction mixtures was either 1.0 or 2.0 ml and the amount of enzyme added corresponded to 0.2–0.8 μg of protein/ml. $MgIDP^-$ was used as the variable substrate and unless free IDP^{3-} was also being varied, this nucleotide species was held constant at 0.1 mM at which concentration it has only a negligible ability to activate the reaction. The total concentrations of $MgCl_2$ and IDP to give the required concentrations of $MgIDP^-$ and free IDP^{3-} were calculated as described by Heyde and Morrison (1970). The stability constant for $MgIDP^-$ was taken to be 4000 M^{-1} (Schramm and Morrison, 1968; O'Sullivan and Perrin, 1964). For studies of the activation of the reaction by the magnesium complexes of the nucleoside and deoxynucleoside triphosphates, it was considered that their stability constants were similar to the value of 70,000 M^{-1} as determined for $MgATP^{2-}$ (O'Sullivan and Perrin, 1964) and that the complexes could be formed by the addition of equimolar amounts of $MgCl_2$ and the nucleoside or deoxynucleoside triphosphate. To ensure that initial velocities were being measured, reactions were run for two or three time periods. After stopping the reaction with acid, the formation of IMP or P_i (as indicated in the legends to the figures) was determined (Schramm and Morrison, 1968).

Analysis of Data. Experimental data were analyzed by the computer programs of Cleland (1963) in conjunction with an IBM 360 computer. Data which gave linear double-reciprocal plots of initial velocity as a function of substrate concentration were fitted to eq 1. Data yielding rectangular hyperbolas that did not pass through the origin were fitted to eq 2.

$$v = \frac{V[A]}{K_A + [A]} \tag{1}$$

SCHEME I



$$v = V\left(\frac{1 + \frac{[M]}{K_N}}{1 + \frac{[M]}{K_D}}\right) \tag{2}$$

Weighted mean values together with their standard errors, as well as the standard errors of sums, products, and quotients were calculated as described by Morrison and Uhr (1966).

Theory

From the results of previous studies (Schramm and Morrison, 1969) using $MgIDP^-$ as the substrate and $MgATP^{2-}$ as an allosteric modifier, it was concluded that the nucleoside diphosphatase reaction proceeds, under rapid equilibrium conditions, *via* the mechanism illustrated in Scheme I where E, A, and M represent enzyme, $MgIDP^-$ and $MgATP^{2-}$, respectively; K_{a1}, K_{a2}, and K_A represent dissociation constants for the reaction of A with E, EA, and AE, and ME, MEA, MAE, and MAEA, respectively; K_{im}, K_{ima1}, and K_{ima2} represent dissociation constants for the reaction of M with E, EA, and AE, and AEA, respectively. The initial rate equation for the mechanism can be expressed as

$$v = \frac{V\left[[A^2] + \frac{k_3}{k_7}K_{a2}[A] + \frac{k_{13}}{k_7}\frac{K_{a1}K_{a2}}{K_AK_{im}}\left(1 + \frac{[A]}{K_A}\right)[MA]\right]}{[A^2] + 2K_{a2}[A] + K_{a1}K_{a2} + \frac{K_{a1}K_{a2}[M]}{K_{im}} + \frac{K_{a1}K_{a2}}{K_AK_{im}}\left(2 + \frac{[A]}{K_A}\right)[MA]} \tag{3}$$

where $V = 2k_7E_t$. When M is present at concentrations which are high relative to the value of K_{im}, eq 3 would simplify to eq 4 which has the same form as eq 1.

$$v = \frac{2k_{13}E_t[A]}{K_A + [A]} \quad \text{or} \quad \frac{V^1[A]}{K_A + [A]} \tag{4}$$

