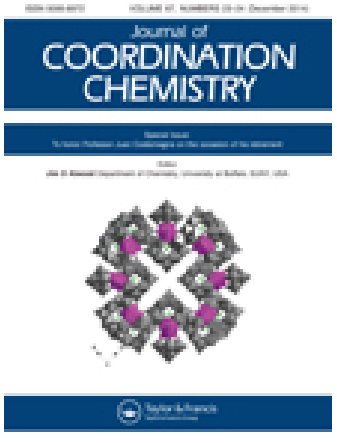

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Impact of divalent metal cations on the catalysis of peptide bonds: a DFT study

Brandall L. Ingle[a] & Thomas R. Cundari[a]
[a] Center for Advanced Scientific Computing and Modeling (CASCaM), Department of Chemistry, University of North Texas, Denton, TX, USA

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Impact of divalent metal cations on the catalysis of peptide bonds: a DFT study

BRANDALL L. INGLE and THOMAS R. CUNDARI*

Center for Advanced Scientific Computing and Modeling (CASCaM), Department of Chemistry, University of North Texas, Denton, TX, USA

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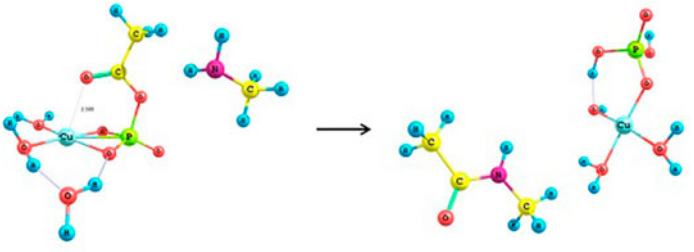


Within the ATP-grasp family of enzymes, divalent alkaline earth metals are proposed to chelate terminal ATP phosphates and facilitate the formation of peptide bonds. Density functional theory methods are used to explore the impact of metal ions on peptide bond formation, providing an insight into experimental metal substitution studies. Calculations show that alkaline earth and transition metal cations coordinate with an acylphosphate reactant and aid in the separation of the phosphate leaving group. The critical biochemical reaction is proposed to proceed through the formation of a six-membered transition state in the relatively nonpolar active site of human glutathione synthetase, an ATP-grasp enzyme. While the identity of the metal ion has a moderate impact on the thermodynamics of peptide bond formation, kinetic differences are much sharper. Simulations indicate that several transition metal ions, most notably Cu^{2+}, may be particularly advantageous for catalysis. The detailed mechanistic study serves to elucidate the vital role of coordination chemistry in the formation of peptide bonds.

Keywords: Metal cations; Peptide bond; Catalysis; ATP-grasp enzymes; DFT

1. Introduction

Coordination chemistry has a large role in many biological processes such as gene regulation, lipogenesis, oxygen transport, and photosynthesis [1–4]. Bioinorganic systems have influenced the design of numerous biomimetic inorganic catalysts, offering inspiration for macrocyclic ligands aimed at small molecule activation, heme-based hydrocarbon oxidation, and photocatalysis, as detailed in reviews by Costamagna and others [5–7]. The ATP-grasp family of enzymes provides an excellent example of biological coordination chemistry; each

*Corresponding author. Email: t@unt.edu

active site has alkaline earth metals complexed with terminal ATP phosphates [8, 9]. Experimental studies indicate that these metal ions are crucial for the activity of ATP-grasp enzymes, but the precise mechanism remains unclear [10, 11].

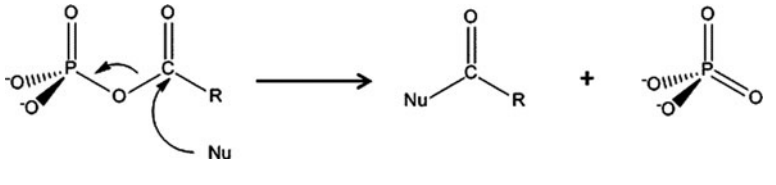
Members of the ATP-grasp family of enzymes participate in a variety of important biological pathways, including purine biosynthesis, lipid metabolism, and cell wall synthesis [8]. Such enzymes are increasingly pursued as targets for antibiotics [12–14]. However, further investigations are required to build a deeper understanding of the mechanisms of catalysis in ATP-grasp enzymes. While all members of the ATP-grasp family of enzymes catalyze the nucleophilic attack of a carboxylic acid, several form peptide bonds, as in the case of D-alanine-D-alanine ligase and glutathione synthetase [8, 12, 15]. Named for a characteristic ATP-binding site, ATP-grasp enzymes couple the cleavage of ATP into ADP and inorganic phosphate with the targeted ligation [16]. The reaction is hypothesized to proceed through the formation of an acylphosphate intermediate, which then undergoes nucleophilic attack, as shown in scheme 1 [17, 18].

Most ATP-grasp enzymes have one to three catalytic Mg^{2+} ions in the active site; the sole exception is synapsin Ia, which instead utilizes a single Ca^{2+} [19]. Crystal structures of several of these enzymes show the Mg^{2+} complexes with the terminal ATP-phosphates, waters, and charged or polar active site residues [20, 21]. An experimental study of glutathione synthetase found that the ATP-grasp enzyme requires a divalent cation to function [10]. Substitution of the native 12.5 mM Mg^{2+} with Mn^{2+} allowed the enzyme to function at a much lower cation concentration (2.25 mM), albeit maintaining only 73% of original activity. Similarly, addition of 5.0 mM Co^{2+} results in 77% activity relative to Mg^{2+}. Clearly, the identity of the metal cation can have a significant impact on the rate of peptide bond formation.

