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# A transition state analog for phosphate diester cleavage catalyzed by a small enzyme-like metal ion complex

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**Abstract**

The values of $K_i$ for methylphosphate dianion ($\mathbf{MP}^{2-}$) inhibition of the cleavage of 2-hydroxypropyl-4-nitrophenyl phosphate (**HpPNP**) catalyzed by 1,3-bis(1,4,7-triazacyclonon-1-yl)-2-hydroxypropane ($\mathbf{Zn_2(1)(H_2O)}$) approach a small limiting value of 6 μM at $pH < pK_a = 7.8$ for deprotonation of the catalyst to form $\mathbf{Zn_2(1)(HO^-)}$. There is a 1600-fold difference in the affinity of a phosphate diester monoanion (diethylphosphate) and phosphate monester dianion ($\mathbf{MP}^{2-}$) for $\mathbf{Zn_2(1)(H_2O)}$. This suggests that the latter is an analog for the transition state dianion for the cleavage reaction of **HpPNP** and other phosphate diesters. The observation that this transition state analog binds selectively to $\mathbf{Zn_2(1)(H_2O)}$ provides strong evidence that this is the active form of the catalyst which binds selectively to the ionized substrate. The efficiency of catalysis of the cleavage of phosphate diesters by $\mathbf{Zn_2(1)(H_2O)}$ and proteins is compared.


*Keywords:* Metal ion catalysis; Phosphate diester; RNA analog

## 1. Introduction

Much has been learned about the mechanism for catalysis of biological reactions through the study of reactions catalyzed by small and medium-sized molecules [1]. Simple Brønsted buffer acids and bases cause an increase in the rate of proton transfer reactions,

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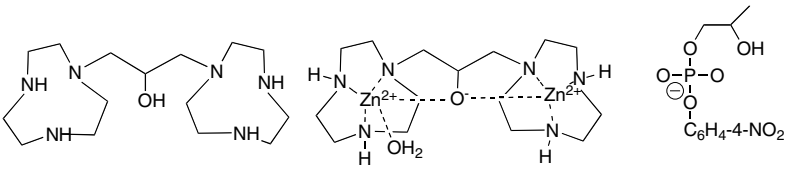
that are sometimes coupled to changes in bonding to heavy atoms, because they provide greater stabilization of the transition state from hydrogen bonding [2,3] and electrostatic interactions [4,5] than is observed for the competing reactions catalyzed by the conjugate acid or base of solvent, and because of the tendency of the strong solvation of hydroxide ion to reduce its reactivity as a specific base catalyst [6–8]. Metal dications provide greater electrostatic stabilization of the anionic transition state for deprotonation of carbon than do cationic buffers or hydronium ion [9]. Catalysis by small Brønsted acids and bases and by metal cations can be enhanced through the incorporation of simple recognition elements in synthetic catalysts. However, the activity of these small-molecule catalysts is far below that observed for large molecular weight protein catalysts.




**1** **$Zn_2(1)(H_2O)$** **HpPNP**

All catalysis results from the development of specific stabilizing binding interactions between the catalyst and the transition state for the catalyzed reaction [10–12]. Very good catalysts bind their respective transition state with an extraordinarily high affinity and this molecular recognition has often been mimicked in the tight binding of stable small molecule analogs of the transition states for protein-catalyzed reactions [10,11]. By comparison, scant attention has been paid to the possibility that transition state analogs might show a high affinity to low molecular weight synthetic catalysts.

