

when electron correlation is included. We thus find that the only energetically reasonable form for CLi_4 to be the T_d singlet, in contrast to the case of SiLi_4 , where the T_d structure exhibits three imaginary vibrational frequencies.¹

VII. Conclusion

We find C_{2v} structures with unusually small Li-X-Li bond angles to be favored for SiLi_4 , GeLi_4 , and SnLi_4 . Delocalization from the $\sigma(\text{X-Li}_{\text{eq}})$ and $\sigma(\text{Li}_{\text{ax}}\text{-X-Li}_{\text{ax}})$ bonding orbitals into the low-occupancy 2s and 2p orbitals of the other lithium atoms results in effective Li-Li attractive interaction at the RHF level, as illustrated by our orbital plots. The importance of the lithium 2p orbitals in acting as acceptors in such interactions is illustrated by our finding that SiLi_4 would be tetrahedral in the absence of Li 2p orbitals. By contrast, the only important structure for CLi_4 is found to be the T_d singlet.

For SiLi_4 , inclusion of electron correlation effects favor even smaller Li-Si-Li bond angles and destabilize the T_d structure with respect to the C_{2v} structure further. The essence of this correlation effect is described at the two-configuration MCSCF (MC-2) level

and involves excitation of $(A_1)^2 \rightarrow (A_1^*)^2$ type. The MC-2 description is unbalanced with respect to Li-Li bonding, however, as the excited configurations contributing most strongly to the four $\text{Li}_{\text{eq}}\text{-Li}_{\text{ax}}$ bonding interactions are omitted. A more balanced treatment of correlation effects is given at the full double excitation MP2 level. More refined treatment of correlation at the full CISD level yields results nearly identical with those of MP2, showing that the simple MP2 perturbational treatment provides a good approximation of the CISD variational result. These correlation effects on molecular geometry are smaller when the 6-31G* basis set is employed instead of 3-21G(*). Similar results are found at the MP2 level for GeLi_4 .

Acknowledgment. This work was supported by the Deutsche Forschungsgemeinschaft, the Fonds der chemischen Industrie, the Volkswagen Stiftung, and Convex Computer Corp. We thank Prof. R. W. F. Bader and Prof. R. J. Lagow for stimulating correspondence and Prof. E. Heilbronner for his interest.

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Interaction of Calcium and Magnesium Ions with Malonate and the Role of the Waters of Hydration: A Quantum Mechanical Study

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Abstract: The structure of malonate interacting with either a Ca(II) or a Mg(II) ion in a chelation bidentate orientation has been studied with ab initio molecular orbital theory. In addition, the optimized chelation bidentate structures have also been determined for the enol tautomer of malonate ion and two different deprotonated forms of the malonate-metal ion complex. All malonate-derived structures were optimized with and without four waters of hydration about the divalent metal ion. The enol tautomer of malonate was found to be less stable than the keto form complexed with either Ca(II) or Mg(II) ions. Computations have demonstrated that removal of a proton from one of the waters of hydration about the divalent metal ion leads to a structure that is calculated to be more stable than the structure resulting from the deprotonation on carbon of the malonate complex. A mechanism for the chemical modification and proton exchange of proteins containing γ -carboxyglutamic acid (e.g., prothrombin), for which malonic acid serves as a model, is proposed.

Introduction

γ -Carboxyglutamic acid (Gla) is formed in a posttranslational, vitamin K mediated carboxylation of specific glutamyl residues.¹ The net result of this modification is the conversion of the substituted acetic acid side chain of Glu into the substituted malonic acid of Gla. It is the malonic acid functionally that is responsible for the ability of coagulation zymogens to bind divalent metal ions [usually Ca(II) or Mg(II)] under physiological conditions.^{2,3} For the vitamin K dependent coagulation proteins [e.g., factors II (prothrombin), VII, IX (Christmas), and X (Stewart) and proteins C, S, and Z], the binding of divalent metal ions is required prior to physiological activity (either formation of binary or ternary protein complexes or the assembly of an active enzyme and co-

enzyme embedded on an acidic phospholipid surface interacting with substrate). Ca(II) ions, but not Mg(II) ions, will support the coagulation cascade at significant rates under physiological conditions.^{2,3} Furthermore, low concentrations of Mg(II) ions in the presence of Ca(II) ions will exert a synergistic influence on the rate of clot formation.² Thus, one of the basic questions of the coagulation protein literature deals with differentiating the roles of these two divalent metal ions. Specifically, are the interactions of Ca(II) and Mg(II) ions with the malonate side chain of Gla similar? Or does the observed metal ion specificity reside in a different portion of the coagulation process (e.g., in the binding of metal ions to either the acidic phospholipid surface or the glycosylated part of the protein)?

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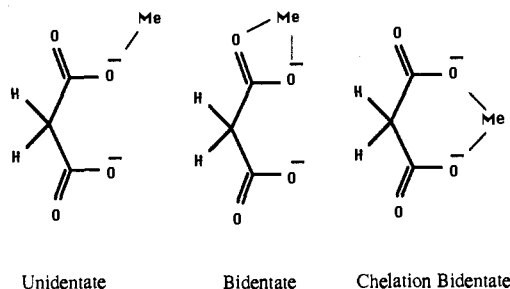


Figure 1. Three of the possible orientations of a metal ion (Me) interacting with a malonate.

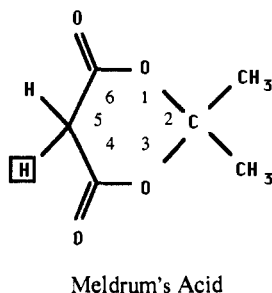


Figure 2. Meldrum's acid. The boxed proton has a pK_a of 5.1

Several experimental studies have investigated the reaction of C_γ of Gla with an electrophile—either a Schiff base^{4,5} or a proton.⁶ All Gla residues in the native protein seem to be equally reactive, with all Gla residues being modified with the Schiff base^{4,5} or experiencing exchange with the γ -proton.^{6,7} But in the presence of a metal ion (usually a trivalent metal ion), the reactivity of the various Gla residues has been attenuated toward the Schiff base (and protonation⁶), and only a small number of Gla residues appear to undergo modification.⁴⁻⁷ The modified residues have been sequenced, and equilibrium dialysis^{4,5} experiments have clearly established that the three modified Gla's are elements of at least one of the three Ca(II) ion tight sites; it can be argued that each modified Gla is a member of a different tight site.⁴ The Mg(II) ion equilibrium dialysis experiment of the modified protein reveals essentially no change of either the affinity or number of sites upon modification [there are no "tight" Mg(II) ion binding sites in either the native or modified protein; instead, all sites are equivalent and noninteracting].