While previous computational studies have probed the role of metal cations in peptide bond formation, none have accounted for the interaction of the metal with the phosphate moiety of the acylphosphate intermediate found in ATP-grasp enzymes [22, 23]. In the present work, density functional theory methods are used to model the formation of a peptide bond from an acylphosphate and an amine. Models of this reaction with eight biologically relevant metal ions (Mg^{2+}, Ca^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+}) are used to assess the impact of the divalent metal cation upon the reaction pathway. The trends in reaction barrier across these metals not only elucidate critical aspects of peptide bond formation in biological systems but also provide a valuable insight into the role of metal ion identity in catalysis.

2. Computational methods

The Gaussian 09 software package was used for all calculations [24]. The B3LYP hybrid density functional was used with the 6-311+G(d) basis set [25–28]. All calculations were



Scheme 1. Peptide bond formation through acylphosphate intermediate.

conducted at 1 atm, 298.15 K. Chloroform ($\varepsilon = 4.7113$) was used as solvent to best approximate the relatively "greasy" nature of the binding sites in ATP-grasp enzymes [21, 29]. All species were modeled as neutrals, with the exception of the metal-free phosphate system (charge of −2). Spin multiplicity are noted for complexes with multiplicities not equal to 1; the high spin state was computed to be the ground state for all species investigated. Free energies are reported in kcal mol^{-1}. Optimized ground- and transition-state geometries contained zero and one imaginary frequencies, respectively, in the energy Hessian. Duplicate calculations were conducted with the B97D3 functional [30, 31]. Geometries mirrored those of B3LYP calculations. Ground state energies were within 10 kcal mol^{-1}, while barriers remained 3–13 kcal mol^{-1} lower than those with B3LYP (table S1, see online supplemental material at http://dx.doi.org/10.1080/00958972.2014.964223). Similar trends as a function of metal were observed with the B3LYP and B97D3 functionals.

3. Results

3.1. *Metal-free system*

A simplified metal-free system was used to highlight the importance of a metal cation on peptide bond formation. Acetic acid served as a generic carboxylic acid, while methylamine was used as a model for the nucleophile (figure 1). A catalytic water molecule was included in the transition state to aid in the transfer of a proton from the amine to the hydroxyl of the carboxylic acid. The reaction was modeled for the product with the methyl groups of acetic acid and methylamine in both *cis* and *trans* relative dispositions.

Calculations show that the *trans* product is more stable, a finding consistent with the preference for *trans* conformations about peptide bonds in nature. The ground states of the *cis* and *trans* products are fairly similar (*cis* product is 2.3 kcal mol^{-1} higher in energy), and

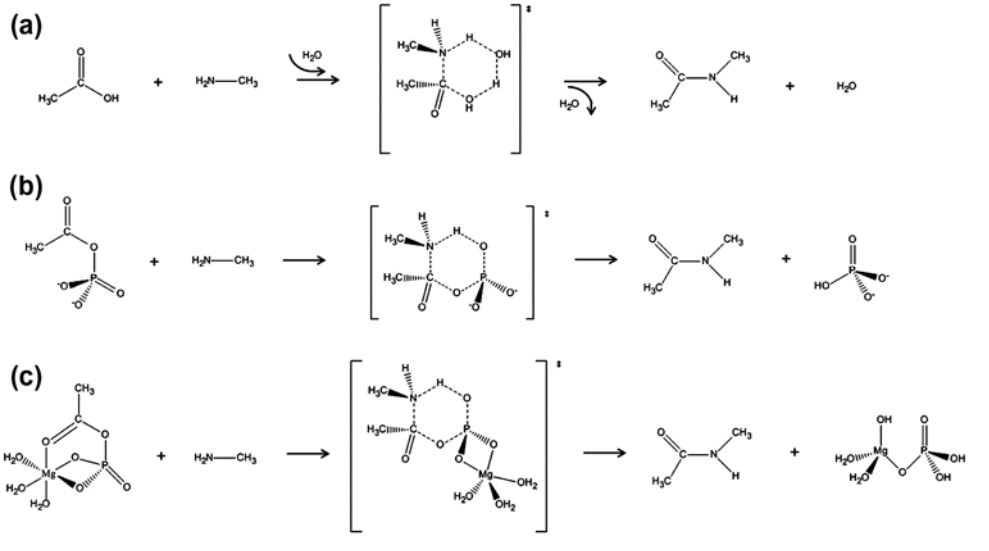


Figure 1. Modeled peptide bond formation in (a) metal free, (b) metal free acylphosphate, and (c) Mg^{2+} systems.

the barrier for the formation of the *cis* product is slightly higher than the *trans* (46.4 *versus* 45.7 kcal mol^{-1}, respectively). Therefore, only the *trans* conformation was considered for the remainder of the calculations. The transition state for the formation of the *trans* peptide bond has a planar six-membered ring with the carboxyl C, methylamine N and H, water O and H, and finally the acetic acid hydroxyl O (figure 1). As the peptide bond is formed between acetic acid and methylamine, a catalytic water shuttles a proton from the amine to the hydroxyl leaving group in an exergonic reaction ($\Delta G_{rxn} = -4.5$ kcal mol^{-1}).