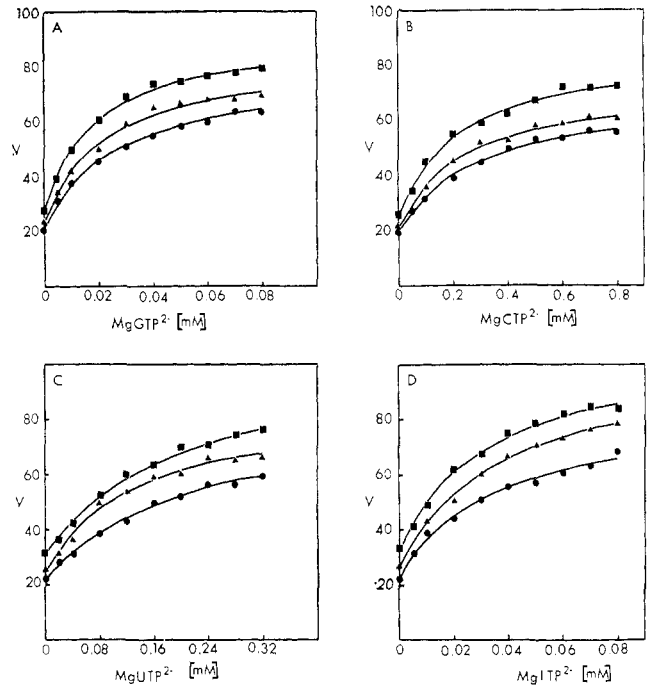


FIGURE 1: Effect of the concentration of magnesium–nucleoside triphosphate complexes on the initial velocity of the reaction. The concentrations of the substrate ($MgIDP^-$) were 0.20 (●), 0.25 (▲), and 0.30 (■) mM while the concentration of IDP^{3-} was held constant at 0.1 mM. The curves were drawn using the kinetic constants obtained by fitting the data to eq 2. Initial velocities are expressed as millimicromoles of IMP per minute per microgram of protein.

With M as the variable reactant, rearrangement of eq 3 would give

$$v = V^1 \left[\frac{1 + \dfrac{[M]}{\left([A] + \dfrac{k_3}{k_7} K_{a2}\right)\left(\dfrac{k_7 K_A 2K_{im}}{k_{13} K_{a1} K_{a2}(K_A + [A])}\right)}}{1 + \dfrac{[M]}{\dfrac{K_A{}^2 K_{im}([A]^2 + 2K_{a2}[A] + K_{a1}K_{a2})}{K_{a1}K_{a2}(K_A + [A])^2}}} \right] \quad (5)$$

where

$$V^1 = V\left[\frac{[A^2] + \dfrac{k_3}{k_7} K_{a2}[A]}{[A^2] + 2K_{a2}[A] + K_{a1}K_{a2}}\right]$$

Equation 5 has the same general form as eq 2. As IDP^{3-} and the magnesium complexes of CTP, GTP, ITP, and UTP had kinetic effects on the reaction similar to those reported for $MgATP^{2-}$ (Schramm and Morrison, 1969) eq 4 and 5 have been used in connection with the calculation of the values of the kinetic constants associated with the reaction of $MgIDP^-$ with the various EM complexes (K_A) and the reaction of the modifiers with different enzyme forms (K_{im}, K_{ima1}, and K_{ima2}).

Results

Kinetics of the Reaction in the Presence of High Concentrations of Modifiers. The initial velocity of the reaction was determined as a function of the concentration of $MgIDP^-$, over the range from 0.2 to 1.0 mM, in the presence of each of the modifiers (M) at fixed concentrations of 1.0, 2.0, and 3.0 mM. When the data, as obtained with the modifiers $MgCTP^{2-}$, $MgGTP^{2-}$, $MgITP^{2-}$, $MgUTP^{2-}$, and IDP^{3-}, were plotted in double-reciprocal form, each set gave three straight lines which were virtually superimposable. These results are in accord with the predictions of eq 4 and hence it may be considered that when any one of the above modifiers is present at a concentration greater or equal to 1.0 mM, its concentration is high relative to the value for K_{im} and that the horizontal intercepts of the plots yield true values for K_A which represents the dissociation constant for the reaction of substrate (A) with the ME, MEA, and MAE complexes (*cf.* eq 3 and 4). Analysis of the data gave values for K_A (Table I) which indicate that the chemical nature of the modifier influences the binding of the substrate to the enzyme–modifier complex. Further, as no inhibition was observed by increasing modifier concentrations, it may be concluded that no dead-end complexes were formed by the addition of modifier to both the allosteric and substrate sites.

