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Gas phase interaction of L-proline with Be^{2+}, Mg^{2+} and Ca^{2+} ions: a computational study

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ABSTRACT: Geometry optimisation and metal ion affinities (MIAs) of the binding configurations of Be^{2+}, Mg^{2+} and Ca^{2+} to L-proline were calculated using the hybrid Density Functional Theory (DFT-B3LYP) and second order Møllet–Plesset perturbation theory (MP2) methods. Be^{2+} was found to bind preferentially in a charge transfer type arrangement through the carbonyl oxygen (—C═O) and the lone pair of the imino-group nitrogen atom (—NH—). On the contrary Mg^{2+} and Ca^{2+} were found to prefer binding in a bi-dentate manner through the carboxylate group of L-proline (OCO) in a zwitterion form. The main types of interactions found to influence the binding preference of M^{2+} ions to L-proline were (i) charge transfer in the case of Be^{2+} ions and (ii) electrostatic interactions in the case of Mg^{2+} and Ca^{2+} ions. Inspection of the IR stretching of the N—H and the O—H groups of L-proline with M^{2+} ions in a chelating configuration (to both O and N atoms) indicated a considerable shift to higher frequency with decreasing MIA. On the other hand, the MIA for the zwitterion L-proline with M^{2+} tracks the reciprocal distance of the M^{2+}—OCO bond further confirming that the nature of the bond is mainly electrostatic. Comparison with other molecules containing the carboxylic function is also included in order to gain more insight on the types of interaction of this amino acid with metal ions in general. Copyright © 2007 John Wiley & Sons, Ltd.
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KEYWORDS: L-proline; metal ion affinity (MIF); free energy (ΔG); zwitterion structure; Ca^{2+}; Mg^{2+}; Be^{2+}; infrared frequency; natural charge; DFT B3LYP; MP2

INTRODUCTION

The study of the interaction of biological molecules with metal ions is of importance as it supplies a simple model system to help in the understanding of basic chemical and physical properties in wide areas extending from biochemical reactions,[1–4] biomedical implants[5] sensors, catalysis and evolution theories.[6] Group 2 metal ions (Be^{2+}, $Mg^{2+}$and Ca^{2+}) are of particular interest as they play important roles within the human body, ranging from the role in enzymatic activity and stabilising biological membranes (Mg^{2+}), their use in helping of blood clot formation, muscle contraction and growth (Ca^{2+}) and their role in fixing proteins into particular conformers.[4] Of particular interest is how group 2 ions interact with the amino acid L-proline. L-Proline has many functions within the human body. First, it is a constituent of collagen (I): a major high tensile structural protein found in bone, teeth and cartilage.[5] Second, proline itself plays an important role in enzymes and peptide hormones.[7] Third, the amine group of L-proline is contained within the pyrrolidene ring hence making conformation more rigid when compared to other amino acids. This rigidity makes L-proline an important residue in the specific function of β and γ turns in polypeptide chains.[8]

L-Proline has been studied experimentally through X-ray crystallography,[9] laser-ablation coupled with Fourier-Transform microwave spectroscopy[10] and nuclear magnetic resonance spectroscopy[11] to elucidate structural information on both the neutral and zwitterionic forms of L-proline. Matrix isolation infrared (IR) spectroscopy has been employed to obtain vibrational frequencies of neutral L-proline.[12] In general, experimental studies involving amino acids interacting with different metal ions have proven difficult to perform in particular because of their low vapour pressure. This has resulted in the increased use of computational modelling to investigate these systems. L-Proline has 18 possible stable conformers,[13] the most stable two are presented in Fig. 1.

Experimental studies to determine the binding energies of metal–amino acid complexes are performed through the use of many techniques including mass spectroscopy,

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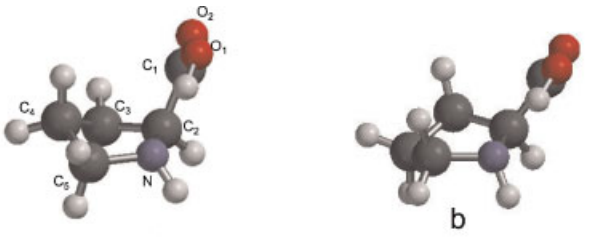

	6-311+G**	6-311++G(2df, 2p)
E_a (a.u.)	-401.2846558	-401.3065817
E_b (a.u.)	-401.2838708	-401.3056631
E_a- E_b (kcal/mol)	0.49	0.58

Figure 1. Structure of L-proline geometry optimised using DFT B3LYP. This figure is available in colour online at www.interscience.wiley.com/journal/poc

IR and kinetic studies to determine either absolute or relative binding energies.[14–16] It is, however, tedious to obtain information on how the metal ion itself binds to the amino acid or the types of interactions that are involved in the bonding process. At present a growing number of experimental work is devoted to probe the possible binding mechanism of amino acids to metal ions.[17–20] Several computational studies involving both amino acids and biological molecules attached to a range of metal ions[1,2,21–34] have been performed. Those involving proline have included metal ions such as Cu^+,[1,2] Ag^{+}[21–23] and group 1 metals (Li^+, Na^+ and K^+),[27] among others.

The interaction of proline with Cu^+ was undertaken to obtain vibrational frequencies and thermodynamic data on the calculated structures.[2] The most stable structures found were (i) co-ordination of Cu^+ through the carboxylate functional (OCO) in a bi-dentate fashion, similar to a Cu^{2+}-amino acid adduct and (ii) Cu^+ positioning itself between the N of the −NH group in the pyrrolidine ring and the O of the carboxyl group, C═O, (charge solvation); unlike the case of Cu^{2+}[25] and Ag^{+}[22] ions.