The different modes of chelation of Ca(II) and Mg(II) (and other metal ions) to carboxylates observed in crystal structures have been examined previously.⁸⁻¹⁰ For the case of a malonate interacting with a metal ion, there are three obvious orientations of the metal relative to the malonate carboxylates (Figure 1). These include the metal ion interacting with one (unidentate or bidentate) or both carboxylates (chelation bidentate, sometimes called malonate chelation). There have been numerous crystal structure determinations of substituted malonates with metal ions, including Na(I),¹¹ Li(I),¹¹ Nd(I),¹² Mg(II),^{13,14} Ca(II),¹⁴⁻²⁰ and

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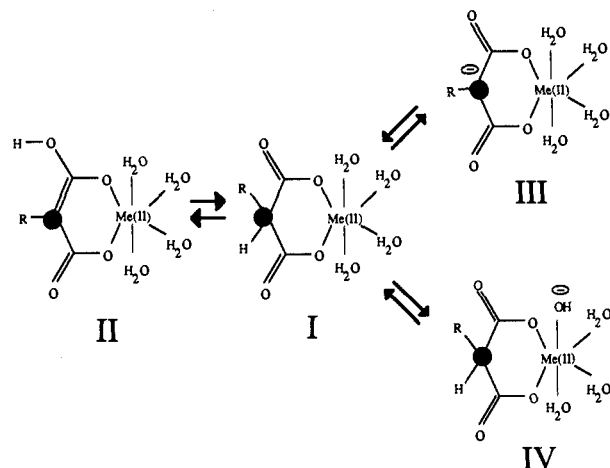


Figure 3. Summary of the structures studied. In this study, malonate ($R = H$, highlighted carbon would be C_2) is used as a model for GLA ($R = CH_2CH(COR')NHR''$, highlighted carbon would be C_γ). Malonate complexed with a divalent metal ion (Me) in a chelation bidentate orientation (I) is the central molecule in this study. The other malonate complexes optimized in this study were the enol tautomer (II), deprotonated on the C_2 carbon (III), and deprotonated on one of the waters of hydration about the metal ion (IV). See Table V for a comparison of the relative energies of these complexes.

Sr(II).¹⁵ For structures determined with malonate interacting with Mg(II), the metal ion is found in a chelation bidentate orientation and six coordinate overall. Great diversity is observed for Ca(II) ion structures. One structure is polymeric,¹⁶ several display only uni- and bidentate interactions,¹⁵⁻¹⁹ and two structures have the metal ion in a chelation bidentate binding mode.^{14,20} The coordination number of the Ca(II) ions in these studies varied from six to eight. Approximately 75% of all crystal structures reported, over 45 different structures to date, contain a malonate and a metal ion in a chelation bidentate interaction.¹⁹

Malonic acid, and the esters of malonic acid, have been the subject of several theoretical studies,²¹⁻²⁶ primarily the cyclic diester Meldrum's acid^{22,23,27-29} which has a very acidic proton ($pK_a = 5.1$; Figure 2). Electrophilic attack at C_5 of Meldrum's acid is facile, and the rate constant for the second alkylation is approximately the same as that for the first alkylation.²⁸ Thus, it is virtually impossible to stop the reaction after the first alkylation. If the chemistry of the malonate-metal ion complex (structure I, Figure 3) is similar to that of Meldrum's acid, then it would not be unreasonable to expect this complex to be readily alkylated at C_γ —accounting for the observed reactivity with either the Schiff base or a proton source. It is thus possible that electrophilic attack at C_γ proceeds via the deprotonated intermediate (structure III,

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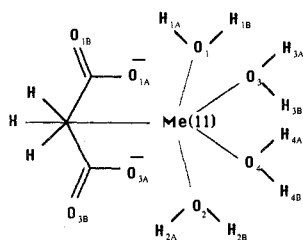


Figure 4. Graphical definition of the atoms in the malonate complex (I) with a divalent metal ion [Me, either Ca(II) or Mg(II) ion] in a chelation bidentate orientation. The complex was minimized both without and with four added waters of hydration about the metal ion (Table I). C_1 is the carbon that O_{1A} and O_{1B} are attached to, C_3 is the carbon that O_{3A} and O_{3B} are attached to, and C_2 is the methylene carbon. This basic framework was also used in the malonate complex deprotonated on oxygen (IV, Table IV) (atom H_{4B} was removed).

Figure 3). However, an alternative pathway would involve the alkylation of the enol form (structure II, Figure 3). The observed acidity of Meldrum's acid ($pK_a = 5.1$) emphasizes that to understand the protonation and alkylation studies performed near pH 5.5, we must consider both possible target forms (structures II and III, Figure 3).

We report here a study aimed at distinguishing between the possible pathways of protonation and electrophilic attack mentioned above as well as exploring divalent metal ion differentiation in the Ca(II) and Mg(II) ion complexes. Whereas previous computational studies have focused on describing the geometry and energetics of the interaction of a Mg(II) or Ca(II) ion with malonate at a minimum basis set level,³⁰⁻³³ this study will concentrate on describing the chemistry of the complex resulting from a single malonate interacting with the divalent metal ion. The metal ion will be placed in the chelation bidentate orientation, the unique mode of chelation of malonate that has been most commonly observed in crystal structures to date. Additionally, waters of hydration will be added about the divalent metal ion to yield hexacoordinated complexes, which can be viewed as a minimal model for solvated surface binding sites for Glu in proteins. Within these assumptions, we shall examine the energies and structures of the parent malonate-divalent metal ion complex (structure I, Figure 3) relative to the deprotonated forms (structures III and IV, Figure 3) and complexes involving the enol tautomer of malonate (structure II, Figure 3).

The following nomenclature will be used in this paper for clarity: Structure I (Figure 3) will be referred to as the **keto** form, structure II (Figure 3) will be referred to as the **enol** form, structure III (Figure 3) will be referred to as the **deprotonated on carbon** form, and structure IV will be referred to as the **deprotonated on oxygen** form. The terms deprotonated on carbon and deprotonated on oxygen refer exclusively to the physical site of deprotonation of the divalent metal ion-malonate complex, specifically, the methylene carbon (C_2) of the malonate and an oxygen from one of the waters of hydration, respectively.