TABLE I: Values of the Dissociation Constants (K_A) for the Combination of $MgIDP^-$ with various Enzyme–Modifier Complexes.

Modifier	K_A[a] (mM)
$MgITP^{2-}$	0.37 ± 0.01
$MgATP^{2-}$[b]	0.69 ± 0.03
$MgUTP^{2-}$	0.86 ± 0.11
$MgGTP^{2-}$	0.94 ± 0.06
$MgCTP^{2-}$	1.12 ± 0.03
IDP^{3-}	1.69 ± 0.08

[a] Values for K_A were determined from fits of the data to eq 1 and represent weighted means values from several experiments. [b] Determined previously by Schramm and Morrison (1969) and included here for comparative purposes.

Effect of the Concentration of Modifiers on the Reaction Velocity. Determination of the effect of increasing concentrations of the modifiers on the initial velocity of the reaction at different fixed, nonsaturating concentrations of the substrate gave the results illustrated in Figure 1. These indicate that the initial velocity increases as a hyperbolic function of the concentration of each of the four magnesium–nucleoside triphosphate complexes and a similar result was obtained with IDP^{3-}. It is apparent (Figure 1) that $MgITP^{2-}$ and $MgGTP^{2-}$ are better activators than either $MgUTP^{2-}$ or $MgCTP^{2-}$ as judged by the range of concentrations necessary to obtain maximum stimulation of the catalytic activity. Since all sets of data gave good fits to eq 2, it may be concluded that only one molecule of modifier undergoes reaction with the enzyme or that multiple molecules react in an independent manner. The results are, therefore, in agreement with the theoretical predictions of the mechanism outlined in Scheme I. The values for K_N and K_D, which were obtained by fitting the data of Figure 1 to eq 2, were used to determine the values of the dissociation constants for the various EM complexes (K_{im}) and the relative rates of product formation from the MAEA and AEA complexes (k_{13}/k_7). The values so obtained are recorded in Table II. It will be noted that the values for K_N and K_D are considerably more precise than those calculated for K_{im} and the k_{13}/k_7 ratio. This is a consequence of the

TABLE II: Values for the Apparent and True Kinetic Constants Associated with the Reaction of Magnesium–Nucleoside Triphosphates with Nucleoside Diphosphatase.

Modifier	Substrate Concn (mM)	K_N (mM)[a]	K_D (mM)[a]	K_{im} (mM)[b]	k_{13}/k_7[b]
$MgGTP^{2-}$	0.20	0.0080 ± 0.0009	0.029 ± 0.003	0.031 ± 0.013	0.61 ± 0.30
	0.25	0.0071 ± 0.0009	0.025 ± 0.003	0.027 ± 0.012	0.73 ± 0.34
	0.30	0.0062 ± 0.0005	0.020 ± 0.002	0.022 ± 0.009	0.87 ± 0.41
$MgCTP^{2-}$	0.20	0.0990 ± 0.0060	0.390 ± 0.027	0.39 ± 0.16	0.45 ± 0.22
	0.25	0.0725 ± 0.0068	0.265 ± 0.025	0.26 ± 0.10	0.64 ± 0.31
	0.30	0.0681 ± 0.0063	0.206 ± 0.019	0.21 ± 0.09	0.71 ± 0.34
$MgUTP^{2-}$	0.20	0.0584 ± 0.0055	0.229 ± 0.028	0.25 ± 0.10	0.61 ± 0.31
	0.25	0.0483 ± 0.0058	0.151 ± 0.024	0.17 ± 0.08	0.77 ± 0.38
	0.30	0.0628 ± 0.0102	0.215 ± 0.041	0.25 ± 0.10	0.62 ± 0.31
$MgITP^{2-}$	0.20	0.0128 ± 0.0013	0.048 ± 0.006	0.083 ± 0.037	0.32 ± 0.15
	0.25	0.0101 ± 0.0006	0.040 ± 0.002	0.076 ± 0.032	0.41 ± 0.20
	0.30	0.0087 ± 0.0009	0.029 ± 0.003	0.060 ± 0.025	0.47 ± 0.22
IDP^{3-}	0.10	0.100 ± 0.016	0.388 ± 0.070	0.37 ± 0.17	1.0 ± 0.57
	0.125	0.106 ± 0.013	0.395 ± 0.055	0.36 ± 0.17	1.0 ± 0.54
	0.167	0.081 ± 0.010	0.306 ± 0.036	0.28 ± 0.12	1.4 ± 0.75