Inclusion of a negatively charged phosphate group has a large impact on the energetics of the reaction. The overall reaction remains exergonic ($\Delta G_{rxn} = -3.5$ kcal mol^{-1}). The transition state no longer requires catalytic water; instead a six-membered ring forms wherein a phosphate oxygen molecule accepts the hydrogen directly (figure 1). It follows that the barrier for the reaction is much lower (31.1 kcal mol^{-1}). As hypothesized in the experimental literature for ATP-grasp enzymes [17, 18], the formation of an acylphosphate reactant makes the ensuing peptide bond formation more highly favored kinetically, with only slight thermodynamic differences.

3.2. *Mg^{2+} complex*

As the native metal for most ATP-grasp systems, Mg^{2+} serves as an ideal baseline for understanding the role of metal cations in the formation of peptide bonds. Consistent with crystal structures [19, 20], the lowest energy arrangement for the Mg^{2+} complexes is a six-coordinate geometry with an acylphosphate and three water molecules as ligands. In the resulting octahedral complex, two O atoms of the phosphate group bind the Mg^{2+} equatorially (2.10 and 2.10 Å), while the carboxyl O binds axially (2.13 Å). The three water molecules fill the remainder of the coordination sites (2.08, 2.08, and 2.10 Å) as shown in figure 2. These interaction lengths are consistent with the bond lengths of Mg^{2+} complexes found in the Cambridge structural database (CSD) [32]. In the product form, Mg^{2+} forms a tetrahedral complex with the phosphate, two waters, and a hydroxide (1.93, 2.04, 2.04, and 1.90 Å, respectively). Hence, a proton transfers from a water ligand to the phosphate group to form the preferred $Mg(H_2PO_4)(OH)(H_2O)_2$ tautomer (figure 3). The overall reaction is exergonic by −4.1 kcal mol^{-1}.

Two transition states were explored for the Mg^{2+} system. First, catalytic water was used as in the metal-free system to shuttle a proton from the amine to the phosphate leaving group. This transition state possesses an eight-membered ring (figure 4), wherein the peptide bond is formed; as the acylphosphate bond breaks, the phosphate group accepts a proton from water, and the water accepts a proton from the amine. The reaction free energy barrier is 45.4 kcal mol^{-1} and the enthalpic barrier is 23.6 kcal mol^{-1}.

In contrast, a six-membered transition state without the catalytic water has a lower free energy barrier (38.1 kcal mol^{-1}). While the six-membered transition state has slightly higher enthalpic barrier (26.4 kcal mol^{-1}) than the eight-membered transition state, the drastic change in entropy suggests that the six-membered state is preferred. Thus, further calculations on other divalent metal ions focused on the six-membered transition state, where formation of the peptide bond between carboxyl C and methylamine N is accompanied by proton transfer to the phosphate and cleavage of the acylphosphate bond (figure 3). While the computed barriers may seem high upon initial observation, it should be noted that consideration of the enzymatic environment necessary for the reaction to occur has been neglected. As such, these barriers provide a reasonable approximation for exploring trends among metal systems.