**$Zn_2$(1)($H_2O$)** is an unusually potent *small molecule* catalyst where the two metal cations function cooperatively [13] to provide a stabilization of the transition state for phosphodiester cleavage in water that is a substantial fraction (−9.6 kcal/mol, ca. 50%) of that possible for a hypothetical protein catalyst of the same reaction [14]. We have proposed that this rate acceleration reflects the large selectivity of the catalyst for binding the transition state dianion compared with the reactant monoanion [15,16]. We report that methyl phosphate dianion (**$MP^{2-}$**) is a very strong competitive inhibitor of **$Zn_2$(1)($H_2O$)**-catalyzed cleavage of 2-hydroxypropyl-4-nitrophenyl phosphate (**HpPNP**) and by this criterion serves as an analog of the transition state for the catalyzed reaction. A comparison of the pH rate profile for **$Zn_2$(1)($H_2O$)**-catalyzed cleavage of **HpPNP** and the corresponding logarithmic profile of values of $K_i$ for inhibition of the reaction by **$MP^{2-}$** provide insight into the reaction mechanism. Finally, we speculate on the explanation for the apparent difference in the affinity of **$Zn_2$(1)($H_2O$)** for binding to the reaction transition state and to the transition state analog **$MP^{2-}$**.

## 2. Experimental

All buffers and **$Zn(NO_3)_2$** were reagent grade from Sigma–Aldrich. Water was distilled and then passed through a Milli-Q purification system. The ligand **1** [13,17] and the barium salt of 2-hydroxypropyl-4-nitrophenylphosphate (**HpPNP**) [18] were synthesized by literature procedures. The solution pH was determined at 25 °C using an Orion digital

pH meter equipped with a temperature compensation probe. Procedures described in earlier work were used to prepare solutions of $\mathbf{Zn_2(1)}$ for kinetic studies [14].

The following buffers were used in these experiments: *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid) (Hepes, pH 7.6–8.0) and 2-(*N*-cyclohexylamino)ethanesulfonic acid (CHES, pH 8.6–9.3). The solutions were prepared and the reactions were initiated as described in earlier work [13,14]. The transesterification of **HpPNP** was monitored by following the increase in absorbance at 400 nm due to the release of 4-nitrophenolate ion. Pseudo-first-order rate constants $k_{obsd}$ ($s^{-1}$) for transesterification of **HpPNP** were determined from the slopes of semilogarithmic plots of reaction progress against time, which were linear for at least three reaction halftimes.

## 3. Results

Fig. 1 shows the decrease in the normalized rate constants $k_{obsd}/k_o$ for the cleavage of **HpPNP** catalyzed by 0.10 mM $\mathbf{Zn_2(1)(H_2O)}$ in the presence of increasing concentrations of methylphosphate dianion ($CH_3OPO_3^{2-}$ or $\mathbf{MP^{2-}}$), where $k_{obsd}$ ($s^{-1}$) is the observed rate constant for the cleavage reaction and $k_o$ is the rate constant for reaction in the absence of the inhibitor dianion. These data show that $\mathbf{MP^{2-}}$ is a potent competitive inhibitor of the $\mathbf{Zn_2(1)(H_2O)}$-catalyzed cleavage reaction. The binding of $\mathbf{MP^{2-}}$ to $\mathbf{Zn_2(1)(H_2O)}$ is so tight that essentially no catalytic activity is observed when $[CH_3OPO_3^{2-}] > 10[\mathbf{Zn_2(1)(H_2O)}] = 10^{-4}$M. Therefore, it was not possible to work under conditions where the inhibitor concentration is very much larger than the catalyst concentration and make