Studies involving group 1 metal ions (Li^+, Na^+ and K^+) binding with L-proline have also been conducted[27] using the DFT-B3LYP method. The most stable configuration was found to involve the Li^+ ion interacting with zwitterionic proline through the carboxylate group in a bi-dentate fashion. This interaction was found to be strongly ionic in nature as very little charge transfer occurred between the Li^+ and the proline molecule (natural charge for $Li^+ = 0.956\,|e|$). Similar results were found when considering the interaction of Na^+ and K^+ with proline. The only difference is in the nature of the bonding, where it was found that the interaction was more ionic than Li^+ with a natural charge of $0.970\,|e|$ on the Na^+ ion and $0.982\,|e|$ for K^+.

Although these studies give a good insight into how metal ions interact with amino acids and with particular emphases on L-proline, there have been no investigations into how L-proline interacts with divalent cations such as Mg^{2+} and Ca^{2+}. This is of interest as both magnesium and calcium are present within the body as bone in the form of calcium carbonate-hydroxyapatite $((Ca, X)_{10}(PO_4HPO_4CO_3)_6(OH,Y)_2$ where $X = Mg^{2+}$, Na^+, Sr^{2+}; $Y = Cl^-$ and F^-).[35]

The effect of alkali earth metal ions (Be^{2+}, Mg^{2+}, Ca^{2+}, Sr^{2+} and Ba^{2+}) on the intramolecular proton transfer of glycine[28] has been studied using the DFT-B3LYP method. The most stable configurations for the various metal ion/amino acid complexes are as follows: For $Be^{2+}$the charge solvation arrangement, where binding occurs between the carbonyl functional and nitrogen of the imino group, was found to be energetically the most favourable. With regards to Mg^{2+}, Ca^{2+}, Sr^{2+} and Ba^{2+}, binding to glycine in a bi-dentate manner through the carboxylate oxygen atoms was preferred. This was attributed to (i) the bi-dentate binding configuration being the only way to accommodate the increasing atomic radius size of M^{2+} ion and (ii) electrostatic and polarisation effects of the metal ions being able to stabilise the zwitterionic form of glycine.

We present in this work a computational study of the interaction of Be^{2+}, Mg^{2+} and Ca^{2+} with L-proline using the DFT-B3LYP and MP2 methods to investigate (i) how M^{2+} ions bind to L-proline and the nature of the binding that occurs (i.e. whether the bonding is ionic or covalent in nature) and (ii) to obtain the metal ion affinities (MIAs) and corresponding IR frequencies. Although not of biological significance, Be^{2+} has been included to investigate any trends that may occur due to such factors as increasing ion or electrostatic field size.

COMPUTATIONAL METHODS

While L-proline has 18 possible configurations, only 4 are present at room temperature.[13] Out of these four configurations, we have restricted the study to only one configuration of L-proline. The conformer chosen, has the carboxylate group twisted to allow intramolecular hydrogen bonding to occur between the hydroxyl proton and the imino group of the pyrrolidine ring in the pucker down position. There are two structures with global minima that lay within a few 10s of a kcal mol^{-1} in energy from each others: these are presented in Fig. 1.[13,27] All the work reported here is with the ring puckering down (Fig. 1b). Also presented in the Fig. 2 basis sets for the DFT/B3LYP computation using: 6-311+G** and 6311++G(2df,2pd). Numerous studies have shown that 6-311+G** is adequate for organic compounds containing nitrogen atoms, such as amino acids and their complexes with monovalent and divalent alkali metal ions[25,27,36–42] and this basis set was the method of choice

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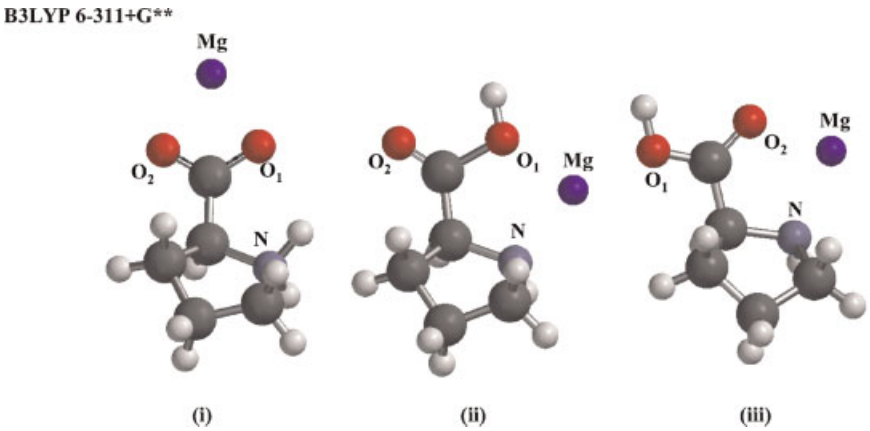

Figure 2. Optimised structures for Mg^{2+} bound to L-proline at the B3LYP 6-311+G**. (i) Zwitterion adduct, (ii) charge solvated structure 1 and (iii) charge solvated structure 2; these structures differs little at the MP2 6-311+G** level, more details are found in Tables 3 and 4. This figure is available in colour online at www.interscience.wiley.com/journal/poc

in the work although some of the results are reported with 6-311++G** (that adds a diffuse function to the 6-311G**) as well as 6-311++G(2df,2pd) (that improve the polarisation of the 6-3111++G**).[43] In general very small variations in the total energy and negligible differences in the bond distance and bond angle is observed. For example, the energy difference of L-proline when using 6-311+G** to 6-311++G(2df,2p) is about 0.02 a.u. for both structures reported in Fig. 1. As indicated below comparison between structures is done at the same basis sets. Although many possible modes of complexation can be studied we have restricted the study to three as they are the most interesting based on many previous work as detailed in the introduction section. These modes are:

(i) Salt Bridge (SB) configuration: bi-dentate co-ordination through a chelating type bonding arrangement to carboxylate group of zwitterionic L-proline,
(ii) Charge solvated 1 (CS1): binding of the metal ion through both the imino N atom and the oxygen atom of the hydroxyl group,
(iii) Charge solvated 2 (CS2): binding of the metal ion through the imino N atom and the oxygen atom of the carbonyl group.