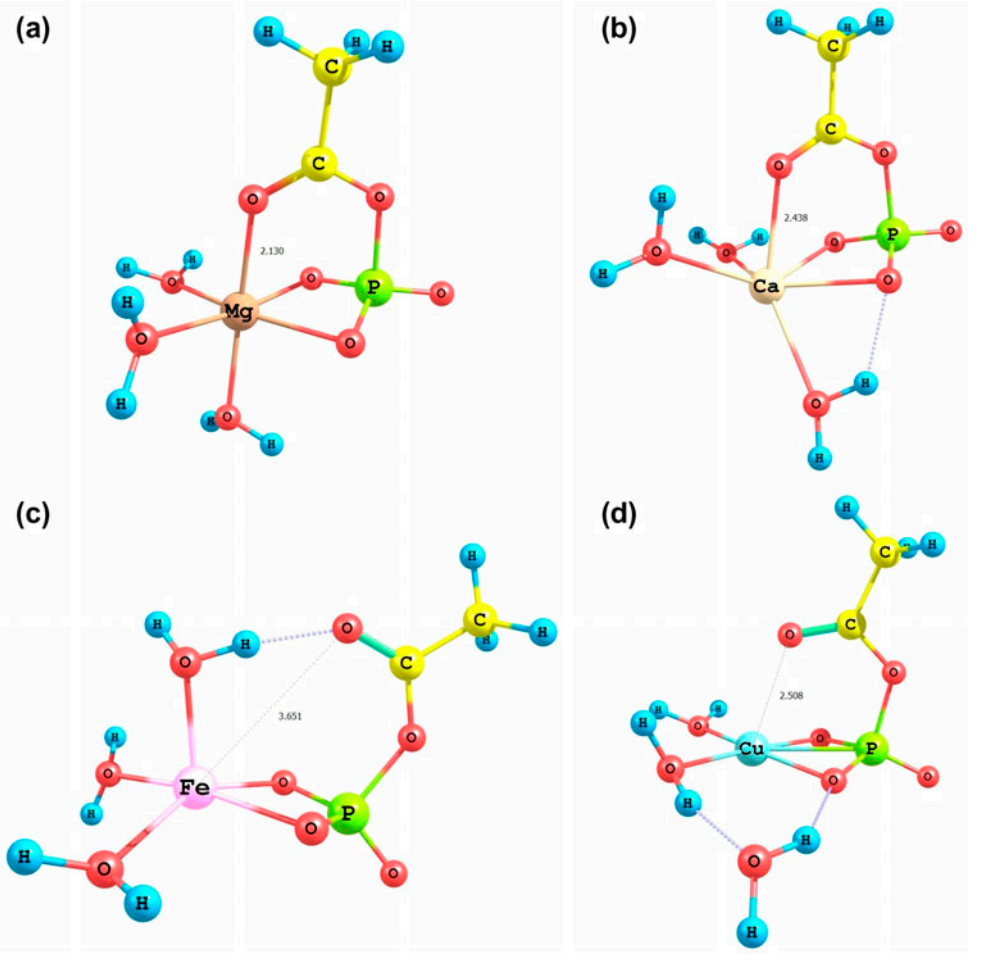


Figure 2. Reactant acylphosphate complexes with (a) Mg^{2+}, (b) Ca^{2+}, (c) Fe^{2+}, and (d) Cu^{2+}.

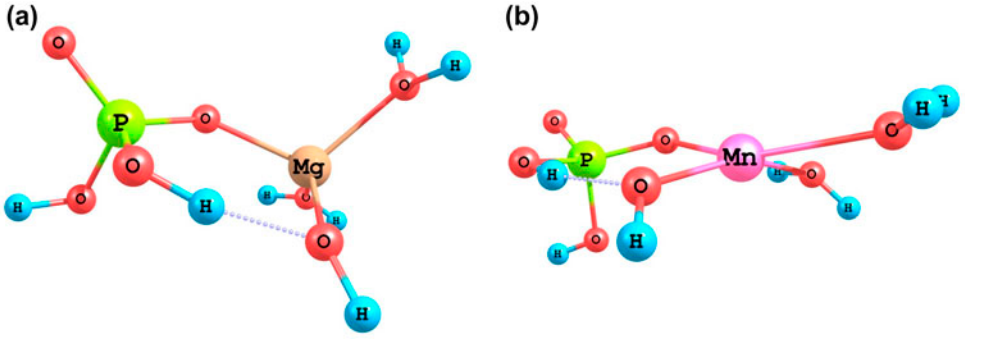


Figure 3. Product metal phosphate complexes with (a) Mg^{2+} and (b) Mn^{2+}.

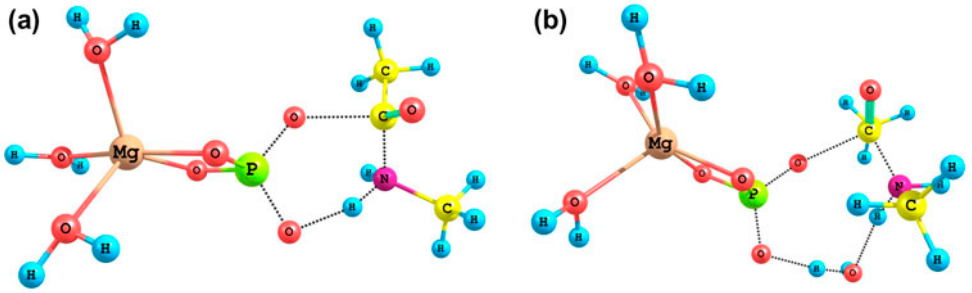


Figure 4. Transition states for Mg^{2+} catalyzed synthesis of a peptide bond with (a) six-membered ring and (b) eight-membered ring with catalytic water. The ring of atoms involved in each transition is shown with dotted black lines. Atom colors are as follows: H is blue, C is yellow, N is purple, O is red, Mg is tan, and P is green (see http://dx.doi.org/10.1080/00958972.2014.964223 for color version).

3.3. *Ca^{2+} complex*

The reaction pathway with a Ca^{2+} complex is remarkably different from the Mg^{2+} pathway, even though this cation serves as the native ion for the ATP-grasp enzyme synapsin Ia. As shown in figure 2, the starting geometry of the acylphosphate Ca^{2+} complex approximates a distorted trigonal prism with two O atoms from the phosphate and the carboxyl O forming one trigonal face and three water molecules forming the other (2.39–2.44 Å). The overall reaction is endergonic by 2.2 kcal mol^{-1}. The product Ca^{2+} complex is a distorted tetrahedral with the phosphate, two waters, and a hydroxide as ligands (2.28, 2.44, 2.41, and 2.18 Å, respectively). As with the Mg^{2+} complex, the $Ca(H_2PO_4)(OH)(H_2O)_2$ tautomer is preferred over the $Ca(HPO_4)(H_2O)_3$ tautomer.

The transition state for peptide bond formation exhibits a six-membered ring similar to that seen with the Mg^{2+} complex. The reaction barrier for the Ca^{2+} system (31.1 kcal mol^{-1}) is lower than that of the Mg^{2+} and metal-free systems. The lowered barrier relative to Mg^{2+} may be due in part to the loose coordination of the acyl O by the Ca^{2+} in the reactant, which allows the system to adopt a more favorable orientation with respect to the methylamine (figure 2).