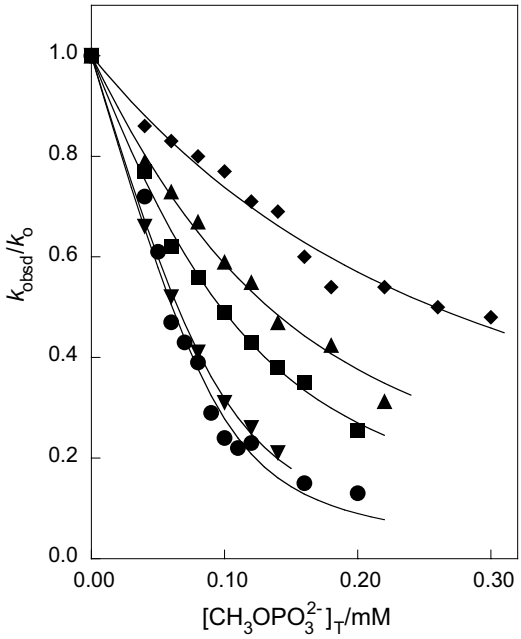



Fig. 1. The effect of increasing total concentration of $CH_3OPO_3^{2-}$ on the normalized rate constant for cleavage of **HpPNP** catalyzed by $[\mathbf{Zn_2(1)(H_2O)}] = [L]_T = 0.1$ mM. Key: (●), reactions at pH 7.6; (▼), pH 8.0; (■), pH 8.6; (▲), pH 8.9; (♦), pH 9.3.

the assumption in fitting these data that formation of the $\mathbf{Zn_2(1)(H_2O)}\bullet\mathbf{MP}^{2-}$ complex does not cause a significant decrease in the concentration of the unbound inhibitor. The solid lines in Fig. 1 show the least squares fit of data at different pH to Eq. (1), derived for a scheme where $\mathbf{MP}^{2-}$ and substrate compete for binding to $\mathbf{Zn_2(1)(H_2O)}$, where $[\mathbf{I}]_T = [CH_3OPO_3{}^{2-}]_T$ and $[L]_T = 10^{-4}$ M are the total concentrations, respectively, of all the different forms of the inhibitor and catalyst. This fitting procedure gave values of the dissociation constant $(K_i)_{obsd}$ for breakdown of the complex between $\mathbf{Zn_2(1)(H_2O)}$ and $\mathbf{MP}^{2-}$ to form the free catalyst.

The values of $(K_i)_{obsd}$ determined by fitting the data from Fig. 1 to Eq. (1) are reported in Table 1. No attempt was made to distinguish between inhibition by methyl phosphate monoanion and dianion, because the inhibitor ($pK_a = 6.3$) [19] was at least 95% ionized at the pH range examined in these experiments [7.6–9.3]. Table 1 also lists the value of $K_i = 16$ mM for inhibition of $\mathbf{Zn_2(1)(H_2O)}$-catalyzed cleavage of **HpPNP** by diethyl phosphate determined in earlier work [14]. This is 1600-fold larger than $K_i$ for inhibition by $\mathbf{MP}^{2-}$ at the same pH. Williams et al. also reported very recently a similar large difference in the values of $K_i = 10$ mM and 5 μM for inhibition by diethylphosphate monoanion and phenyl phosphate dianion, respectively, of **HpPNP** cleavage catalyzed by a highly reactive aqua form of a mononuclear Zn(II) complex [20]. Diethylphosphate inhibition of $\mathbf{Zn_2(1)}$-catalyzed cleavage of **HpPNP** was fit in earlier work to a scheme where the catalyst forms a complex with 2-molecules of inhibitor [14], consistent with independent binding of the two metal-coordinated macrocycles of $\mathbf{Zn_2(1)}$ to the inhibitor. By comparison the data in this work were fit to a scheme where $\mathbf{Zn_2(1)}$ binds a single molecule of $\mathbf{MP}^{2-}$. This provides evidence for *cooperativity* of the macrocycles in binding of both the dianionic inhibitor and the transition state for cleavage of **HpPNP** [13].

$$\frac{k_{obsd}}{k_o} = \frac{[L]_T - [I]_T - (K_i)_{obsd} + \sqrt{[L]_T^2 + [I]_T^2 + (K_i)_{obsd}^2 - 2[L]_T[I]_T + 2[L]_T(K_i)_{obsd} + 2[L]_T(K_i)_{obsd}}}{2[L]_T} \quad (1)$$

## 4. Discussion

The large decrease in $k_{obsd}$ for $\mathbf{Zn_2(1)(H_2O)}$-catalyzed cleavage of **HpPNP** observed as the concentration of $\mathbf{MP}^{2-}$ is increased (Fig. 1) shows that $\mathbf{Zn_2(1)(H_2O)}$ combines with