Method

The computations were performed by using the ab initio molecular orbital program Gaussian88³⁴ on the Cray Y-MP8/432 at the North Carolina Supercomputing Center and on the Cray Y-MP8/832 at the Pittsburgh Supercomputing Center. All calculations were performed by using the Hartree-Fock method within the 3-21G* basis set, which is of split valence quality. The optimizations were started by using eigenvalue-

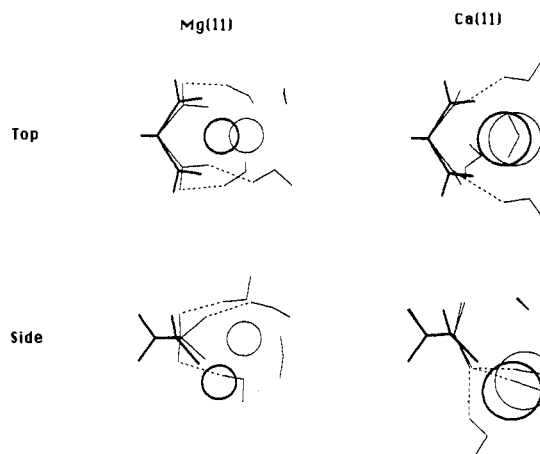


Figure 5. Graphical representation of the ab initio optimized complex of malonate interacting with a divalent metal ion in a chelation bidentate orientation (I). The Mg(II) ion structure is the left pair (top and side views), and the Ca(II) ion structure is the right pair. The complexed optimization with the added waters of hydration is the thin-lined structure, and the complex without the waters is the thick-lined structure. See Table I for the optimized geometrical parameters and Figure 3 for the atom definitions.

following methods,^{35,36} but near convergence it was often helpful to restart the job by using analytically calculated force constants. The calcium 3-21G* basis set was taken from the literature,³⁷⁻³⁹ while the internal basis set for magnesium was used. While this level of theory cannot be expected to yield results of great accuracy, it should be adequate for obtaining qualitative information. It will be interesting at some future date when computer resources are sufficient to repeat key parts of these calculations with larger basis sets and methods that better describe electron correlation. All calculations are for 0 K structures; thus, the possible influences of temperature and entropy are not included.

Results

Malonate in a Chelation Bidentate Structure with either a Mg(II) or Ca(II) Ion (Structure I). The malonate-Mg(II) ion complex (without waters of hydration) was fully optimized (Figure 4) with the crystal structure¹⁰ providing the starting structure. The final optimized structure had C_s symmetry [this is also observed in the Mg(II)^{13,14} and Ca(II) ion crystal structures with malonate in a chelation bidentate complexation.^{14,19} Therefore, all the subsequent malonate-divalent metal ion complexes (structure I) were defined with a reflection plane that passed through the methylene carbon (C_2) (and protons) and the metal ion.

When added, the oxygen of the added waters of hydration (Figure 4) was either orthogonal to or in the symmetry plane ($O_1 = O_2$, $H_{1A} = H_{2A}$, $H_{1B} = H_{2B}$, O_3 and O_4 were in the reflection plane, $H_{3A} = H_{3B}$, and $H_{4A} = H_{4B}$). After the minimum was found with these symmetry constraints, the constraints were removed and the structure was completely minimized. Vibrational analysis was performed on the resultant structures and revealed no imaginary frequencies, thus proving that the minimized structures are at a local minimum. Upon relaxation of the symmetry constraints, ONLY one of the waters of hydration in the Ca(II) ion structure ($H_{4A}-O_4-H_{4B}$) moved a significant distance to establish an additional hydrogen bond. This is compared to the Mg(II) ion-malonate complex, where all four of the waters of hydration moved during the complete geometry optimization. The symmetry observed in the Curry et al.¹⁴ crystal structure and maintained in the initial malonate-hydrated divalent metal ion

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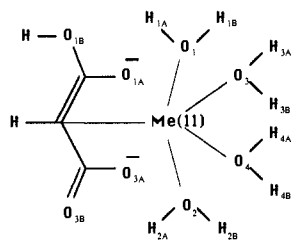


Figure 6. Graphical definition of the atoms in the enol tautomer of malonate complex with a divalent metal ion [Me, either Ca(II) or Mg(II)] in a chelation bidentate orientation (II). The complex was minimized both without and with four added waters of hydration about the metal ion (Table II). C_1 is the carbon that O_{1A} and O_{1B} are attached to, C_3 is the carbon that O_{3A} and O_{3B} are attached to, and C_2 is the carbon that H_2 is attached to.

optimizations was completely destroyed during the full optimization. This is especially evident in the conformation of the carboxylates. In the initial structures, the relative orientation of the carboxylates was defined by a mirror plane. In the final structure, the planes of the two carboxylates are almost orthogonal.

A comparison of the malonate–Mg(II) ion without and with the added waters of hydration, along with the Ca(II) ion structures, is given in Figure 5. In the complexes without the added waters of hydration about the metal ion, the metal ion was essentially in the plane containing C_1 , C_3 , O_1 , and O_{3A} . After the waters of hydration were added to the metal ion complexes, the metal moved above this plane (C_1 , C_3 , O_{1A} , and O_{3A}). The resultant Ca(II) ion hydrated metal ion complex is in what is best described as a chair conformation, and the analogy to Meldrum's acid becomes evident. The conformation of the Mg(II) ion complex is best described as a flattened twist boat. But there still appears to be reasonable overlap between the "axial-like" proton on the methylene and one of the carboxylates and, overall, the chelation bidentate structure has remained intact. Both hydrated metal ion structures contained a number of hydrogen bonds. In the Mg(II) ion structure, the metal ion's van der Waals surface is completely covered by ligands, whereas a portion of the Ca(II) ion's surface is still exposed.

Enol Tautomer of Malonate in a Chelation Bidentate Structure with either a Mg(II) or Ca(II) Ion (Structure II). Because the complex did not naturally contain a symmetry element, the complexes of the enol tautomer of malonate and the divalent metal ions were fully optimized (Figure 6). The initial starting structure for the metal ion complexes without waters of hydration had all atoms of the malonate in a plane with the initial metal ion positioned below the malonate plane. The waters of hydration about the metal ion were then added to the optimized structures in an octahedral orientation with the plane defined by the waters either in the plane of or orthogonal to the plane of C_1 – C_2 – C_3 . A significant amount of movement of the waters of hydration occurred during the optimization, but the total energy was relatively insensitive to these displacements. The Ca(II) ion complex was optimized without geometry constraints (Figure 6). All of these structures were determined to be minima by calculating the vibrational frequencies.

A comparison of the enol tautomer of malonate complexed with a Mg(II) ion without and with the added waters of hydration, along with the Ca(II) ion structures, is given in Figure 7. In both divalent metal ion complexes without the added waters of hydration, the metal ion is essentially in the plane of the enol tautomer of malonate (all atoms of the enol tautomer were coplanar). Upon addition of the waters of hydration to the Mg(II) ion complex, the metal ion moved below the plane of the enol tautomer (all malonate atoms remained essentially coplanar) and three hydrogen bonds were formed between the waters of hydration and the carboxylate oxygens along with a possible interaction of a proton from the fourth water and C_2 of malonate. Even after the addition of the waters of hydration to the Ca(II) ion complex, the metal ion remained essentially coplanar with all of the atoms in the enol tautomer. All four waters of hydration about the Ca(II)