3.4. *Mn^{2+} complex*

Several multiplicities of Mn^{2+} complexes were modeled. The high spin complexes are calculated to be 30–60 kcal mol^{-1} lower in energy than the quartet and doublet complexes. Therefore, high spin sextet complexes are the focus of further discussion. The overall reaction pathway for the sextet Mn^{2+} system is exergonic by −8.8 kcal mol^{-1}. The reactant acylphosphate Mn^{2+} complex is a distorted octahedral with the carboxyl O, bidentate phosphate, and three waters (2.14–2.30 Å). Bond distances are consistent with Mn^{2+} complexes in the CSD [32]. Upon formation of the peptide bond, square planar Mn^{2+} with phosphate, a hydroxide, and two waters coordinated (1.97–2.22 Å) act as the leaving group (figure 3).

Calculations show that the reaction proceeds through a six-membered transition state similar to that of the Mg^{2+} system. The free energy barrier for this reaction is 36.1 kcal mol^{-1}. The Mn^{2+} system has a more favorable reaction pathway than the Mg^{2+} system in terms of barriers and overall energetics (Mg^{2+}: $\Delta G_{rxn} = -4.1$ kcal mol^{-1}, $\Delta G^{\ddagger} = 38.1$ kcal mol^{-1}). The

Table 1. Energetics of peptide bond formation in the presence of metal cations. All values are free energies in kcal mol^{-1}. Calculations with B3LPY//6-311+G(d).

System	ΔG_{rxn} (B3LYP)	ΔG^{t} (B3LYP)
Metal free	−4.51	45.66
Metal free PO_4^{2-}	−3.47	31.11
Mg complex	−4.07	38.07
Ca complex	2.16	31.06
Mn complex	−8.82	36.14
Fe complex	−12.50	32.58
Co complex	−7.78	34.76
Ni complex	0.04	38.49
Cu complex	−7.28	25.20
Zn complex	−11.67	34.40

finding that the Mn^{2+} pathway is more favorable than the Mg^{2+} pathway is consistent with the experimental studies of cation substitution in glutathione synthetase, which found that Mn^{2+} serves as a more potent ion in the catalysis of peptide bond formation [10].

3.5. *Fe^{2+} complex*

Of the systems studied, the Fe^{2+} reaction pathway was the most thermodynamically favored, with a ΔG_{rxn} of −12.5 kcal mol^{-1} (table 1). The quintet acylphosphate Fe^{2+} complex displays square pyramidal coordination about the metal cation. In the equatorial positions, two water molecules and bidentate phosphate coordinate around the Fe^{2+} (2.00–2.21 Å). The axial water molecule forms a hydrogen bond to the carboxyl O as shown in figure 2. The quintet Fe^{2+} phosphate product is a tetrahedral complex, with two waters, a hydroxide, and phosphate complexed around the metal ion (1.90–2.14 Å). Consideration of triplet and singlet spin states yielded geometries that are 35–50 kcal mol^{-1} higher in energy than the quintet for the Fe^{2+} models.

The peptide bond transition state for the Fe^{2+} system mirrors the six-membered ring of the Mg^{2+} model. The barrier for the reaction is 32.6 kcal mol^{-1}. It is hypothesized that the lower barrier for Fe^{2+} relative to Mg^{2+} and Mn^{2+} systems may be due in part to the lack of interaction between Fe^{2+} and the carboxyl O, which would decrease the energy needed to properly orient the acylphosphate relative toward the amine.

3.6. *Co^{2+} complex*

The reactant acylphosphate forms an octahedral complex with the quartet Co^{2+} cation. The carboxyl O and a water molecule occupy axial positions (2.14 and 2.14 Å, respectively), while a bidentate phosphate and two waters fill the equatorial sites (2.08–2.15 Å). In the product, the phosphate chelates the quartet Co^{2+} in mondentate manner, with two waters and a hydroxide filling the other coordination sites of the tetrahedral-like complex (1.89–2.08 Å). The reaction is exergonic by –7.8 kcal mol^{-1}. Consideration of low spin (doublet) states found structures with ~15 kcal mol^{-1} higher in energy than the corresponding quartets.

The transition state for the Co^{2+} complex follows the pattern of the other transition metal complexes. The barrier of 34.8 kcal mol^{-1} for the Co^{2+} system is lower than that of the Mg^{2+} system, a finding supported by experimental cation substitution studies [10]. The barrier to

peptide bond formation is ~3.5 kcal mol^{-1} lower in the Co^{2+} system than in the Mg^{2+}, a difference that may explain the high rates of catalysis at lower Co^{2+} concentrations, which is shown experimentally [10].

3.7. *Ni^{2+} complex*

Calculations indicate that the reaction of a complexed Ni^{2+} acylphosphate with amine to form a peptide bond is nearly energetically neutral (ΔG_{rxn} = 0.0 kcal mol^{-1}). The triplet reactant octahedral Ni^{2+} complex includes an equatorially coordinated bidentate phosphate, an axially coordinated carboxyl O, and three water molecules (2.08–2.10 Å). Among all the metal ions studied, Ni^{2+} has the shortest bond to the carboxyl O (2.09 Å). In the product form, a monodentate phosphate binds a triplet Ni^{2+} with two waters and a hydroxide in a tetrahedral complex (1.89–2.05 Å). Singlet ground states are 10–15 kcal mol^{-1} higher in energy than the triplet states and thus are deemed to be chemically irrelevant.