Table 1
Values of $(K_i)_{obsd}$ Determined for Inhibition of $\mathbf{Zn_2(1)(H_2O)}$-catalyzed cleavage of **HpPNP** at 25 °C and $I = 0.10$ maintained with $NaNO_3$

| pH | $(K_i)_{obsd}$ (mM) | |
|---|---|---|
| | $CH_3OPO_3{}^{2-}$ [a] | $(EtO)_2PO_2{}^-$ [b] |
| 7.6 | 0.010 ± 0.011 | 16 |
| 8.0 | 0.015 ± 0.0001 | |
| 8.6 | 0.047 ± 0.002 | |
| 8.9 | 0.083 ± 0.003 | |
| 9.3 | 0.22 ± 0.01 | |

[a] Values of $(K_i)_{obsd}$ determined by fitting the data from Fig. 1 to Eq. (1). The quoted errors are standard deviations.
[b] Ref. [14].

$\mathbf{MP}^{2-}$ to form an inactive complex. The 1600-fold difference in the values of $K_i$ at pH 7.6 for inhibition of $\mathbf{Zn_2(1)(H_2O)}$-catalyzed cleavage of **HpPNP** by diethylphosphate monoanion, a reactant analog, and by $\mathbf{MP}^{2-}$ is due to the much stronger stabilizing electrostatic interactions between the cationic dinuclear catalyst and the dianion compared to the monoanion. We propose that the tight binding of the dianion mimics the tight binding of $\mathbf{Zn_2(1)(H_2O)}$ to the dianionic transition state for the cleavage of **HpPNP**, and so by this criterion methylphosphate dianion is a transition state analog [10,11]. The value of $K_i = 6\ \mu M$ determined at low pH corresponds to an unusually strong binding affinity for small charged ions in water, because intermolecular electrostatic interactions in this solvent are normally strongly attenuated by the strong solvation of the uncomplexed ions.

Fig. 2 presents the pH profile of values of log $(K_i)_{obsd}$ for inhibition of $\mathbf{Zn_2(1)(H_2O)}$-catalyzed cleavage of **HpPNP** by $\mathbf{MP}^{2-}$. The solid line shows the least-squares fit of the data to Scheme 1 determined using Eq. (2), $K_a = 10^{-7.8}$ M [14] and $K_i = 6\ \mu M$, the limiting value for inhibition at low pH (see below), where the catalyst is fully protonated. Fig. 2 also presents the pH rate profile of second-order rate constants $k_{Zn}$ for $\mathbf{Zn_2(1)(H_2O)}$-catalyzed transesterification of **HpPNP** (Fig. 2) taken from earlier work [14]. The solid line shows the fit of these data to Eq. (3) determined using $K_a = 10^{-7.8}$ M, where $k_c$ is the limiting second-order rate constant at high pH.