[a] Values for the apparent constants, K_N and K_D, are weighted means of the values obtained by fitting to eq 2 at least two sets of data for each magnesium–nucleoside triphosphate including the sets of Figure 1. [b] Values for K_{im} were calculated from the given values for K_D using eq 5, the appropriate values for K_A (Table I) and the fixed substrate concentration (Figure 1), as well as values for K_{a1} and K_{a2} of 1.10 ± 0.38 and 1.98 ± 0.16 mM, respectively (Schramm and Morrison, 1969). The resulting values for K_{im} were then used, together with the values for the fixed substrate concentration (Figure 1), K_N, K_{a1}, K_{a2}, K_A (Table I), and a k_3/k_7 ratio of 0.17 ± 0.07 (Schramm and Morrison, 1969), to determine values for the various k_{13}/k_7 ratios from the relationship given in eq 5.

TABLE III: Summary of the Values for the Dissociation Constants Associated with the Reaction of Modifiers with Various Enzyme Forms and for the Ratios of the Maximum Velocities in the Presence and Absence of Modifiers.

Modifier	K_{im} (mM)[a]	K_{ima1} (mM)[b]	K_{ima2} (mM)[b]	k_{13}/k_7[a]	V^1/V[c]
$MgGTP^{2-}$	0.026 ± 0.006	0.022 ± 0.009	0.011 ± 0.004	0.71 ± 0.20	0.86 ± 0.12
$MgITP^{2-}$	0.070 ± 0.017	0.024 ± 0.010	0.004 ± 0.002	0.38 ± 0.11	0.43 ± 0.06
$MgATP^{2-}$ [d]	0.039 ± 0.010	0.024 ± 0.011	0.008 ± 0.004	0.42 ± 0.14	0.47 ± 0.02
$MgCTP^{2-}$	0.26 ± 0.06	0.26 ± 0.11	0.15 ± 0.06	0.56 ± 0.16	0.96 ± 0.15
$MgUTP^{2-}$	0.22 ± 0.05	0.17 ± 0.07	0.08 ± 0.03	0.65 ± 0.19	0.59 ± 0.06
IDP^{3-}	0.32 ± 0.08	0.49 ± 0.21	0.42 ± 0.18	1.10 ± 0.35	0.49 ± 0.05

[a] Values for K_{im} and k_{13}/k_7 are weighted means of those given in Table II. [b] Values for K_{ima1} and K_{ima2} were calculated from the relationships: $K_{a1}K_{ima1} = K_AK_{im}$ and $K_{a2}K_{ima2} = K_AK_{ima1}$ which must hold when the reactions of Scheme I occur under rapid equilibrium conditions. [c] The ratio of the maximum velocities in the presence and absence of modifiers V^1/V also gives a measure of the value for k_{13}/k_7 (*cf.* eq 3 and 4). The values represent weighted means of the ratios of V^1 and V obtained by fitting to eq 1 two sets of data for each modifier including each set of Figure 2. [d] Values of the constants associated with $MgATP^{2-}$ are taken from Schramm and Morrison (1969).