The free energy barrier for the Ni^{2+}-mediated peptide bond formation is 38.5 kcal mol^{-1}, the highest among all the metal systems studied. As with other metals, the transition state adopts a six-membered ring for the formation of the peptide bond. Overall, peptide bond formation in the presence of Ni^{2+} is computed to be the least favored both thermodynamically and kinetically among the metal systems studied, perhaps in part due to the tight carboxyl coordination.

3.8. *Cu^{2+} complex*

The square planar doublet Cu^{2+} acylphosphate complex contains two waters and a bidentate phosphate (2.02, 2.00, 2.00, and 1.98 Å). The carboxyl group is directly above the Cu^{2+} at a distance of 2.51 Å (figure 2). A third water is bound to a coordinated water and phosphate through hydrogen bonds. Similarly, the doublet Cu^{2+} phosphate product is square planar around the Cu^{2+} with a monodentate phosphate, two waters, and a hydroxide (1.89–2.08 Å). The reaction is exergonic by −7.3 kcal mol^{-1}.

As in the previously described systems, the transition state for the formation of the peptide bond is associated with the simultaneous loss of the acylphosphate bond and the transfer of the amine hydrogen. The Cu^{2+} ion in the transition state maintains the same square planar geometry exhibited by the ground state complexes. A relatively low free energy barrier of 25.2 kcal mol^{-1} may be associated with the lack of interaction between the metal cation and the carboxyl group, as the rotated carboxyl group is the sterically favored conformation for the association of the amine preceding peptide bond formation. Of all the metal ions studied, the Cu^{2+} system has the lowest barrier by 5.9 kcal mol^{-1}. When coupled with the overall reaction energetics, the simulation suggests that Cu^{2+} may be the best cation for catalyzing the reaction, and certainly is an ion that warrants further experimental scrutiny for ATP-grasp enzyme catalysis of peptide bonds.

3.9. *Zn^{2+} complex*

The octahedral Zn^{2+} reactant complex includes a bidentate phosphate and two waters in equatorial positions, while a water and carboxyl O coordinate axially (2.11–2.19 Å). The product phosphate is complexed through a single O with the tetrahedral Zn^{2+} with two waters and hydroxide coordinated (1.90–2.12 Å). The reaction is exergonic by −11.7 kcal mol^{-1}.

The transition state for the Zn^{2+} system mimics that of the Mg^{2+} system with a six-membered ring central to the reaction. The free energy barrier for this reaction (34.4 kcal mol^{-1}) lies in the middle of the metal systems studied. It is interesting to note that while the ground state geometries of the Zn^{2+} system differ from the Fe^{2+} system, these cations have similar energetics.

3.10. *Additional coordination*

Interestingly, there is a correlation between the ionic radii of the divalent metal cation and the ability of the complex to coordinate an additional water molecule. Complexes of larger ions (Mg^{2+}, Ca^{2+}, Mn^{2+}, and Fe^{2+}) accept a fourth water, which replaces one of the phosphate O atoms, thus maintaining the geometry about the metal of the tris–aqua reactant complexes (figure 5). Acylphosphate complexes with smaller ions (Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+}) do not ligate the additional water. For each metal system, the tris- and tetra-aqua complexes were within 4 kcal mol^{-1} of each other. Due to the small energetic differences, only tris–aqua complexes were considered to provide a consistent means of comparison across different metal cations.

In several of the reactant acylphosphate complexes studied, the carboxyl O binds the metal cation axially. In the process of the reaction, the O must rotate before the amine can associate and form the peptide bond. The energetic impact of the coordination was considered by modeling both the Mg^{2+} reactant with the carboxyl O bound to Mg^{2+} and rotated away from the metal (figure 5). The resulting square pyramidal structure is 9.9 kcal mol^{-1} higher in energy than the bound form. Therefore, a significant portion of the transition state barrier may arise from the carboxyl rotation. Consistent with this proposal, the complexes that lack carboxyl O coordination (Fe^{2+} and Cu^{2+}) have some of the lowest calculated barriers.

4. Discussion

The native metal involved in peptide bond formation in most ATP-grasp enzymes is Mg^{2+}. Calculations show that reaction of Mg^{2+} acylphosphate reactant with an amine yields a simple peptide in an exergonic reaction. Relative to a simple, metal- and phosphate-free

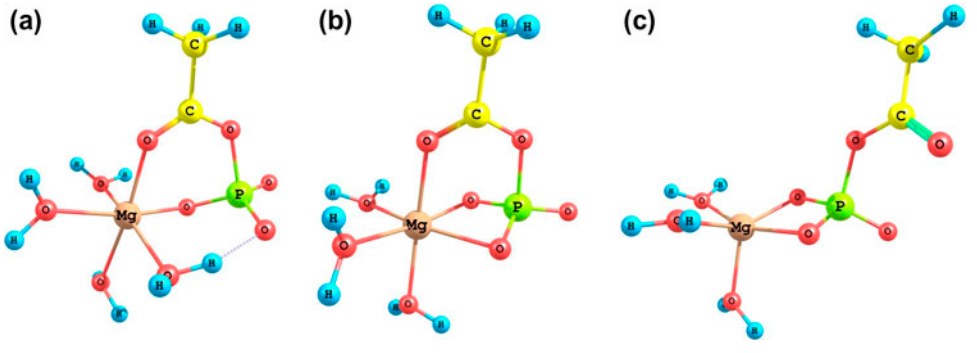


Figure 5. Reactant acylphosphate Mg^{2+} complexes studied: (a) tetra-aqua complex, (b) tris-aqua complex, and (c) rotated carboxyl complex.