The various binding modes investigated for M^{2+} to L-proline is presented in Fig. 2, where that of Mg^{2+} is given as an example.

Calculations performed in this study used Spartan 04, Spartan 06 and Gaussian 03 programs.[43,44] The equilibrium geometries and energies of the various metal ion–proline complexes were calculated first using the restricted Hartree-Fock method with the 3-21G* basis set. These geometries were then further refined using the B3LYP[34,35] and/or MP2 methods with the 6-31G**, 6-311+G** and 6-311++G** (for some data in particular those involved Li^+, Na^+ and K^+ ions complexes to L-proline to compare with previous work[27]) basis sets to investigate the effects of using hydrogen polarisation (**) and diffuse (+) basis functions on the various metal complexes. Most of the work presented here is using the 6-311+G** and 6-311++G**. Using the 6-31+G** with the MP2 method was found in general inadequate for this study as the result often deviated from those found using the 6311+G** or the 6-311++G** basis set. Negligible structural and energy differences were observed between these last two basis sets (6-311+G** and 6-311++ G**) at both levels of theories. Moreover, it was found that the DFT B3LYP method gave accurate representation of the nature of bonding and its energy when compared to other work on L-proline or monometallic ions and thus it was preferred than the MP2 method, since the former method is computationally less demanding. All calculations were performed without symmetry constraints and then vibrational frequency calculations were performed to confirm that all structures had reached a minimum.

Thermodynamic calculations were performed to obtain enthalpy (H) and Gibbs free energy (G) values for each individual system at standard conditions ($T = 298.15$ K, 1 atmosphere) to determine the MIAs for each individual system. MIA is considered to be the negative of the enthalpy ($-\Delta H$) from the following reaction:

$$\mathrm{Pro} + \mathrm{M}^{2+} \rightarrow \mathrm{Pro} - \mathrm{M}^{2+}$$

where Pro denotes the amino acid L-proline and M^{2+} the alkali earth metal ion under investigation.

RESULTS AND DISCUSSION

Table 1 in supplementary materials (SMT1) presents the structure parameters for the isomer of L-proline shown in Fig. 1b using both DFT(B3LYP) and MP2 theories and comparison with previous work. Good agreement is particularly noticed between both DFT and MP2 methods with the 6-311+G** basis set.

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Table 1. The relative stability for M^{2+} to L-proline

	B3LYP 6-311+G**	ΔE (kcal mol^{-1})	MP2 6-311+G**	ΔE (kcal mol^{-1})
Be^{2+}				
	(iii)	0	(iii)	0
	(i)	7.63 (7.67)	(i)	5.80
	(ii)	10.14	(ii)	7.05
Mg^{2+}				
	(i)	0	(i)	0
	(iii)	10.59 (10.81)	(iii)	10.87
	(ii)	29.91	(ii)	27.83
Ca^{2+}				
	(i)	0	(i)	0
	(iii)	17.42 (16.42)	(iii)	15.12
	(ii)	36.37	(ii)	31.53

The numbers (i) to (iii) are the same as in Figure 2. (i) SB, (ii) CS1, (iii) CS2. Numbers in ( ) are using the 6-311++G** basis set.

M^{2+} to L-proline complexes

The three possible binding configurations considered for the Be^{2+}–L-proline complexes, such as those shown in Fig. 2 for Mg^{2+} have been optimised and were found to be true minima (i.e. no imaginary vibrational frequencies were present). From the geometry optimisations at the B3LYP and MP2 levels, the order of stability for the three conformers is given in Table 1. Be^{2+} favours binding to L-proline in a CS2 arrangement through the carbonyl oxygen on the carboxylate group and imino N atom on the pyrrolidine ring independent of the method, basis set used and ring puckering (SM1). This configuration is preferred by 7.6 and 5.8 kcal mol^{-1} at the DFT-B3LYP and MP2 levels (both with 6-311+G** basis set), respectively (Table 1). In SM Fig. 1 (SMF1) results for Be^{2+} in CS2 and SB for the pucker up position are included for comparison at the DFT/B3LYP and MP2 levels of theory both with 6-311+G** basis set.

As with Be^{2+}, the three initial bonding arrangements were considered for the attachment of Mg^{2+} and Ca^{2+} to L-proline. However, upon optimisation with the B3LYP and MP2 methods, some clear differences became evident from comparing the relative stabilities of the different structures. Both levels of theory (at all investigated basis sets: 6-31G**, 6-311+G** and 6311++G**) predict that the zwitterionic bonding arrangement (structure (i)) is the most stable mode of binding for the Mg^{2+} and Ca^{2+} ion. Presented in SMT2 are the structure parameters for the zwitterion complexes.

The change in relative stability from the CS structure in the Be^{2+}–L-proline complex to the zwitterionic structure for Mg^{2+} and Ca^{2+} might be due to at least two possible reasons (i) as one moves down the alkali earth metal series, one finds an increase in ionic volume ($V_{Be^{2+}} = 7.89$ Å^3, $V_{Mg^{2+}} = 10.51$ Å^3, $V_{Ca^{2+}} = 14.98$ Å^3, volume computed using DFT/B3LYP 6-31G**). This makes incorporating the metal ion between the imine N and the carboxylate group increasingly difficult as evidence by the increasing M—O_1 distances presented in Table 3, and (ii) the preference for the metal ion to adopt a particular binding arrangement could be based on whether charge transfer or electrostatic type interaction is the preferred method of binding. The importance of these electrostatic and polarisation interactions on the geometries of metal ion-adducts has been explained in a previous work involving the co-ordination of Na^+ to various adducts.[31]