$$(K_i)_{obsd} = \left(\frac{K_i(K_a + [H^+])}{[H^+]}\right) \tag{2}$$

$$k_{Zn} = \left(\frac{k_c K_a}{K_a + [H^+]}\right) \tag{3}$$

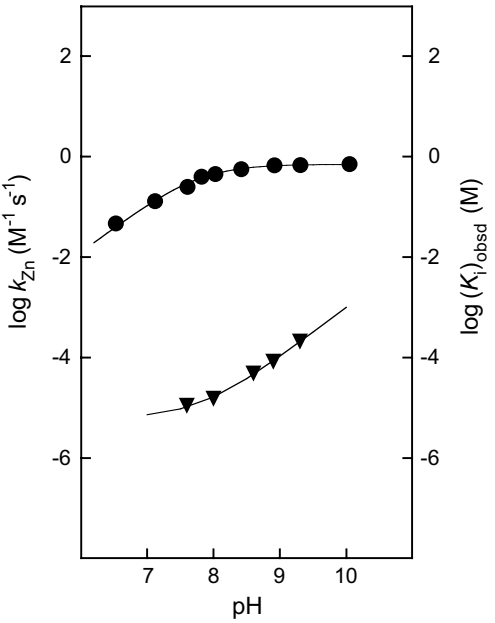
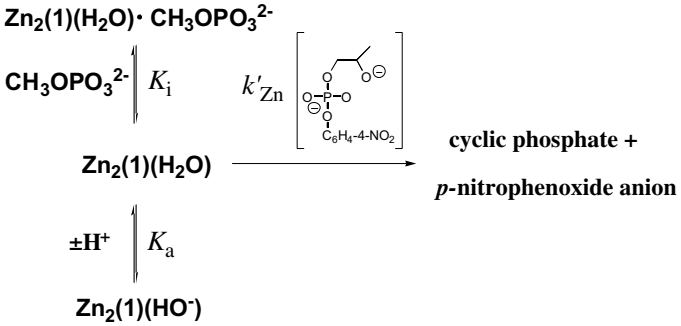



Fig. 2. The pH profiles for: (a) (▼), the observed inhibition constants $(K_i)_{obsd}$ for inhibition by methylphosphate dianion of $\mathbf{Zn_2(1)(H_2O)}$-catalyzed transesterification of **HpPNP**. (b) (●), second-order rate constants $k_{Zn}(M^{-1}\ s^{-1})$ for $\mathbf{Zn_2(1)(H_2O)}$-catalyzed cleavage of **HpPNP** determined in earlier work [14].




Scheme 1.

The pH profiles from Fig. 2 show breaks at $pH = pK_a = 7.8$ for ionization of $\mathbf{Zn_2(1)(H_2O)}$ to form $\mathbf{Zn_2(1)(HO^-)}$ [14]. This provides strong support for a mechanism in which $\mathbf{Zn_2(1)(H_2O)}$ is the active form of catalyst that is selective for binding and catalysis of the reaction of ionized substrate $\mathbf{HpPNP^-}$ (Scheme 1) [15,20]. The active catalyst $\mathbf{Zn_2(1)}(H_2\mathbf{O})$ shows optimal affinity for binding to the transition state analog methylphosphate dianion and this binding affinity falls off with deprotonation of the catalyst to form $\mathbf{Zn_2(1)(HO^-)}$. By comparison, there is an increase in the concentration of the ionized C-2 alkoxide form of $\mathbf{HpPNP^-}$ (a $pK_a \approx 12.8$ was estimated for the corresponding hydroxyl at uridine-3′-4-nitrophenyl phosphate and an even higher $pK_a$ is expected for **HpPNP**) [21] throughout the entire range of pH studied in these experiments, so that $k_{Zn}$ increases with increasing pH when the catalyst is fully protonated, but becomes pH independent at $pH > pK_a = 7.8$ because of the falloff in the fraction of the catalyst in the active form. The microscopic rate constant $k'_{Zn}$ must be much larger than the observed rate constant $k_{Zn}$, because only a small fraction of the total substrate is present in the reactive oxygen-ionized form.

The mechanism shown in Scheme 1 is also strongly supported by the absence of a primary solvent deuterium isotope effect on the cleavage of uridine 3′-4-nitrophenyl phosphate (**UpPNP**) [15]. Together these results show that the high catalytic activity of $\mathbf{Zn_2(1)(H_2O)}$ is due mainly to the strong stabilization of the transition state from electrostatic interactions between the cationic catalyst and the dianionic transition state for cleavage of phosphate diesters [13–15,22,23].

### 4.1. Transition state binding energy

Compared to enzymes, small molecules are relatively poor catalysts of organic reactions in water, because they do not normally develop strong interactions with their bound ligands. The complex that forms between $\mathbf{MP^{2-}}$ and $\mathbf{Zn_2(1)(H_2O)}$ ($K_i = 6\ \mu M$) represents an extraordinarily tight association of two small charged ligands in the polar solvent water. The large binding energy for association of $\mathbf{MP^{2-}}$reflects the great power of $\mathbf{Zn_2(1)(H_2O)}$ as a catalyst of phosphate diester cleavage through a dianionic transition state—the observed binding energy of −7.1 kcal/mol is 75% of the estimated −9.6 kcal/mol stabilization of the transition state for the cleavage of **HpPNP** by interaction with the $\mathbf{Zn_2(1)(H_2O)}$ catalyst [14].