reaction, the Mg^{2+} system is more thermodynamically and kinetically favored. The calculations also indicate that the reaction pathways of the Mn^{2+} and Co^{2+} systems are more favorable than the Mg^{2+} system, which correlates with experimental metal substitution studies [10]. Not only are overall reaction free energies lower by 3–4 kcal mol^{-1} with Mn^{2+} and Co^{2+}, but the transition state barriers are also lower in energy by 2–3 kcal mol^{-1}. Despite the neglect of protein environment, the model presented herein correctly predicts that an acylphosphate reactant in preferred reactions with Mg^{2+} is more favorable than those without a metal ion, and more specifically, that Mn^{2+} and Co^{2+} serve as more efficient catalysts than Mg^{2+}. Based on these findings, it is reasonable to assume that the calculated trends would apply to related biological systems.

Consideration of the acylphosphate in metal-free systems shows that the addition of the phosphate group lowers the transition state barrier by ~15 kcal mol^{-1}, without a large impact on overall reaction thermodynamics. Within the active site of an ATP-grasp protein, the negatively charged phosphate moiety would likely interact with polar or charged amino acids, thus impacting the barrier. The resulting dispersion of electron density would decrease the ability of the acylphosphate to accept the amine H. Therefore, the present simulations suggest that the protein environment is likely to have a larger impact on the energetics of the metal-free acylphosphate system than any of the other reactions studied, which makes it difficult to draw comparisons to the metal complexes.

Calculations of the Ca^{2+} reaction pathway show that while the kinetic barrier is fairly low (31.1 kcal mol^{-1}), the overall reaction is endergonic. Despite disparate geometries, Fe^{2+} and Zn^{2+} exhibit similar energetics in the formation of peptide bonds. Thermodynamically, these reactions are 3–13 kcal mol^{-1} more exergonic than the other metal systems, and the moderate transition state barriers are within 2.2 kcal mol^{-1}. Among the metals studied, Ni^{2+} has the shortest metal-carboxyl distance and is the least favored. The endothermic overall reaction coupled with the high barrier makes the Ni^{2+} system an unlikely choice for catalysis of peptide bonds. In contrast, Cu^{2+} exhibits a very favorable reaction pathway. Thermodynamically, the Cu^{2+} reaction path is exergonic by 7.3 kcal mol^{-1}, which is near the median for the metals studied. With the lowest transition state barrier by 5.9 kcal mol^{-1}, the Cu^{2+} system shows a significant kinetic drive to catalyze the reaction.

It seems likely that the ability of the metal cation to accept electron density impacts the barrier of peptide bond formation. Among six-coordinate aqua complexes of the divalent metals studied, waters associated with octahedral Cu^{2+} have the lowest pK_as [33–37]. Perhaps, the ability of Cu^{2+} to easily accept electron density allows the complex to more easily cleave the bond between the carboxyl C and the phosphate O. The relatively high reduction potential of Cu^{2+} also supports the role of the metal in accepting electron density in this reaction [38]. Finally, the loose coordination of the carboxyl O may also contribute to the low barrier in the Cu^{2+} system. With a relatively low reaction barrier and favorable thermodynamics, the Cu^{2+} system warrants further biological study and offers promise as a potential biomimetic catalyst.

The research presented herein highlights the importance of metal cations in the synthesis of peptide bonds by ATP-grasp enzymes. Calculations indicate that the divalent metal ion complexes with the acylphosphate to facilitate peptide bond formation. While the identity of the metal ion has a moderate impact on the thermodynamics of the reaction, the kinetic barriers are highly sensitive to the metal complex. A survey of several metals found that Mn^{2+} and Co^{2+} have more favorable reaction pathways than Mg^{2+}, as predicted by experiments. Simulations also suggest that Fe^{2+} and Zn^{2+} may be auspicious metals ions. Interestingly, the Cu^{2+} system seems to be the most promising reaction, with a low barrier and

exergonic reaction. It seems that the ability of metal ions to accept electron density in the transition state plays a significant role in the catalysis of peptide bonds. Further research, both computational and experimental, of these metal ions is needed to fully elucidate the precise mechanism by which ATP-grasp enzymes harness the power of coordination chemistry to catalyze the synthesis of peptide bonds.