Comparison of the natural charges was used in conjunction with calculated bond lengths for the binding configurations of Be^{2+}, Mg^{2+} and Ca^{2+} to L-proline to determine the nature of the binding occurring in each of the structures (Table 2). Natural charges were computed from Natural Atomic Orbital (NAO) populations analysis (also known as natural population analysis) conducted within Spartan and Gaussian codes. These analyses are based on the method reported by Reed *et al*.[45,46] Unlike Mulliken population analyses where half of the overlap population is arbitrary assigned to each basis function, the method of refs. is based on computing for a set of orthonormal NAOs that are then used to compute for a set of natural bond orbitals representing the type orbital (core, bond or lone pair orbital).[47] The natural charges on structure (i) showed that as the Van der Waal's radius of the M^{2+} ion increases, the amount of' positive charge on the M^{2+} ion increases (natural charge zwitterion B3LYP: 6-311+G** $Be^{2+} = 1.75$ |e|, $Mg^{2+} = 1.82$ |e| and $Ca^{2+} = 1.87$ |e|). This increase in the amount of charge on the M^{2+} is also accompanied by an increase in the M^{2+}—O bond lengths. Thus, with the Mg^{2+} and Ca^{2+} ions retaining more of the charge, this tends to suggest that their bonding is more electrostatic in nature. This trend is also visible in structures (ii) and (iii).

From this reasoning, the binding of Be^{2+} to L-proline involves higher contribution of charge transfer rather than electrostatic effects, when compared to Mg^{2+} to L-proline and Ca^{2+} to L-proline. This can be seen in the electrostatic potential (charges) map (the energy of interaction, ε_p, of a unit positive charge at some point in space p with the nuclei and the electrons of a molecule) of L-proline with

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Table 2. Calculated natural charges and bond lengths for the lowest energy configuration for M^{2+} to L-proline

	Be^{2+} to L-proline			Mg^{2+} to L-proline			Ca^{2+} to L-proline		
	Natural charge	Bond type	Bond lengths (Å)	Natural charge	Bond type	Bond lengths (Å)	Natural charge	Bond type	Bond lengths (Å)
Structure (i)									
B3LYP 6-31G**									
M	1.75			1.79			1.84		
O_1	−0.85	M–O_1	1.57	−0.32	M–O_1	2.00	−0.83	M–O_1	2.22
O_2	−0.81	M–O_2	1.56	−0.78	M–O_2	1.98	−0.79	M–O_2	2.20
N	−0.64			−0.64			−0.64		
B3LYP 6-311+G**									
M	1.75			1.82 (1.83)			1.87		
O_1	−0.84	M–O_1	1.57	−0.84 (0.82)	M–O_1	2.02 (2.03)	−0.84	M–O_1	2.23
O_2	−0.80	M–O_2	1.56	−0.78 (0.77)	M–O_2	1.99 (2.00)	−0.79	M–O_2	2.28
N	−0.57			−0.57 (0.67)			−0.57		
MP2 6-311+G**									
M	1.72			1.83			1.94		
O_1	−0.61	M–O_1	1.59	−0.63	M–O_1	2.03	−0.95	M–O_1	2.30
O_2	−0.56	M–O_2	1.58	−0.56	M–O_2	2.01	−0.91	M–O_2	2.26
N	−0.49			−0.49			−0.60		
Structure (iii)									
B3LYP 6-31G**									
M	1.75			1.81			1.86		
O_1	−0.56			−0.60			−0.61		
O_2	−0.88	M–O_2	1.502	−0.83	M–O_2	1.93	−0.82	M–O_2	2.167
N	−1.03	M–N	1.626	−0.97	M–N	2.08	−0.88	M–N	2.392
B3LYP 6-311+G**									
M	1.76			1.84			1.86		
O_1	−0.53			−0.57			−0.58		
O_2	−0.87	M–O_2	1.503	−0.82	M–O_2	1.94	−0.82	M–O_2	2.19
N	−0.97	M–N	1.625	−0.88	M–N	2.08	−0.85	M–N	2.42
MP2 6-311+G**									
M	1.73			1.85			1.95		
O_1	−0.41			−0.45			−0.67		
O_2	−0.60	M–O_2	1.52	−0.56	M–O_2	1.96	−0.92	M–O_2	2.23
N	−0.78	M–N	1.64	−0.71	M–N	2.10	−0.89	M–N	2.43

Numbers in ( ) are for glycine–Mg^{2+}.

the three ions investigated of Fig. 3; more information on the electrostatic potential grid method can found in Reference [43] The figure presents M^{2+}–L-proline in CS2 and SB configurations as well as the SB configuration for the Li^+ and Na^+ ions. Few points can be extracted from this figure. (i) The electrostatic charges are much localised in the case of Li^+ and Na^+ ions. (ii) while Be^{2+} ion is smaller than the Li^+ ion the charge is less localised, compare the SB structures for both ions. This is due in part to a considerable electronic interaction with the oxygen atoms of the carboxylic group. (iii) The small size of the Be^{2+} ion together with its capacity in accepting electrons from the electronegative atoms (O and N) has resulted in a stable chelating adduct when compared to Mg^{2+} and Ca^{2+}.

The preference for Be^{2+} to bind in a charge transfer type arrangement has been shown previously.[48] Strittmatter *et al*. have shown that Be^{2+} prefers to bind to glycine in a CS bonding arrangement as there is a large amount of electron density transfer from the electrons lone pair of carbonyl oxygen and the amino nitrogen atom to the empty Be^{2+} 2s orbital (16.8 kcal mol^{-1} greater when compared to zwitterion bonding arrangement). The high charge density on Be^{2+} compared to the other alkali earth metals enhances the ability of Be^{2+} to act as a good electron pair acceptor thus increasing the amount of charge transfer that can occur and hence stabilising the CS structure. Similar reasoning has also been purposed to explain the binding of Cu^+ to proline.[2]

With regards to the binding of the other alkali metal ions in the series, electrostatic effects are the influential factor in determining the bonding configuration adopted by the metal ion.