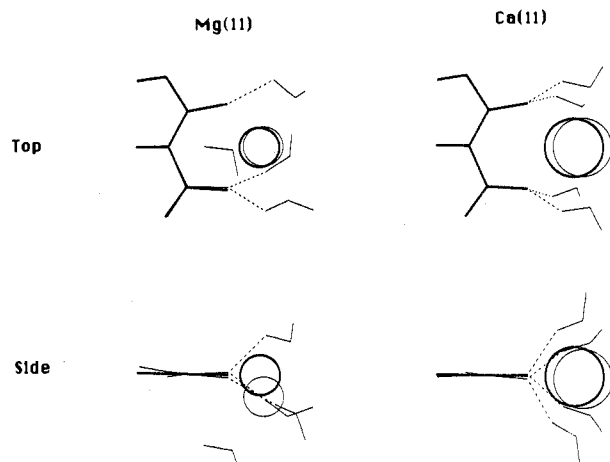


Figure 7. Graphical representation of the ab initio optimized complex of the enol tautomer of malonate (II) interacting with a divalent metal ion in a chelation bidentate orientation. The Mg(II) ion structure is the left pair (top and side views), and the Ca(II) ion structure is the right pair. The complexed optimized with the added waters of hydration is the thin-lined structure, and the complex without the waters is the thick-lined structure. See Table II for the optimized geometrical parameters and Figure 5 for the atom definitions.

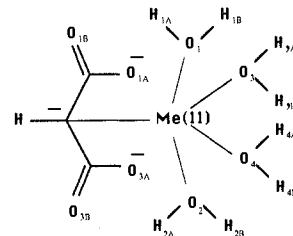


Figure 8. Graphical definition of the atoms in the malonate complex deprotonated on carbon (C_2) with a divalent metal ion [Me, either Ca(II) or Mg(II)] in a chelation bidentate orientation (III). The complex was minimized both without and with four added waters of hydration about the metal ion (Table I). C_1 is the carbon that O_{1A} and O_{1B} are attached to, C_3 is the carbon O_{3A} and O_{3B} are attached to, and C_2 is the carbon that H_2 is attached to.

ion formed hydrogen bonds with the enol tautomer of malonate.

Deprotonation on Carbon (C_2) of the Malonate Complex in a Chelation Bidentate Structure with either a Mg(II) or Ca(II) Ion (Structure III). Two different starting structures were employed for the optimization of the malonate deprotonated on carbon form (C_2) and a divalent metal ion without the waters of hydration. The first starting structure had the entire complex planar, and the second starting structure was essentially a deprotonated form of the malonate–divalent metal ion complex (the proton attached to C_2 was placed in the plane defined by C_1 – C_2 – C_3 , with all other parameters identical with those of the final optimized structure I). The natural symmetry present in this complex was used (it is the reflection plane used in the optimization of the malonate–metal ion chelation bidentate structure). Both starting structures optimized to the same final structure (Figure 8). The waters of hydration about the divalent metal were then added in an octahedral arrangement with the reflection plane symmetry intact (thus, $O_1 = O_2$, $H_{1A} = H_{2A}$, $H_{1B} = H_{2B}$, $H_{3A} = H_{3B}$ and $H_{4A} = H_{4B}$). During the optimization of this complex, the protons (H_{1A} and H_{2A}) attached to the symmetry equivalent water molecules (O_1 and O_2) "jumped" from the water molecule to the two carboxylate oxygens of the deprotonated malonate (although the protons did remain hydrogen bonded to their respective oxygens). It was feared that the symmetry constraints applied during the minimization were responsible for this apparently anomalous behavior. Thus, the symmetry constraints were removed and the structure was fully optimized. Analysis of the vibrational frequencies revealed that the resultant structures were local minima. A comparison of the deprotonated Ca(II) and Mg(II) ion com-

plexes with and without the waters of hydration is given in Figure 9. A significant reorganization of the waters of hydration about both of the divalent metal ions was observed during the minimization. The two protons that had jumped during the symmetry-constrained minimization of the Mg(II) ion complex remained associated with the carboxylates and hydrogen-bonded to the hydroxides (the former waters that had donated the protons). This is in contrast with the Ca(II) ion complex, where one of the symmetry equivalent protons that had jumped to the carboxylate "returned" to the hydroxide. The second proton that had jumped remained associated with the carboxylate while the hydroxide that had donated this proton drifted to the "backside" of the Ca(II) ion. Thus, a significant difference between the Mg(II) and Ca(II) ion structures was noted. The waters of hydration about the Mg(II) ion "donated" two protons to the complexed malonate anion (malonic acid trianion), whereas the waters of hydration about the Ca(II) ion donated only one proton to the malonate anion. This is consistent with the increased acidity of Mg(II) ion solutions as compared to Ca(II) ion solutions. In addition, the Mg(II) ion shifts further under the C₂ carbon of the deprotonated malonate so that the major contact of the Mg(II) ion is with the C₂ carbon—NOT the carboxylate oxygens.⁴⁰ Thus, the net result of the Mg(II) ion and proton movement is to have the Mg(II) ion with two hydroxides and two waters of hydration interacting with the deprotonated carbon of malonic acid. Presumably, this would significantly reduce the nucleophilicity of this anion. In the hydrated Ca(II) ion complex, the metal ion remains complexed

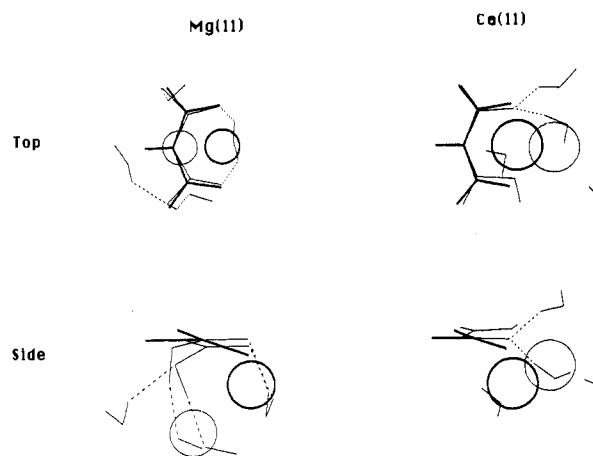


Figure 9. Graphical representation of the ab initio optimized complex of malonate deprotonated on carbon (III) interacting with a divalent metal ion in a chelation bidentate orientation (I). The Mg(II) ion structure is the left pair (top and side views), and the Ca(II) ion structure is the right pair. The complex optimized with the added waters of hydration is the thin-lined structure, and the complex without the waters is the thick-lined structure. See Table III for the optimized geometrical parameters and Figure 7 for the atom definitions.