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References

[1] C.O. Pabo, E. Peisach, R.A. Grant. *Annu. Rev. Biochem.*, **70**, 313 (2001).
[2] C.T. Supuran. *Nat. Rev. Drug Discovery*, **7**, 168 (2008).
[3] J.P. Collman, R. Boulatov, C.J. Sunderland, L. Fu. *Chem. Rev.*, **104**, 561 (2004).
[4] F. Rappaport, B.A. Diner. *Coord. Chem. Rev.*, **252**, 259 (2008).
[5] J. Costamagna, G. Ferraudi, B. Matsuhiro, M. Campos-Vallette, J. Canales, M. Villagrán, J. Vargas, M.J. Aguirre. *Coord. Chem. Rev.*, **196**, 125 (2000).
[6] L. Que Jr., W.B. Tolman. *Nature*, **455**, 333 (2008).
[7] S. Rau, D. Walther, J.G. Vos. *Dalton Trans.*, 915 (2007).
[8] M.V. Fawaz, M.E. Topper, S.M. Firestine. *Bioorg. Chem.*, **39**, 185 (2011).
[9] W. Wang, T.J. Kappock, J. Stubbe, S.E. Ealick. *Biochemistry*, **37**, 15647 (1998).
[10] L. Oppenheimer, V.P. Wellner, O.W. Griffith, A. Meister. *J. Biol. Chem.*, **254**, 5184 (1979).
[11] D. Ye, M. Wei, M. McGuire, K. Huang, G. Kapadia, O. Herzberg, B.M. Martin, D. Dunaway-Mariano. *J. Biol. Chem.*, **276**, 37630 (2001).
[12] G. Triola, S. Wetzel, B. Ellinger, M.A. Koch, K. Hübel, D. Rauh, H. Waldmann. *Bioorg. Med. Chem.*, **17**, 1079 (2009).
[13] V. Škedelj, T. Tomašić, L.P. Mašič, A. Zega. *J. Med. Chem.*, **54**, 915 (2011).
[14] S. Halouska, R.J. Fenton, D.K. Zinniel, D.D. Marshall, R.G. Barletta, R. Powers. *J. Proteome Res.*, **13**, 1065 (2014).
[15] A. Dinescu, T.R. Cundari, V.S. Bhansali, J.-L. Luo, M.E. Anderson. *J. Biol. Chem.*, **279**, 22412 (2004).
[16] A.G. Murzin. *Curr. Opin. Struct. Biol.*, **6**, 386 (1996).
[17] P.K. Chakravarty, W.J. Greenlee, W.H. Parsons, A.A. Patchett, P. Combs, A. Roth, R.D. Busch, T.N. Mellin. *J. Med. Chem.*, **32**, 1886 (1989).
[18] B. Eroglu, S.G. Powers-Lee. *Arch. Biochem. Biophys.*, **407**, 1 (2002).
[19] L. Esser, C.R. Wang, M. Hosaka, C.S. Smagula, T.C. Sudhof, J. Deisenhofer. *EMBO J.*, **17**, 977 (1998).
[20] G. Polekhina, P.G. Board, R.R. Gali, J. Rossjohn, M.W. Parker. *EMBO J.*, **18**, 3204 (1999).
[21] G.J. Miller, M.P. Wilson, P.W. Majerus, J.H. Hurley. *Mol. Cell*, **18**, 201 (2005).
[22] M. Remko, B.M. Rode. *Chem. Phys. Lett.*, **316**, 489 (2000).
[23] M. Remko, B.M. Rode. *Struct. Chem.*, **15**, 223 (2004).
[24] M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G.A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H.P. Hratchian, A.F. Izmaylov, J. Bloino, G. Zheng, J.L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J.A. Montgomery Jr., J.E. Peralta, F. Ogliaro, M. Bearpark, J.J. Heyd, E. Brothers, K.N. Kudin, V.N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J.C. Burant, S.S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J.M. Millam, M. Klene, J.E. Knox, J.B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, R.L. Martin, K. Morokuma, V.G. Zakrzewski, G.A. Voth, P. Salvador, J.J. Dannenberg, S. Dapprich, A.D. Daniels, Ö. Farkas, J.B. Foresman, J.V. Ortiz, J. Cioslowski, D.J. Fox. *Gaussian 09, Revision D.01*, Gaussian, Inc., Wallingford, CT (2009).
[25] A.D. Becke. *Phys. Rev. A*, **38**, 3098 (1988).
[26] A.D. Becke. *J. Chem. Phys.*, **98**, 5648 (1993).
[27] P.J. Stephens, F.J. Devlin, C.F. Chabalowski, M.J. Frisch. *J. Phys. Chem.*, **98**, 11623 (1994).
[28] M.P. Andersson, P. Uvdal. *J. Phys. Chem. A*, **109**, 2937 (2005).
[29] C.N. Schutz, A. Warshel. *Proteins*, **44**, 400 (2001).

[30] A.D. Becke. *J. Chem. Phys.*, **107**, 8554 (1997).
[31] S. Grimme. *J. Comput. Chem.*, **27**, 1787 (2006).
[32] F.H. Allen, O. Kennard. *Chem. Des. Autom. News*, **8**, 31 (1993).
[33] J.A. Duffy, M.D. Ingram. *Inorg. Chem.*, **17**, 2798 (1978).
[34] J. Li, C.L. Fisher, J.L. Chen, D. Bashford, L. Noodleman. *Inorg. Chem.*, **35**, 4694 (1996).
[35] W. Grzybkowski. *Polish J. Environ. Stud.*, **15**, 655 (2006).
[36] R. Gilson, M.C. Durrant. *Dalton Trans.*, 10223 (2009).
[37] E. Biondi, R.R. Poudyal, J.C. Forgy, A.W. Sawyer, A.W.R. Maxwell, D.H. Burke. *Nucleic Acids Res.*, **41**, 3327 (2013).
[38] S.G. Bratsch. *J. Phys. Chem. Ref. Data*, **18**, 1 (1989).