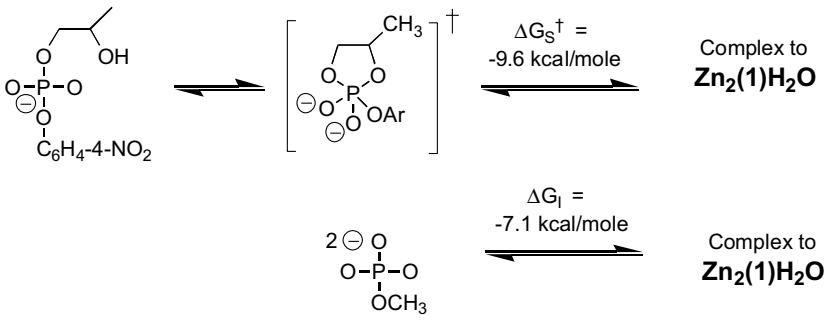
The 2.5 kcal/mol difference in the binding energies for stable $\mathbf{MP}^{2-}$ compared with the metastable dianionic phosphorane transition state is intriguing. The difference is probably not due to the smaller bulk of methylphosphate dianion compared with the transition state for cleavage of **HpPNP**, because optimal transition state stabilization is observed for catalysis of the cleavage of the minimal substrate **HpPNP** and falls off by 2.4 kcal/mol for the more bulky substrate **UpPNP** [23]. We have proposed that this falloff reflects steric hindrance to the interaction between **UpPNP** and the densely charged catalytic "core" of $\mathbf{Zn_2(1)(H_2O)}$ [23]. By comparison, there will be minimal steric hindrance to the approach of $\mathbf{MP}^{2-}$ to the metal cations in the complex with $\mathbf{Zn_2(1)(H_2O)}$.

The negative charge at methylphosphate dianion is delocalized over three oxygens at free $\mathbf{MP}^{2-}$. However, it is not clear that this should reduce the affinity of $\mathbf{MP}^{2-}$ for the formation of a divalent complex to $\mathbf{Zn_2(1)(H_2O)}$ compared with the localized dianion at an oxyphosphorane intermediate or oxyphosphorane-like transition state. This is because the three oxygen atoms at $\mathbf{MP}^{2-}$ will not remain equivalent in a complex to $\mathbf{Zn_2(1)(H_2O)}$. Rather, the negative charge will become localized at the two oxygens that interact most strongly with the metal cations, in order to ensure optimal electrostatic interactions.

We suggest that the 2.5 kcal/mol difference between the binding energy for the metastable transition state and the stable inhibitor (Scheme 2) is due to the stronger full "equilibrium" solvation of charge at $\mathbf{MP}^{2-}$ compared with the dianionic transition state, because reorganization of the solvation shell of the substrate monoanion that is required for optimal solvation is only partly complete [7,24,25]. This would lead to a *utilization* of a larger part of the intrinsic ligand binding energy to "pay the price" associated with *desolvation* of $\mathbf{MP}^{2-}$ [26], and a corresponding more favorable free energy of transfer of the incompletely solvated *metastable* transition (Scheme 2). In other words, the small catalyst $\mathbf{Zn_2(1)(H_2O)}$ may resemble enzymes by providing a mechanism to reduce the barrier to the solution reaction that arises from incomplete reorganization of solvent at the reaction transition state, by providing optimal stabilization of charge at a *preorganized* active site [27,28].

### 4.2. Analogies with enzyme-catalyzed reactions

An important goal of studies of small molecule catalysts such as $\mathbf{Zn_2(1)(H_2O)}$ is to obtain insight into the explanation for the differences in the catalytic activity of




Scheme 2.

large and small catalysts. The results reported here support the following generalizations.