Metal ion affinity (MIA)

MIAs were calculated for the three considered binding arrangements of Be^{2+}, Mg^{2+} and Ca^{2+} and are given in Table 3. As seen from the data there is a significant

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Table 3. Calculated thermodynamic data and metal ion affinities (MIAs) for M^{2+} to L-proline

	Conformer	Metal ion affinity ($-\Delta H$) (kcal mol^{-1})	ΔH difference (kcal mol^{-1})	$\Delta G^{298\,K}$ (kcal mol^{-1})
Be^{2+}	B3LYP 6-31G**			
	(iii)	301.44	0	−298.55
	(i)	296.07	5.37	−294.11
	(ii)	282.68	18.76	−283.48
	B3LYP 6-311+G**			
	(iii)	300.11	0	−290.07
	(i)	292.62	7.49	−283.72
	(ii)	288.68	11.43	−281.13
	B3LYP 6-311++G**			
	(iii)	301.52	0	−321.30
	(i)	294.48	7.04	−293.63
	MP2 6-311+G**			
	(iii)	290.90	0	−279.62
	(i)	285.10	5.80	−274.41
	(ii)	283.85	7.05	−277.03
Mg^{2+}	B3LYP 6-31G**			
	(i)	193.78	0	−186.10
	(iii)	182.62	11.16	−175.03
	(ii)	160.94	32.84	−154.78
	B3LYP 6-311+G**			
	(i)	186.36	0	−197.24
	(iii)	175.77	10.59	−186.79
	(ii)	156.45	29.91	−168.88
	B3LYP 6-311++G**			
	(i)	184.29 (162.69)	0	−207.17 (−155.93)
	(iii)	174.49	9.80	−196.63
	MP2 6-311+G**			
	(i)	176.07	0	−166.01
	(iii)	165.21	10.86	−155.12
	(ii)	148.25	27.82	−139.83
Ca^{2+}	B3LYP 6-31G**			
	(i)	150.64	0	−143.70
	(iii)	133.03	17.61	−126.16
	(ii)	111.67	38.97	−106.66
	B3LYP 6-311+G**			
	(i)	141.88	0	−131.74
	(iii)	124.46	17.42	−97.24
	(ii)	105.51	36.37	−95.75
	B3LYP 6-311++G**			
	(i)	139.95	0	−163.09
	(iii)	123.53	16.42	−146.22

Numbers in ( ) are for glycine–Mg^{2+} using B3LYP/6311++G**.

decrease in the calculated MIA value from Be^{2+} to Ca^{2+}. At present there are no experimental values involving group 2 metal ions which can be used to compare to the calculated values presented in this paper. Talley *et al.*[49] have published experimental MIA values for Li^+, Na^+, K^+ and Cs^+ bound to various amino acids and their corresponding methyl esters. However, no absolute values were reported. With regards to theoretical studies performed, Ai *et al.*[28] have reported absolute MIAs for alkali earth metals bound to glycine in the SB configuration. With the DFT-B3LYP (and LanI2dz basis set) method these energies were found as follows: Be^{2+} = 259.5 kcal mol^{-1}, Mg^{2+} = 161.6 kcal mol^{-1} and Ca^{2+} = 111.8 kcal mol^{-1}; Fig. 4 compares these figures to those reported in this work for L-proline. The MIAs are slightly higher for the L-proline complexes, the trend is however very similar. The authors of Reference [28] have studied the effect of larger basis sets such as 6-311+G* (compared to LanI2dz and 6-31G*) in the case of Be^{2+}–glycine and found a decrease of about 15 kcal mol^{-1}. The only theoretical study for the MIAs of L-proline involved its complexes with Li^+, Na^+ and K^+ ions.[27] The same decreasing trend in MIA values was observed for Li^+ to K^+ as for Be^{2+} to Ca^{2+}. However, the MIA values are much larger in the Be^{2+}, Mg^{2+} and Ca^{2+} L-proline complexes (Li^+ = 64.21 kcal mol^{-1}, Na^+ = 47.32 kcal mol^{-1} and K^+ = 34.62 kcal mol^{-1}). This large difference in MIA values can be attributed to the increased charge and charge density present on the M^{2+} ions as compared with the M^+ ions investigated by Marino *et al.*[27] Figure 5

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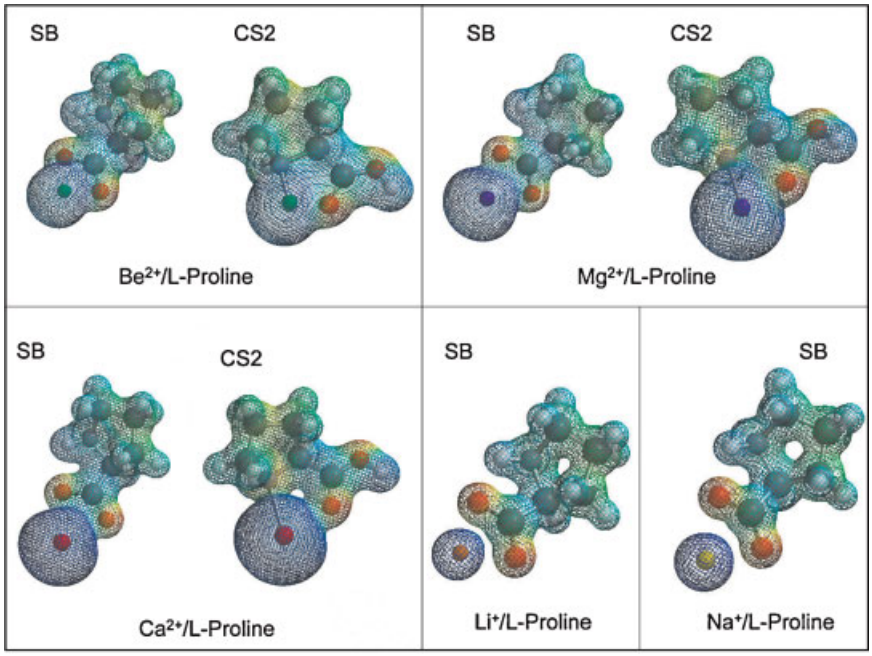