(40) It is tempting to describe structure III as an enolate, but there are several criteria that must be evaluated prior to using this distinction. The first criterion would be structural; specifically, whether the methine-carboxylate distance (C₁-C₂ and C₂-C₃) of the deprotonated on carbon form (structure III, Figure 3) is consistent with what one would expect if the enolate valence structure was dominant. This is apparently the case when appropriate bond lengths in this study are compared to what was obtained with the various forms of acetic and malonic acid:

| | keto | enol | enolate |
|--|------|------|---------|
| Mg(II) ion complex w/waters of hydration | 1.53 | 1.35 | 1.41 |
| acetic acid | 1.50 | 1.31 | 1.36 |
| malonic acid | 1.51 | | 1.39 |

The keto, enol, and enolate are structures I, II, and III from this study for the Mg(II) ion complex (C₂-C₃ optimized bond length); the lower two lines are the appropriate 3-21G optimized C-CO₂ bond length for acetic and malonic acid (26). The second criterion is how the additional negative charge is distributed among the various atoms upon deprotonation (whether C₂ or the carboxylate oxygens become more negative). The change of the charge on selected atoms during the deprotonation of structure II (removal of H₁ from O_{1B}) to give structure III was investigated. This sequence was chosen because the basic carbon backbone and number of protons attached to C₂ do not change during this deprotonation. Since O_{1B} is the site of deprotonation, the change on the charges on O_{3A} and O_{3B} was evaluated. The Mullikan populations of the various structures studied are

| Structure | C ₁ | O _{1A} | O _{1B} | H ₁ | C ₂ | H _{2A} | H _{2B} | C ₃ | O _{3A} | O _{3B} | Me(II) |
|-------------------------|----------------|-----------------|-----------------|----------------|----------------|-----------------|-----------------|----------------|-----------------|-----------------|--------|
| Magnesium Ion Complexes | | | | | | | | | | | |
| I | 0.876 | -0.593 | -0.780 | | 0.686 | 0.293 | 0.271 | 0.876 | -0.593 | -0.780 | 1.115 |
| I w/ water | 0.824 | -0.764 | -0.664 | | -0.616 | 0.246 | 0.264 | 0.824 | -0.764 | -0.664 | 0.665 |
| II | 0.903 | -0.805 | -0.651 | 0.407 | -0.563 | 0.227 | | 0.866 | -0.789 | -0.728 | 1.131 |
| II w/ water | 0.875 | -0.855 | -0.682 | 0.400 | -0.628 | 0.208 | | 0.884 | -0.800 | -0.733 | 0.673 |
| III | 0.803 | -0.747 | -0.717 | | -0.737 | 0.200 | | 0.803 | -0.747 | -0.717 | 0.857 |
| III w/ water | 0.821 | -0.797 | -0.728 | | -0.625 | 0.190 | | 0.783 | -0.802 | -0.712 | 0.657 |
| IV | 0.884 | -0.723 | -0.724 | | -0.645 | 0.250 | 0.231 | 0.874 | -0.758 | -0.708 | 0.548 |
| Calcium Ion Complexes | | | | | | | | | | | |
| I | 0.893 | -0.832 | -0.630 | | -0.667 | 0.244 | 0.272 | 0.893 | -0.832 | -0.630 | 1.289 |
| I w/ water | 0.851 | -0.789 | -0.673 | | -0.613 | 0.247 | 0.267 | 0.842 | -0.816 | -0.669 | 0.844 |
| II | 0.921 | -0.859 | -0.668 | 0.397 | -0.565 | 0.201 | | 0.875 | -0.842 | -0.732 | 1.292 |
| II w/ water | 0.911 | -0.887 | -0.689 | 0.398 | -0.563 | 0.203 | | 0.880 | -0.857 | -0.734 | 0.860 |
| III | 0.787 | -0.820 | -0.736 | | -0.692 | 0.197 | | 0.787 | -0.820 | -0.736 | 1.034 |
| III w/ water | 0.887 | -0.872 | -0.721 | | -0.657 | 0.208 | | 0.792 | -0.737 | -0.732 | 0.627 |
| IV | 0.926 | -0.809 | -0.692 | | -0.648 | 0.241 | 0.232 | 0.881 | -0.732 | -0.750 | 0.640 |

During the deprotonation of the enol form (structure II) to give the deprotonated on carbon form (structure III) in the absence of waters of hydration about the metal ion, one finds that C₂ and the Me(II) ion gain the most charge [$\Delta(\text{charge units})_{\text{Mg(II)}}: \text{C}_2 = -0.17, \text{Mg(II)} = -0.28; \Delta(\text{charge units})_{\text{Ca(II)}}: \text{C}_2 = -0.13, \text{Ca(II)} = -0.26$] while the charge on the carboxylate atoms changed slightly. This general trend is also noted in the deprotonation of the hydrated Ca(II) ion complex [$\Delta(\text{charge units})_{\text{Ca}^*(\text{H}_2\text{O})_6}: \text{C}_2 = -0.10, \text{Ca(II)} = -0.26$]. However, there is noted little change in the charge distribution during the deprotonation of the hydrated Mg(II) ion complex, perhaps reflecting the significant amount of conformational changes upon the addition of the waters of hydration. With this exception, the charge on C₂ and the Me(II) ion became more negative and the charge on the carboxylate oxygens either remained the same or slightly decreased (more positive), which is not totally consistent with the dominate valence structure being an enolate.

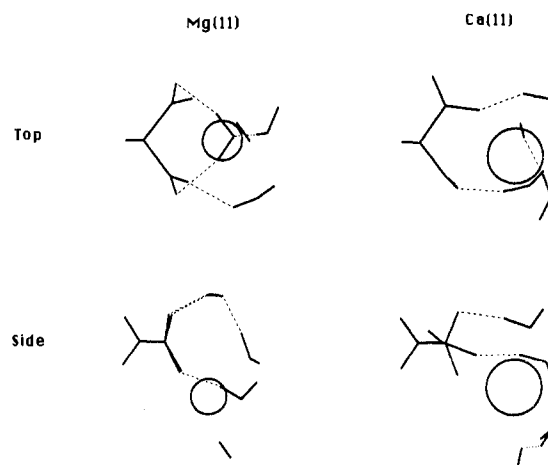


Figure 10. Graphical representation of the ab initio optimized complex of malonate deprotonated on oxygen (IV) interacting with a divalent metal ion in a chelation bidentate orientation (I). The Mg(II) ion structure is the left pair (top and side views), and the Ca(II) ion structure is the right pair. The complex optimized with the added waters of hydration is the thin-lined structure, and the complex without the waters is the thick-lined structure. See Table IV for the optimized parameters and Figure 3 (the proton labeled H_{4A} was removed from that structure) for the atom definitions.

with the oxygens of the malonate, although one of the protons has jumped from the waters of hydration to a carbonyl oxygen of one of the carboxylates. Thus, C₂ of the Ca(II) ion complex would appear to be more nucleophilic, analogous to Meldrum's acid.