(1) Effective, albeit nonspecific catalysis [16], is obtained by packaging $Zn^{2+}$ cations in a densely charged core where the interaction with dianions is strong, even in the polar solvent water where electrostatic interactions are normally strongly attenuated. A related and equally successful strategy is to enhance electrostatic catalysis by a single $Zn^{2+}$ through the incorporation of multiple hydrogen bond donors to stabilize the dianionic transition state for cleavage of **HpPNP** [20,29].
(2) Since $\mathbf{Zn_2(1)(H_2O)}$ derives its impressive catalytic activity essentially entirely from electrostatic interactions, even more impressive catalytic activity might be obtained through the incorporation into the catalyst of a fragment to provide binding recognition. In the same vein, effective catalysis can be obtained through the modular design of enzyme active sites so as to obtain additive interactions with several different portions of the substrate. This is the case for members of the enolase superfamily, which possess a common catalytic core that provides effective stabilization of related carbanion reaction intermediates, and a variable region to provide recognition of groups attached to the carbanion [30].

It has been proposed that enzymes with catalytic proficiencies of $([k_{cat}/K_m]_{cat}/k_{uncat}) > 10^{11}$ "*achieve over 15 kcal/mol of "transition state binding" not merely by concatenation of noncovalent effects but by covalent bond formation…*" [31]. This statement is controversial, in part because it invites debate about whether partly or largely electrostatic interactions, such as hydrogen-bonding interactions between enzyme and substrate or interactions between an enzyme-bound metal cation and liganded transition state, should be classified as covalent or noncovalent. We are reluctant to become involved in this debate, which places greater emphasis on the semantics for *classification* of transition state binding interactions than on the more informative *determination* of the magnitude of these interactions. However, we note: (1) A hypothetical catalyst with modules that show −10 kcal/mol and −5 kcal/mol interactions, respectively, with the transition state dianion and just a single free single uracil of the diribonucleoside uridylyl-3′5′-uridine (the −5 kcal/mol interaction for uracil was calculated from the value of $K_i = K_d \approx 0.1$ mM reported for the binding of uracil to uridine phosphorylase) [32], would provide a total binding energy of $(-10) + (-5) + (-6) = -21$ kcal/mol [−6 kcal/mol is the "connection" Gibbs free energy that represents the more favorable binding of tethered compared with individual ligands] [33] to the transition state for cleavage of this phosphodiester. (2) Interactions between a protein catalyst and dianionic substrate fragment of >10 kcal/mol can be obtained without the recruitment of a metal ion cofactor. Intrinsic substrate binding energies of 14 and 12 kcal/mol, respectively, have been estimated for the phosphate groups of D-glyceraldehyde 3-phosphate and orotidine 5′-monophosphate in reactions catalyzed without the assistance of metal cations by triosephosphate isomerase [34] and orotidine 5-monophosphate decarboxylase [35]. We suggest that stabilization of the enzyme-bound transition state for phosphate diester cleavage in excess of 15 kcal/mol is possible through transition state binding by electrostatic interactions with a metal cofactor or the potentially equally strong interactions of the transition state dianion with catalytic side-chains and the peptide

backbone of the protein catalyst, in combination with hydrophobic and other interactions with the nonreacting portions of the substrate.