Figure 3. Potential (charges) map of L-proline $-M^{+,2+}$ for the zwitterion (SB) and charge solvated (CS2) structures. Blue: positive charges, red: negative charges. This figure is available in colour online at www.interscience.wiley.com/journal/poc

presents a comparison of the computation results of M^{2+}–L-proline from this work to that of L-proline–M^{+} from Reference [27] for two structures: the SB and CS2. The relative energy (the numbers on the *y*-axis are those of the relative interaction energy) of SB over CS2 is plotted on the *y*-axis as a function of the reciprocal distance between the metal ion and one of the oxygen of the carboxyl group. In the case of CS2 this oxygen is that of the carbonyl group. In the case of SB, the two oxygen atoms of the carboxyl group are within few % of similar distance from the metal ion although for consistency the O atom in the trans position with respect to the N atom of the ring is used as the reference, as indicated in the figure.

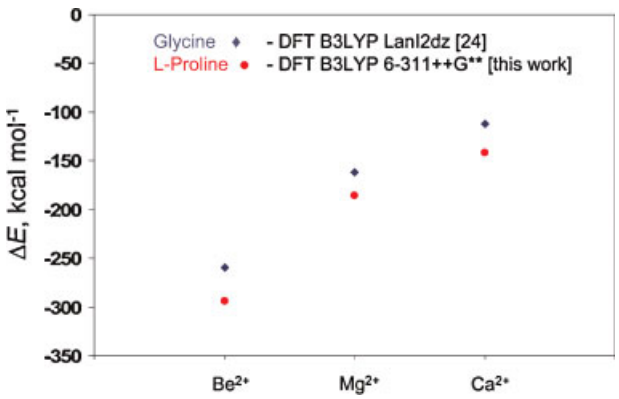

Figure 4. Comparison between the electronic energy of glycine and L-proline complexes with Be^{2+}, Mg^{2+} and Ca^{2+} for the SB structure. This figure is available in colour online at www.interscience.wiley.com/journal/poc

From this figure the following observations are noted:

- A co-relation of the energy with the inverse of distance suggests electrostatic interaction.
- The distance between O and M in ionic bonding should equal the sum of the ionic radii of O and M.
- Extrapolation of the group 1 results from Reference [27] finds the same *x* intercept as the group 2 results.

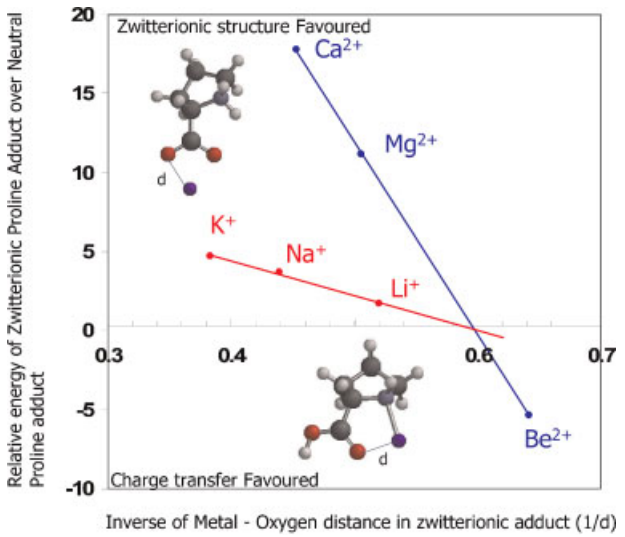

Figure 5. Relative energy of zwitterionic over neutral L-proline adduct as a function of inverse of metal–oxygen distance in zwitterionic adduct (1/d) for the Li^{+}–K^{+} and Be^{2+}–Ca^{2+} series. Data for the Li^{+}–K^{+} series are taken from Reference [27]. This figure is available in colour online at www.interscience.wiley.com/journal/poc

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This suggests that the ionic radii determines which type of interaction is preferred, and that net charge does not affect the type of interaction which is preferred, beyond its influence on the ionic radii.
The differing gradient can probably be accounted for by the differing charge.

Infrared frequencies

IR frequencies were calculated in an attempt to understand the effects of the bonding of M^{2+} ion to L-proline and other related structures.

OH and NH IR frequencies. Figure 6 presents the O—H and N—H IR frequencies for the CS2 complexes with Be^{2+}, Mg^{2+} and Ca^{2+} ions at the DFT B3LYP level. The IR frequencies for the isolated L-proline molecule were determined in two different configurations:

(i) the one used for all calculation in this work (Fig. 1) where the H atom of the O—H group is twisted close to the N atom of the ring and
(ii) L-proline where the H atom of the O—H group is twisted away from the N atom; although this structure is less stable it is included to make the comparison with the CS2 structure clearer.