Deprotonation of the Malonate Complex on Oxygen (O₄) in a Chelation Bidentate Structure with either a Mg(II) or Ca(II) Ion (Structure IV). The observation that protons from the waters of hydration jumped during the minimization of the structure that was deprotonated on carbon suggests that there is a more favorable deprotonation site. Thus, we examined the deprotonation of one of the waters of hydration. In the optimized hydrated malonate-metal ion complex, three of the water molecules contained hydrogen-bonding interactions with the malonate. However, one water molecule was largely exposed. It was from this water molecule that a proton was removed. Other than this single change (the removal of H_{4B}), the complex was optimized with the same restraints that were placed on the malonate complex (vide supra, Figure 4). Upon completion of the restrained minimizations, the

Table V. Summary of the Relative Energies of the Various Malonate-Divalent Metal Ion Complexes Studied^a

| complex charge: description: structure: | neutral | | deprotonated | |
|---|--------------------------------|-----------------------|------------------------|------------------------|
| | keto tautomer I | enol tautomer II | on carbon III | on oxygen IV |
| | Malonate-Calcium Ion Complex | | | |
| without water (ΔE) | -1085.455 293 0.0 | -1085.432 560 14.3 | -1084.819 628 398.9 | |
| with water (ΔE) | -1388.890 242 0.0 | -1388.061 791 15.8 | -1387.487 752 377.4 | -1387.544 392 341.9 |
| | Malonate-Magnesium Ion Complex | | | |
| without water (ΔE) | -610.520 073 0.0 | -610.499 306 13.0 | -609.923 418 374.4 | |
| with water (ΔE) | -913.166 839 0.0 | -913.127 473 24.7 | -912.532 503 398.0 | -912.600 645 355.3 |

^aThe divalent metal ion was in a chelation bidentate orientation relative to the malonate. Each malonate-divalent metal ion complex (except the deprotonated on oxygen complex) was optimized without and with four waters of hydration about the divalent metal ion. Frequency calculations on the hydrated metal ion complexes confirmed that these complexes were minimas. The energy of each individual complex is given in au, and the difference in relative energy (ΔE) is given in kcal mol⁻¹. See Figure 3 for the structures of each of these complexes.

symmetry constraints were removed and the complexes were fully optimized (Figure 10). Calculation of the vibrational frequencies demonstrated that these final structures were local minima. After the optimization, it was found that this deprotonated on oxygen form of the malonate complex was more stable than the malonate structure deprotonated on carbon form for both Ca(II) and Mg(II).

A comparison of the deprotonated on oxygen malonate complexed with a Mg(II) ion without and with the added waters of hydration, along with the Ca(II) ion structures, is given in Figure 10. These hydrated metal ion structures are very similar to the malonate-hydrated metal ion structures (structure I, Figure 3). In both complexes, the metal ion is above the plane defined by C₁-C₂-C₃, and the overall conformation of the malonate-metal ion is best described as a chair form.

In the computations reported here, several trends were noted. First, after complexation with the divalent metal ion, the carboxylate groups reorganized into what could be considered a "metallo ester". This is especially noticeable in the complexes without the waters of hydration. The "etheral oxygen-carbonyl carbon" bond lengthened and the "carbonyl oxygen-carbonyl carbon" bond shortened (Table VI) when compared to the equilibrium carboxylate bond lengths for malonate itself (1.257 Å using the 3-21 G basis set.²⁶ Furthermore, the unhydrated Mg(II) ion complexes show greater differentiation between these two bond lengths than does the Ca(II) ion complex. The direct interaction of both divalent metal ions with C₂ in the deprotonated on carbon forms is clearly seen in the significantly shorter C-Me distance as compared to the other structures. All of these trends are somewhat mitigated in the hydrated metal ion complexes. In addition, the carboxylate oxygen-metal ion distance (the etheral oxygen-metal ion distance) was always shorter than the average water oxygen-metal ion distance.

The implications of the existence of the complex Mg(OH)⁺ [and Ca(OH)⁺] forms in enzymatic biochemistry have recently been examined by Herschlag and Jencks.⁴² It is thus perhaps not so surprising that these metal ion forms appear naturally in the present study.

Discussion

Comparison of selected structural parameters for the appropriate malonate-divalent metal ion crystal structures^{14,19} and the results for the optimizations of structure I are given in Table VII. It is somewhat difficult to directly compare calculated structures with those obtained from crystallographic studies, especially those crystallographic studies of small molecule complexes. The carboxylate oxygens have at least one additional crystal interaction (either ionic or hydrogen bond) that is not present in these computations. Furthermore, crystal packing forces can lead to dis-

Table VI. Selected Distances from Each of the Optimized Structures Reported in This Study (Figure 3) without and with the Waters of Hydration about the Divalent Metal Ion^a

| | structure | | | |
|-------|-------------------------------|--------------------|---------------------|------------|
| | I without/with | II without/with | III without/with | IV with |
| | Malonate-Mg(II) Ion Complexes | | | |
| C—O | 1.346/1.306 | 1.359/1.342 | 1.391/1.402 | 1.285 |
| C=O | 1.202/1.238 | 1.209/1.220 | 1.223/1.238 | 1.238 |
| C—Me | 2.987/3.384 | 2.989/3.194 | 2.287/2.576 | 3.314 |
| O—Me | 1.788/2.000 | 1.787/1.958 | 1.806/1.937 | 1.986 |
| Me—OW | 2.075 | 2.065 | 1.992 | 2.008 |
| | Malonate-Ca(II) Ion Complexes | | | |
| C—O | 1.322/1.305 | 1.336/1.338 | 1.359/1.340 | 1.287 |
| C=O | 1.228/1.228 | 1.218/1.221 | 1.234/1.229 | 1.238 |
| C—Me | 3.480/3.664 | 3.465/3.742 | 2.697/3.622 | 3.737 |
| O—Me | 2.096/2.395 | 2.096/2.325 | 2.118/1.986 | 2.334 |
| Me—OW | 2.348 | 2.339 | 2.352 | 2.389 |

^aC—O is the average of the two etheral oxygen-carbonyl carbon (C₁-O_{1A} and C₃-O_{3A}) bond distances of the metallo ester (except in the enol form, where there is only one ester), C=O is the average of the two carbonyl oxygen-carbonyl carbon (C₁-O_{1B} and C₃-O_{3B}) bond distances of the metallo ester (except in the enol form, where there is only one ester), C—Me is the optimized distance from C₂ to the divalent metal ion, O—Me is the average of the two etheral oxygen-metal ion (O_{1A} and O_{3A} to Me) bond distances, and Me—OW is the average distance from the divalent metal ion to the oxygen of each of the four waters of hydration (O₁, O₂, O₃, and O₄).

ortion relative to the gas-phase (or computed) structure. Overall, the malonate-Ca(II) ion optimized and experimentally observed structures are qualitatively similar with no major differences noted. The symmetry-constrained malonate-Mg(II) ion complex is very similar to the crystal structure of Curry et al.¹⁴ After removal of the symmetry constraints, a significant reorganization of the complex was noted and the symmetry observed in the crystal structure of the complex was broken. Thus, the fully optimized structure in this study differs from the experimentally observed small molecule crystal structure.