complexity of the calculations which involve the use of the values for three or four kinetic constants each of which has a standard error. Nevertheless, from the weighted mean values for K_{im} (Table III) it is clear that the magnesium complexes of the purine nucleoside triphosphates combine more readily with free enzyme than the magnesium complexes of the pyrimidine nucleoside triphosphates whose dissociation constants are similar to that for IDP^{3-}. The weighted mean values for the k_{13}/k_7 ratio (Table III) do not permit definitive conclusions to be reached about the effect of the modifiers on the rates of product formation in the presence of saturating concentrations of $MgIDP^-$. However, it does appear that when the magnesium complexes of ITP, CTP or UTP are present on the enzyme, the maximum velocity of the reaction is less than that obtained in their absence.

Maximum Velocities of the Reaction in the Absence and Presence of Modifiers. In order to obtain more direct evidence for the effect of modifiers on the reaction kinetics, the maximum

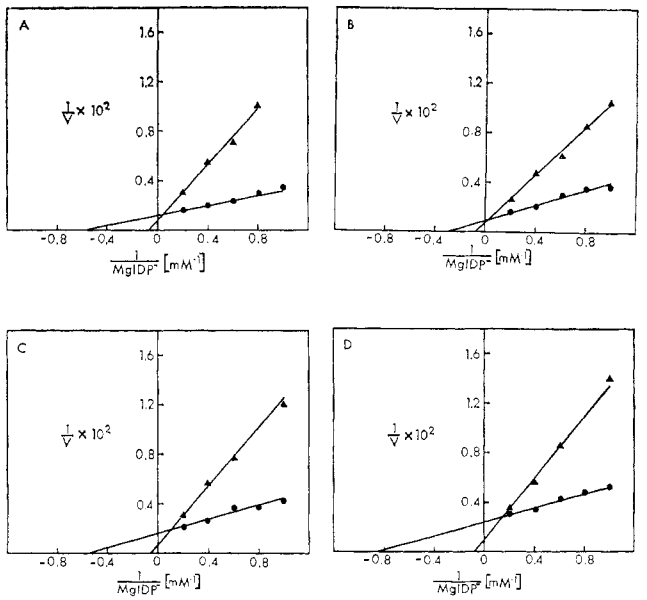


FIGURE 2: Determination of the maximum velocities of the reaction in the absence and presence of saturating concentrations of various magnesium-nucleoside triphosphate complexes. The concentrations of $MgGTP^{2-}$ (A), $MgCTP^{2-}$ (B), $MgUTP^{2-}$ (C), and $MgITP^{2-}$ (D) were varied with the substrate ($MgIDP^-$) in a constant ratio of 1:1 while free IDP^{3-} was held constant at a concentration of 0.1 mM. The initial velocity values in the presence (●) and absence (▲) of modifiers were corrected for the inhibition by free Mg^{2+}, as described in the text, before the data were fitted to eq 1. The resulting constants were used in connection with the drawing of the lines. Initial velocities are expressed as millimicromoles of inorganic phosphate per minute per microgram of protein.