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## References

[1] W.P. Jencks, Catalysis in Chemistry and Enzymology, Dover Publications, New York, 1987.
[2] E. Hartwell, D.R.W. Hodgson, A.J. Kirby, J. Am. Chem. Soc. 122 (2000) 9326–9327.
[3] W.P. Jencks, Acc. Chem. Res. 9 (1976) 425–432.
[4] A. Rios, T.L. Amyes, J.P. Richard, J. Am. Chem. Soc. 122 (2000) 9373–9385.
[5] J.P. Richard, W.P. Jencks, J. Am. Chem. Soc. 106 (1984) 1396–1401.
[6] M.W. Washabaugh, W.P. Jencks, J. Am. Chem. Soc. 111 (1989) 683–692.
[7] W.P. Jencks, S.R. Brant, J.R. Gandler, G. Fendrich, C. Nakamura, J. Am. Chem. Soc. 104 (1982) 7045–7051.
[8] A.J. Kresge, Chem. Soc. Rev. 2 (1973) 475–503.
[9] J. Crugeiras, J.P. Richard, J. Am. Chem. Soc. 126 (2004) 5164–5173.
[10] G.E. Lienhard, Science 180 (1973) 149–154.
[11] R. Wolfenden, Nature 223 (1969) 704–705.
[12] L. Pauling, Nature 161 (1948) 707–709.
[13] O. Iranzo, T. Elmer, J.P. Richard, J.R. Morrow, Inorg. Chem. 42 (2003) 7737–7746.
[14] O. Iranzo, A.Y. Kovalevsky, J.R. Morrow, J.P. Richard, J. Am. Chem. Soc. 125 (2003) 1988–1993.
[15] M.-Y. Yang, O. Iranzo, J.P. Richard, J.R. Morrow, J. Am. Chem. Soc. 127 (2005) 1064–1065.
[16] A. O'Donoghue, S.Y. Pyun, M.-Y. Yang, J.R. Morrow, J.P. Richard, J. Am. Chem. Soc. 128 (2006) 1615–1621.
[17] T.J. Atkins, J.E. Richman, W.F. Oettle, Org. Syn. 58 (1978) 86–98.
[18] D.M. Brown, D.A. Usher, J. Chem. Soc. (1965) 6558–6564.
[19] W.P. Jencks, J. Regenstein, Third Ed., in: G.D. Fasman (Ed.), Handbook of Biochemistry and Molecular Biology, Physical and Chemical Data, vol. 1, CRC Press, Cleveland, OH, 1976, pp. 305–351.
[20] G. Feng, J.C. Mareque-Rivas, R.T. Martin de Rossales, N.H. Williams, J. Am. Chem. Soc. 128 (2005) 13470–13471.
[21] Footnote 10 of Ref. [16].
[22] O. Iranzo, J.P. Richard, J.R. Morrow, Inorg. Chem. 43 (2004) 1743–1750.
[23] M.-Y. Yang, J.P. Richard, J.R. Morrow, Chem. Commun. (2003) 2832–2833.
[24] C.F. Bernasconi, Pure Appl. Chem. 54 (1982) 2335–2348.
[25] C.F. Bernasconi, Acc. Chem. Res. 25 (1992) 9–16.
[26] W.P. Jencks, Adv. Enzymol. Relat. Areas Mol. Biol. 43 (1975) 219–410.
[27] W.R. Cannon, S.J. Benkovic, J. Biol. Chem. 273 (1998) 26257–26260.
[28] A. Warshel, J. Biol. Chem. 273 (1998) 27035–27038.
[29] G. Feng, J.C. Mareque-Rivas, N.H. Williams, Chem. Commun. (2006) 1845–1847.
[30] P.C. Babbitt, M.S. Hasson, J.E. Wedekind, D.R.J. Palmer, W.C. Barrett, G.H. Reed, I. Rayment, D. Ringe, G.L. Kenyon, J.A. Gerlt, Biochemistry 35 (1996) 16489–16501.
[31] X. Zhang, K.N. Houk, Acc. Chem. Res. 38 (2005) 379–385.
[32] A. Kraut, E.W. Yamada, J. Biol. Chem. 246 (1971) 2021–2030.
[33] W.P. Jencks, Proc. Natl. Acad. Sci. USA 78 (1981) 4046–4050.
[34] T.L. Amyes, A.C. O'Donoghue, J.P. Richard, J. Am. Chem. Soc. 123 (2001) 11325–11326.
[35] T.L. Amyes, J.P. Richard, J.J. Tait, J. Am. Chem. Soc. 127 (2005) 15708–15709.