The Mg(II) and Ca(II) ion structures without the added waters of hydration were very similar for all complexes studied. The addition of the waters of hydration to the structures with C₂ saturated resulted in a similar Ca(II) ion structure after optimization, but very different for the Mg(II) ion structure (the chair conformation was distorted). In the structures with C₂ as a methine, the optimized structure for the hydrated Mg(II) ion is substantially different from that for the hydrated Ca(II) ion. Even with the significant movement and differences found for the Ca(II) ion upon addition of the waters of hydration, only modest differences in the relative energies of the various complexes were found, whereas the relative energy for the keto form of the Mg(II) ion complex was lowered considerably upon the addition of the waters of hydration (Figure 3; Table V). It is clear that the

(41) Deerfield, D. W., II; Fox, D.; Head-Gordon, M.; Hiskey, R. G.; Pedersen, L. G. *J. Am. Chem. Soc.* **1990**, to be submitted for publication.

(42) Herschlag, D.; Jencks, W. P. *Biochemistry* **1990**, *29*, 5172-5279.

Table VII. Comparison of Selected Geometrical Parameters from the ab Initio Optimization of Malonate in a Chelation Bidentate with either Mg(II) or Ca(II) without or with (Fully Geometry Optimization) the Added Waters of Hydration about the Metal Ion Compared with Those Obtained in Crystallographic Studies of Complexes between Substituted Malonates and either Mg(II)¹⁴ or Ca(II)^{14,19a}

| | Mg(II) ion complexes | | | Ca(II) ion complexes | | | |
|---|----------------------|------------|-------------------|----------------------|------------|--------------|--------|
| | calcd | | crystal ref 14 | calcd | | crystal | |
| | without water | with water | | without water | with water | ref 14 | ref 19 |
| C ₂ -C ₁ | 1.5471 | 1.5274 | 1.533 | 1.5486 | 1.5422 | 1.512 | 1.531 |
| C ₁ -O _{1A} | 1.3452 | 1.3033 | 1.260 | 1.3219 | 1.3008 | 1.270 | 1.264 |
| C ₂ -C ₁ -O _{1A} | 114.9985 | 113.6729 | 117.5 | 114.9432 | 114.1761 | 117.9 | 119.1 |
| C ₁ -O _{1B} | 1.2016 | 1.2357 | 1.246 | 1.2106 | 1.2278 | 1.243 | 1.260 |
| C ₂ -C ₁ -O _{1B} | 121.4193 | 122.2041 | 119.8 | 120.3076 | 120.5627 | 118.6 | 119.7 |
| C ₂ -C ₃ | 1.5449 | 1.5275 | 1.533 | 1.5486 | 1.5337 | | 1.537 |
| C ₃ -O _{3A} | 1.3458 | 1.3077 | 1.260 | 1.3219 | 1.3096 | | 1.265 |
| C ₂ -C ₃ -O _{3A} | 114.9018 | 113.6420 | 117.5 | 114.9432 | 115.3280 | | 117.6 |
| C ₃ -O _{3B} | 1.2017 | 1.2324 | 1.246 | 1.2106 | 1.2281 | | 1.260 |
| C ₂ -C ₃ -O _{3B} | 121.5463 | 122.5240 | 119.8 | 120.3076 | 120.6256 | | 118.1 |
| Me-O ₃ | | 2.0721 | | | 2.3523 | 2.384, 2.431 | |

^a See Figure 4 for the atom definitions and Figure 5 for the minimized structures of these complexes.

inclusion of the waters of hydration is essential for the proper definition of the acidic proton of the malonate-hydrated metal ion complex.

The enol forms of the malonate-Ca(II) ion complex with and without the waters of hydration about the Ca(II) ion (structure II, Figure 3) were found to be approximately 15 kcal mol⁻¹ less stable (Table V) than the keto tautomer (structure I, Figure 3). The enol form of the unhydrated malonate-Mg(II) ion complex was similarly destabilized as compared to the keto form. This difference was increased upon the addition of the waters of hydration to the Mg(II) ion (by greater than 24 kcal mol⁻¹). This is compared to malonate anion alone where, at this basis set level, the enol form was calculated to be slightly more stable than the keto form.²⁶ Thus, the enol tautomer is destabilized upon complexation with either divalent metal ion, and the amount of destabilization is dependent upon the nature of the metal ion. The origin of this destabilization appears to be primarily steric in nature. This is evident by opening of the C₂-C₁-O_{1A} angle upon going from the keto form (113.7°) to the enol tautomer (128.1°).

The calculated energies to deprotonate on carbon (structure III, Figure 3) are similar for the two metal ion complexes [Mg(II), 374.4 and 397.8 kcal mol⁻¹; Ca(II), 398.9 and 377.4 kcal mol⁻¹; without and with the waters of hydration, respectively] and compare favorably with the first two calculated deprotonation energies for malonic acid.²⁶ These energies are substantially less than the similar deprotonation on carbon to form the trianion ($\delta E_1 = 354.8$ kcal mol⁻¹, $\delta E_2 = 464.1$ kcal mol⁻¹, $\delta E_3 = 630.6$ kcal mol⁻¹). Thus, the calculated energy to deprotonate on carbon in a Meldrum's acid form relative to malonic acid itself has been reduced by about 235 kcal mol⁻¹. The deprotonated on carbon form was found to be substantially less stable than the enol form (the $\Delta E_{\text{Mg(II)}}$ was 341.4 and 373.3 kcal mol⁻¹, and the $\Delta E_{\text{Ca(II)}}$ was 384.6 and 361.6 kcal mol⁻¹, without and with the waters of hydration about the metal ion, respectively). These energy differences are probably substantially greater than the possible difference in stabilization that would occur upon solvation. The calculated energies to deprotonate one of the waters of hydration situated about the metal ion (IV, Figure 3) were similar for the two metal ions studied ($\Delta E_{\text{Mg(II)}} = 355.3$ kcal mol⁻¹, $\Delta E_{\text{Ca(II)}} = 341.9$ kcal mol⁻¹; the deprotonated on oxygen form was found to be more stable than the deprotonated on carbon form ($\Delta \Delta E_{\text{Mg(II)}} = 42.7$ kcal mol⁻¹; $\Delta \Delta E_{\text{Ca(II)}} = 25.5$ kcal mol⁻¹).

We now discuss possible relationships between our model calculations and the mechanism of electrophilic attack at the C_γ position of Gla in the coagulation zymogens. Such attack can proceed in either a one-step mechanism through electrophilic attack on the enol form of a two-step mechanism involving the deprotonation at C_γ to form the reactive deprotonated intermediate, which is then alkylated (or protonated). As described above, the model deprotonated structure is over 300 kcal mol⁻¹ less stable than the model enol. The 300 kcal mol⁻¹ difference does not directly relate to the relative rates via the two pathways, since this is a function of the total activation energy of each pathway.