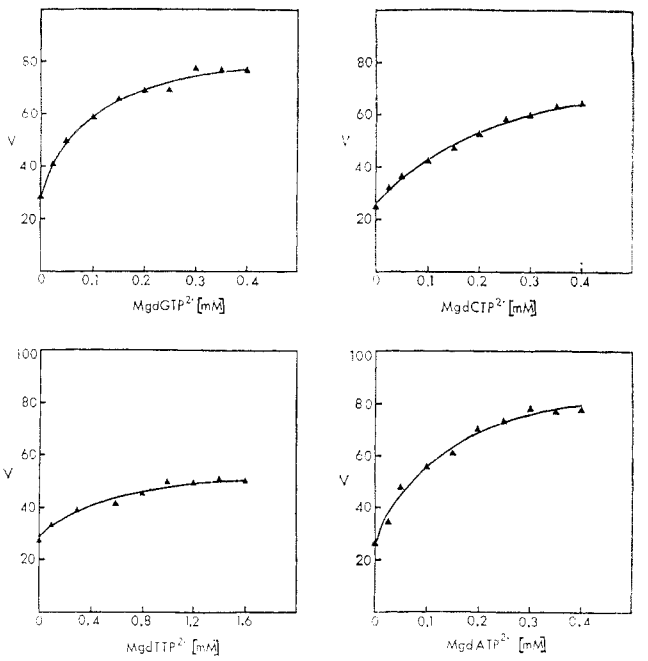


FIGURE 3: Effect of the concentration of magnesium–deoxynucleoside triphosphate complexes on the initial velocity of the reaction. The concentrations of the substrate ($MgIDP^-$) and free IDP^{3-} were held constant at 0.3 and 0.1 mM, respectively. The data were fitted to eq 2 and the constants obtained were used to draw the curves. Initial velocities are expressed as millimicromoles of IMP per minute per microgram of protein.

velocity of the reaction in the presence and absence of saturating concentrations of the modifiers was determined. For the determination of the maximum velocity in the absence of modifiers, initial velocities were measured over a range of higher substrate concentrations from 1.0 to 5.0 mM. Similar experiments were carried out using the same range of substrate concentrations and varying the substrate and modifier concentrations in constant ratio to determine the maximum velocity of the reaction in the presence of a saturating concentration of the modifier. The resulting data were then corrected for the inhibition by free Mg^{2+} which would be present at concentrations 2.5 times that of $MgIDP^-$ when free IDP^{3-} is held constant at 0.1 mM. It was taken that free Mg^{2+} acts as a noncompetitive inhibitor in the absence of modifiers and as a competitive inhibitor in the presence of each modifier since such results were obtained from studies with $MgATP^{2-}$ (Schramm and Morrison, 1970). The values previously determined for the inhibition constants associated with free Mg^{2+} were used to obtain uninhibited initial velocity values (Schramm and Morrison, 1969) which were then plotted in double-reciprocal form to give the results shown in Figure 2. These indicate that all the magnesium–nucleotide complexes are capable of activating the enzyme at lower substrate concentrations while they differ in their ability to influence the maximum velocity of the reaction.

Values for the maximum velocity of the reaction in the presence (V^1) and absence (V) of modifiers were obtained by fitting the data of Figure 2 to eq 1 and the values for k_{13}/k_7 given in Table III (column 6) were determined from the ratio of $V^1:V$ (*cf.* eq 3 and 4). In general, these values for k_{13}/k_7 are of a similar magnitude to those calculated from the data of Figure 1 but have lower standard errors (Table III, column 5). From these two sets of results and previous work with ATP (Schramm and Morrison, 1969), it may be concluded that $MgATP^{2-}$, $MgITP^{2-}$, and $MgUTP^{2-}$ have significant effects in lowering the maximum velocity of the reaction.

Kinetics of the Reaction in the Presence of Magnesium Deoxynucleoside Triphosphates. The magnesium complexes of deoxynucleoside triphosphates were also capable of functioning as modifiers of nucleoside diphosphatase. Increasing concentrations of these complexes in the presence of a relatively low fixed substrate concentration (0.3 mM) increased the initial velocity in a manner similar to that given by the magnesium nucleoside triphosphates (Figure 3). However, qualitative inspection of the data indicates that higher concentrations of the magnesium deoxynucleotide complexes are required to give activation equivalent to that achieved by the magnesium nucleotide complexes (*cf.* Figure 1). Further, it would appear that the apparent maximum velocity of the reaction in the presence of $MgdTTP^{2-}$ is much less than that observed with

TABLE IV: Apparent Kinetic Constants as Determined from the Results of the Activation of the Reaction by Magnesium–Deoxynucleoside Triphosphates.[a]

Modifier	K_N (mM)	K_D (mM)
$MgdGTP^{2-}$	0.033 ± 0.006	0.10 ± 0.02
$MgdATP^{2-}$	0.037 ± 0.008	0.14 ± 0.03
$MgdCTP^{2-}$	0.17 ± 0.02	0.63 ± 0.10
$MgdTTP^{2-}$	0.32 ± 0.19	0.66 ± 0.03

[a] Values for the constants were obtained by fitting the data of Figure 3 to eq 2.

the other modifiers. The apparent kinetic constants associated with the combination of the deoxynucleotide complexes are presented in Table IV.