Initially, in free L-proline with a similar configuration to that found in the CS2 structure (i.e. without the ions) the stretching of N—H and the O—H bond is found at 3761 and 3554 cm^{-1}, respectively. Upon complexation with the divalent ions, in the CS2 structure, the N—H stretching frequency (where the N atom is directly involved in bonding with the metal ion) decreases considerably. While the bonding is conducted between the metal ion and the O atom of the carbonyl, it appears

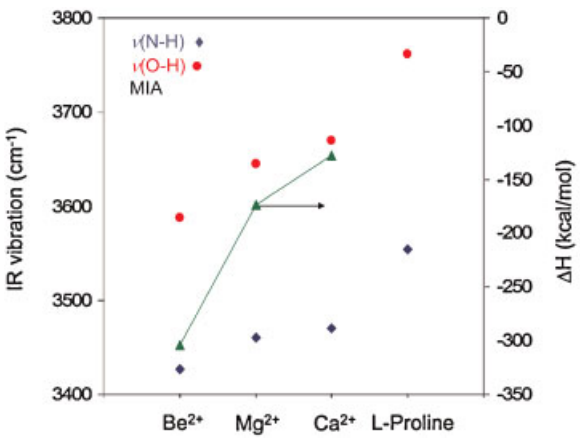

Figure 6. Change in the ν(N–H) and ν(O–H) IR stretching for M^{2+}–L-proline in SC2 (charge solvated) structure, also included is the corresponding metal ion affinity (MIA = $-\Delta H$). This figure is available in colour online at www.interscience.wiley.com/journal/poc

that the oxygen atom of the hydroxyl group is also affected by the bonding and as a result the O—H stretching shifts to lower frequencies in presence of any of the three ions. The effect on the oxygen atom of the hydroxyl can also be seen in the natural charge analyses. Although, the natural charge of the O_1 atom of the hydroxyl, in the case of the CS2 structure iii, increases slightly from −0.53e for Be^{2+} to −0.58e Ca^{2+} at the B3LYP/6-311+G** or from −0.41 to −0.67e for the same ions at the MP2/6-311+G** (Table 4), the natural charge on the same oxygen of free L-proline is more negative (−0.69e). Thus, the decrease in the natural charge of oxygen indicates further involvement in the bonding, the extent of which depends on its strength. It is worth noting that for the free L-proline in its minimum structure, where bonding between the hydrogen of the hydroxyl group and the N atom of the ring occurs, the O—H stretching is considerably low (3425 cm^{-1}). The increase in the stretching frequencies of the OH and NH groups from Be^{2+} to Ca^{2+} is interpreted as due to weakening of the bonding between the metal ion and the oxygen atom of the carbonyl, as illustrated in Fig. 6 by the ΔH values, data from Table 3.

Symmetric and asymmetric stretching of the $COOM^{2+}$ zwitterion complexes. The interaction of the metal ions with L-proline in the zwitterion structure offers a rich example for IR studies. Because of the considerable coupling found in the IR modes, we have opted to compare the IR frequency of the ions/L-proline to a number of other structures starting with the simplest one: M^{2+}-formate. The results are illustrated in Table 4 although most of the computation work was done using several basis sets and the one presented here was determined using the DFT B3LYP (6-311++G**). The clearest example of a chelating (O—M^{2+}—O) type of interaction can be seen in the case of the formate ion with Be^{2+}, Mg^{2+} and Ca^{2+} ions. The difference between the symmetric and asymmetric stretching experimentally observed for formate metal depends on the binding mode and the nature of the metal as well as its oxidation state. In general a separation of 150 cm^{-1} or less is a clear indication of a chelating mode[50,51] such as in this case. Coupling with the CH_2 bending modes is noticeable in the case of acetates and becomes important in the case of propanoates of Be^{2+}. The MIA ($-\Delta H$) decreases as expected from Be^{2+} to Ca^{2+} for formate, acetate and propanoates. ΔE of HCOO—Mg^{2+} has been computed previously using B3LYP/6-31G* equal to −367.1 kcal mol^{-1} and in this work it is found equal to −376.9 kcal mol^{-1} (B3LYP/6-311++G**). There is, however, no particular trend for the MIA between C1 and C3 with one metal cation represented by Be^{2+}. A glycinate ion with the divalent cations was also investigated. The MIA is very close to that of acetate where the difference of 2.4 kcal mol^{-1} is seen between acetate-Be^{2+} and glycinate-Be^{2+}. This is understood by the fact that both

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Table 4. Calculated IR frequencies (cm^{-1}) for M^{2+} bound to carboxylates

Compound	Be^{2+} freq. (cm^{-1})	Mg^{2+} freq. (cm^{-1})	Ca^{2+} freq. (cm^{-1})
Formate (η^2 OO)			
ν C–H	3155	3148	3088
ν_{as} COO	1465	1498	1503
ν_s COO	1365 ($\Delta = 100$)	1351 ($\Delta = 147$)	1364 ($\Delta = 139$)
MIA and (ΔG) kcal mol^{-1}	478.8 (−470.1)	375.4 (−367.0)	323.9 (−315.8)
Acetate (η^2 OO)			
ν_{as} C–H	3173		
ν_s C–H	3032		
ν_{as} COO	1507		
ν_s COO	1449		
ν_s COO, CH_2 (bend)	1356		
MIA and (ΔG) kcal mol^{-1}	497.5 (−490.8)		
Propanoate (η^2 OO)			
ν_{as} CH_3	3146, 3140, 3127		
ν_s CH_2	3069		
ν_s CH_3	3056		
ν_{as}COO, CH_2(bend)	1406		
ν_s COO, CH_2 (bend)	1393		
MIA and (ΔG) kcal mol^{-1}	505.4 (−489.4)		
Glycinate (η^2 OO)			
ν_{as} NH_2	3663	3650	3610
ν_s NH_2	3567	3558	3528
ν_{as} CH_2	3063	3078	3092, 2910
ν_s CH_2	3009	3041	
Bending NH_2	1670	1674	1662
ν_s COO, νC–C, *cis* CH_2	1457	1451	1496
ν_{as} COO, CH_2(bend)	1420	1482	1474
ν_s COO, *cis* CH_2	1352	1326, 1343	1373
ν C–N	1178	1172	1101
MIA and (ΔG)) kcal mol^{-1}	495.9 (−488.0)	413.9 (−408.6)	359.6 (−352.1)