But it is not unreasonable to assume that the two activation energies for the actual alkylation steps are similar, suggesting that the path involving the enol form is the preferred alkylation path. Additionally, solvation at the carbon and oxygen is likely to stabilize the intermediate anion, although this should be on the order of tens of kilocalories. Thus, neither of these corrections seems large enough to eliminate the calculated 300 kcal mol⁻¹ energy differences.

A second factor which also indirectly suggests that electrophilic attack at C_γ proceeds via the enol form follows from a comparison of the changes in relative rate of reaction of the model compounds upon complexation with the metal ion. In particular, the calculations suggest that the enol form is destabilized relative to the keto form upon complexation with the divalent metal ion, so that if the relative activation energy of the actual alkylation (or protonation) step was also similarly affected, then the relative rate of the electrophilic attack would be reduced by this complexation. This is what is observed experimentally.^{4,5} By contrast, the calculations suggest that the species deprotonated at C_γ is dramatically stabilized upon complexation. Thus, the rate should increase if III were the reactive intermediate in the electrophilic attack.

The inherent "protection" of the apparent "loose" metal ion binding sites in the vitamin K dependent proteins to modification and proton exchange is consistent with the calculations presented here. Upon complexation to either divalent metal ion in a chelation bidentate mode, the enol tautomer (structure II) is significantly destabilized relative to the keto form (structure I) when compared to malonic acid. Thus, one would expect a significant retardation of either modification or proton exchange if the reaction pathway involved the enol tautomer. Furthermore, the computations have also clearly demonstrated that the other possible active intermediate—the deprotonated on carbon form (structure III)—is probably not a viable intermediate since deprotonation of one of the waters of hydration about the metal ion (structure IV) leads to a more stable structure. Prothrombin has 10 Gla residues and binds 7 Ca(II) ions—4 loose sites [presumably a single Ca(II) ion interacting with a single Gla residue] and 3 tight sites [presumably the 6 remaining Gla residues interacting with the 3 remaining Ca(II) ions].³ Upon reaction of the Tb(II) ion—prothrombin fragment 1 complex with a Schiff base, three Gla residues are modified, with at least one of these modified Gla residues resident in a Ca(II) ion tight site.^{17,18} From these experimental results and the known chemical reactivity of the proteins, one is tempted to propose that each of the tight metal ion binding sites contains two Gla residues—one in a chelation bidentate orientation to the metal ion and the other in either a unidentate or bidentate orientation. With this view, the unidentate or bidentate coordinated Gla residue would undergo the subsequent modification. However, the Gla residues interacting with a metal ion in a chelation bidentate orientation (the four loose sites and the three Gla residues in the tight sites that are in chelation bidentate orientation) would remain protected from modification.

Conclusion

We have used ab initio molecular orbital theory at the HF/3-21G* level to study the structures and relative energies of the complexes formed by the chelation bidentate binding of Mg(II) and Ca(II) ions to malonate. These calculations can be viewed as simple models for the interaction between these ions and the malonate side chain of Gla residues in coagulation zymogens. The calculations have several limitations: entropic effects are necessarily absent, and finite computer resources prevent us from fully solvating the complexes or using basis sets and correlation treatments capable of yielding quantitative accuracy. We believe that these limitations will not fundamentally affect our main conclusions, which are summarized below.

(1) We report optimized structures and relative energies for the parent complexes, their enol forms, and the deprotonated forms of these complexes. We also report the first ab initio calculations on the effect of hydration on the malonate-metal complexes. We include four water molecules to allow the metal ion to become hexacoordinated. Consideration of hydration is shown to be very important in the study of deprotonated reactions: we find the most acidic proton is from one of the coordinated water molecules, not from the methylene carbon of the malonate.

(2) Comparison of the relative energies of the keto, enol, and deprotonated structures with and without complexed metal ions suggests that the mechanism of electrophilic attack at C_γ on the malonate side chain of Gla occurs via the alkylation of the enol form. Furthermore, the metal ion complexed enol form is de-

stabilized relative to the uncomplexed form. This appears to be consistent with the reductions in the observed rate of alkylation of the Gla containing proteins upon complexation with metal ions.

(3) On the basis of our calculations, we suggest that the experimental results showing reduced reactivity at most Gla sites are consistent with protection of only those sites in which the divalent metal ion is bound in a chelation bidentate orientation.

(4) Physiologically, there are critical differences between Ca(II) ion-malonate and Mg(II) ion-malonate complexes. Thus, the origin of the Ca(II) ion specificity in the coagulation cascade does not appear evident in the chelation bidentate binding of a single metal to malonate. Future work will consider the influence of waters of hydration on other possible metal ion-malonate modes of coordination.⁴¹

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Supplementary Material Available: Tables (I-IV) of the ab initio optimization results (4 pages). Ordering information is given on any current masthead page.

Nature of Bonding in Phosphazoylides. A Comparative Study of N₂H₄, NPH₄, and P₂H₄[†]

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Abstract: Structures, energies, and electronic properties of the ylides H₃NNH (2), H₃PPH (5), H₃PNH (8), and H₃NPH (10) of hydrazine (1), diphosphine (4), and aminophosphine (7) have been studied with ab initio molecular orbital theory by use of a split valence plus polarization basis set and incorporating electron correlation and zero-point energy corrections. The results show all ylides to be equilibrium structures. The Staudinger tautomer H₃PNH (8) is 29.7 kcal/mol less stable than aminophosphine H₂NPH₂ with a 50.0 kcal/mol barrier for hydrogen migration. The very short P-N bond of 8 is ascribed to result mainly from the large Coulombic interaction between the NH and PH₃ groups, augmented with a partial triple bond contribution that results from π and σ interactions. The properties of the H₃PPH ylide 5 are similar to the Staudinger complex 8 but less pronounced. The chemical bonding in these "hypervalent" molecules 5 and 8 is dominated by ionic bonding and conjugative stabilization with little if any d-orbital participation.

Phosphazo compounds have become widely applied synthons since their first synthesis by Staudinger and Meyer in 1919.¹ For six decades primary amines and amides have been synthesized by the Staudinger reaction,² which recently also has been used for polymerizations.³ The applicability and varied reactivity of the key intermediates, the iminophosphoranes, are of experimental and theoretical interest. In this paper we describe the nature of bonding in the parent phosphazo compound.

The extensive literature and detailed understanding on the nature of P=C (alkylidenephosphoranes)⁴ P=O bonding⁵ contrasts the limited information on the iminophosphorane P=N bond. The Staudinger intermediate signifies the importance of the iminophosphorane R₃P=NR, which is isoelectronic with the well-studied Wittig reagent R₃P=CR₂ and phosphine oxide R₃P=O. The reactivity of the Wittig reaction is considered to

result from zwitterionic (ylide) character. In analogy, this may also be an important resonance form for the iminophosphoranes.

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