Discussion

The results of the present investigation have shown that the magnesium complexes of a variety of nucleoside and deoxynucleoside triphosphates, as well as IDP^{-3}, can function as activators of nucleoside diphosphatase. Further, they indicate that the kinetic effects of these nucleotide species are similar to those exhibited by $MgATP^{2-}$ which has been demonstrated to act as an allosteric modifier of the enzyme (Schramm and Morrison, 1968, 1969). Thus the experimental data are consistent with the mechanism illustrated in Scheme I which allows for the reaction of two molecules of substrate at equivalent catalytic centers and the combination of one molecule of modifier at a distinct modifier site so as to affect the binding of substrate and the rate of product formation. It follows then that the data may be analyzed in terms of the initial rate equation (eq 3) describing the mechanism which occurs under rapid equilibrium conditions. From such analyses values may be obtained for the dissociation constants which give a measure of the binding of modifiers to free enzyme and enzyme–substrate complexes as well as the binding of substrate to the various enzyme–modifier complexes.

From the values obtained for the various dissociation constants it becomes apparent that the strength of binding of $MgIDP^-$ (A) to the different enzyme–modifier complexes (K_A) is dependent on the chemical nature of the modifier molecule. Indeed, it appears (Table I) that the binding of substrate can vary by a factor of four to five according to whether the modifier is $MgITP^{2-}$ or IDP^{3-}. The structure of the modifier also influences its combination with free enzyme (K_{im}) and the maximum velocity of the reaction (Table III). It is of interest that the magnesium complexes of the purine nucleoside triphosphates bind more strongly than the magnesium complexes of the pyrimidine nucleoside triphosphates and that none of the nucleotides tested are able to increase the maximum velocity of the reaction. From the values calculated for K_{ima1} and K_{ima2}, which represent dissociation constants for the reaction of a modifier with the EA (or AE) and AEA complexes, respectively, it is clear that there are differences in the degree to which the various nucleotide species are bound to the two enzyme–substrate forms (Table III).

The present work and that of Yamazaki and Hayaishi (1968) establish the broad specificity of nucleoside diphosphatase with respect to its reactions with modifiers while previous work (*cf.* Schramm and Morrison, 1968) has shown that the enzyme exhibits a broad substrate specificity. Because of the similarity in the chemical structure of the modifiers and substrates, it might have been expected that there would be certain similarities in the structures of the modifier and catalytic sites so that modifiers would react at the catalytic site to form dead-end complexes. As this type of reaction does not occur, it must be concluded that there are distinct differences in the structures of the modifier and catalytic sites.

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References

Cleland, W. W. (1963), *Nature (London) 198*, 463.
Heyde, E., and Morrison, J. F. (1970), *Biochim. Biophys. Acta 212*, 288.
Hurlbert, R. B. (1957), *Methods Enzymol. 11*, 238.
Morrison, J. F., and James, E. (1965), *Biochem. J. 97*, 37.
Morrison, J. F., and Uhr, M. L. (1966), *Biochim. Biophys. Acta 122*, 57.
O'Sullivan, W. J., and Perrin, D. D. (1964), *Biochemistry 3*, 18.
Schramm, V. L., and Morrison, J. F. (1968), *Biochemistry 7*, 3642.
Schramm, V. L., and Morrison, J. F. (1969), *Biochemistry 8*, 3821.
Schramm, V. L., and Morrison, J. F. (1970), *Biochemistry 9*, 671.
Yamazaki, M., and Hayaishi, O. (1965), *J. Biol. Chem. 240*, PC2763.
Yamazaki, M., and Hayaishi, O. (1968), *J. Biol. Chem. 243*, 2934.