Glycinate ion (η^2 OO)		Glycine molecule	
ν_{as} NH_2	3499	ν_{as} NH_2	3618
ν_s NH_2	3417	ν_s NH_2	3542
ν_{as} CH_2	3057	ν OH	3480
ν_s CH_2	3000	ν_{as} CH_2	3102
Bending NH_2	1677	ν_s CH_2	3059
ν_s COO	1655	ν C═O	1842
δ-CH_2 in plane	1469	Bending NH_2	1662
ν_s COO, C–C, NH_2 (bend)	1369	δ-CH_2 in plane	1464
ν_s COO	1354	Bending O–H	1441
δ-CH_2 out of plane	1300	δ-CH_2 out of plane	1348
ν C–N	982	δ OH, ν C–C, C–O	1207
		ν C–N	1067

the CH_3 (in the case of acetate) and the NH_2 (in the case of glycinate) are further away from the centre of the bonding with the metal cation. The N—H stretching is, however, still affected by the bonding, with weaker bonding (moving from Be^{2+} to Ca^{2+}) resulting in weaker IR stretching. The effect is mainly noticed in the case of Ca^{2+}. Inspection of the geometry of each complex indicates that the dihedral angle (NCCO) is close to zero in the case of Be^{2+} and Mg^{2+} but considerably distorted (26°) in the case of Ca^{2+}. As a result one of the H of the NH_2 group is at a distance of 2.6 Å from the carboxyl oxygen while both hydrogen atoms in the case of Mg^{2+} and Be^{2+} are at a distance of 3.0–3.06 Å. This small interaction of the oxygen atom of the carboxyl might explain the slight weakness in the N—H bond observed in the case of Ca^{2+} complex. It is worth comparing these to the N—H stretch of the glycine molecule and the glycinate ion in the absence of metal ions. The NH_2 stretch of the glycine molecule is found at 3618 cm^{-1}, lower than that found when the ion is complexed with Be^{2+} and Mg^{2+} while that of glycinate is found at 3499 cm^{-1} or a red shift of 119 cm^{-1} from that of the glycine molecule. The red shift is simply explained by the considerable bonding between the H of the NH_2 group and the O of the carboxylate ion.

Results of the zwitterionic structures of L-proline with Be^{2+}, Mg^{2+}, Ca^{2+}, Li^+ and Na^+ as well as that of glycine with Mg^{2+} are presented in Table 5. The N—H

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Table 5. Calculated IR frequencies (cm^{-1}) for M^{2+} bound to L-proline in a zwitterionic bonding

L-proline–(η^2 OO) (glycine–(η^2 OO))	Be^{2+}		Mg^{2+}		Ca^{2+}		Li^+		Na^+	
ν_{as} NH_2	3451		3465 (3445)		3475		3492		3498	
ν_s NH_2	3398		3395 (3373)		3385		3213		3147	
ν_{as} CH_2		ν_s CH_2		ν_s CH_2		ν_s CH_2		ν_s CH_2		ν_s CH_2
C5 (C2)	3164	3097	3136 (3129)	3097	3164 (3079)	3096	3153	3093	3150	3091
C3	3140	3079	3140	3069	3139	3067	3137	3068	3137	3069
C4	3125	3070	3128	3059	3127	3056	3121	3053	3120	3054
ν CH (C2)	3079		3095		3097		3108		3109	
δ NH_2 (δ NH_3)	1656		1658 (1659) (1654)		1657		1638		1638	
ν_s COO, νC–C, bend CH_2, *cis* CH_2	1515		1456 (1487) (1443) (1381)		1461		1435		1411	
ν_{as} COO, NH_2, CH_2(bend)	1447		1536 (1568)		1554		1667		1681	
ν C–N	1027		1019 (998)		1017		1016		1017	
MIA (kcal mol^{-1})	292.20			184.29		139.95		55.46		42.23

Numbers in ( ) are for glycine–Mg^{2+}.

stretch of SB in the case of Li^+ and Na^+ is almost identical to that of the free glycine ion, an indication of the localised point charge created by the Li or Na ions as presented in Fig. 3 by the electrostatic potential map. The MIA of Li^+ and Na^+ with L-proline is very close to that observed in Reference [27]. It is also worth noting that the bending mode of the NH_2 is consistently different in the case of the mono-valent cations (1638 cm^{-1}) and in the case of the divalent cations (1656–1658 cm^{-1}); both bending modes are lower than that found for the free glycine ion (1677 cm^{-1}) and glycine molecule (1662 cm^{-1}).

CONCLUSIONS

Calculations performed using various theoretical methods and basis sets have revealed that Be^{2+} has a preference to bind to L-proline in a CS type manner through the carbonyl oxygen and imino nitrogen atom. With regards to the binding of Mg^{2+} and Ca^{2+} ions to L-proline, a bi-dentate (SB) type arrangement is preferred where the metal ion is stabilised through the carboxylate group of the L-proline molecule. The main factor of influence in determining the type of bonding arrangement adopted by the metal ion is whether charge transfer or electrostatic effects are the dominate interaction in the bonding process. This is related to the ionic radius of the metal ion. Be^{2+} was found to prefer binding in a charge transfer type 2 arrangements. This was of preference as the small ionic radius of Be^{2+} allows for effective charge transfer of electron density from the electron pair on the imino N to the empty 2s orbital on Be^{2+}. With increasing ionic radius this process becomes less effective. Thus, electrostatic effects become more dominate and hence, Mg^{2+} and Ca^{2+} adopting a SB bonding arrangement with L-proline. Calculated MIAs show a stability order $Be^{2+} > Mg^{2+} > Ca^{2+}$. This is consistent across all the theoretical methods and basis sets used in this investigation and follows similar MIA trends for group 2 ions bound to glycine[28] and for group 1 metal ions bound to L-proline.[27] Comparison with other complexes was also conducted for both the ionic and zwitterionic forms. Formates and glycinates of Be^{2+}, Mg^{2+} and Ca^{2+} also present a decreasing trend for MIA. Analysis of the calculated IR frequency for many of the complexes was also conducted. In the case of the CS2 (chelating) structures the N—H and O—H stretch decrease when compared to the free L-proline molecule. The decrease appears proportional to the MIA.

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