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Acidity and metal (Mg^{2+}, Ca^{2+}, Zn^{2+}) affinity of L-γ-carboxyglutamic acid and its peptide analog

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ABSTRACT

Density functional theory methods with the B3LYP and B97D functionals with triple-zeta 6-311++G(d,p) basis set have been used to study the acidity, basicity and metal affinity of L-γ-carboxyglutamic acid (GLA) and its peptide derivative [2-acetylamino-3-(methylamino)-3-oxopropyl]malonic acid (AMD-GLA). The Gibbs interaction energies of the $GLA^{2-}...M^{2+}$ and AMD-$GLA^{2-}...M^{2+}$ (M = Mg, Ca, Zn) complexes show an increasing binding affinity in the order $Ca^{2+} < Mg^{2+} < Zn^{2+}$ The transition metal Zn^{2+} is most effectively recognized by the dianions of GLA and AMD-GLA. Of the dianions studied the AMD-GLA dianion is the strongest Lewis base. Computations that include the effect of solvation showed that in water the relative stability of $GLA^{2-}...M^{2+}$ and AMD-$GLA^{2-}...M^{2+}$ ionic bonds is rapidly diminished. The computed interaction Gibbs energy in water is small and negative.

1. Introduction

Gamma-carboxyglutamic acid (or the conjugate base, γ-carboxyglutamate (GLA) is a naturally occurring amino acid with a dicarboxylic acid side chain [1]. It is introduced into proteins by a post-translational carboxylation of glutamic acid residues [2]. This modification introduces an affinity for calcium ions. In the blood coagulation cascade, vitamin K is required to introduce gamma-carboxylation of coagulation factors II, VII, IX, X and XIV (vitamin K-dependent protein C) [2,3]. GLA has also been studied in regulatory proteins (proteins C and S), and proteins of mineralized tissue (bone GLA-protein and matrix-GLA protein). Both proteins possess gamma-carboxyglutamate residues that confer metal binding properties on these proteins. The GLA domain in these proteins is responsible for the high-affinity for calcium ions [4]. The addition of calcium ions to these proteins induces conformational changes in the GLA moiety, which are necessary for proper folding of the GLA domain [4,5]. Calcium accounts for 1–2% of adult human body weight, and is the most abundant mineral in our body and needed by the body for healthy bones, teeth, and proper function of the heart, muscles, and nerves. Ca(II) ions play also a key part in blood clotting [6,7]. However, structural research in the complex function of calcium and other bivalent metal cations in blood clot formation and lysis, is still a relatively new field owing to the complexities involved in simultaneously controlling the protein fold and defining the metal coordination environment [8–15]. For this reason, calcium binding in proteins containing GLA has been modeled by small molecules [16–19].

With respect to the dicarboxylic acid side chain, GLA exhibits some unique metal binding properties. Metallic complexes containing Mg^{2+}, Ca^{2+}, and Zn^{2+} ions and ethylmalonic acid (a model of GLA) have been investigated using quantum chemical methods [16,17]. Pedersen et al. introduced the concept of metal cation subvalence for investigation of electronic effects of cation–protein interaction [18], and most recently Meng and Lin investigated complexation of Li^+, Na^+, K^+, Mg^{2+} and Ca^{2+} with gaseous glutamic acid [19].

The present letter reports in detail the structural parameters for GLA and [2-acetylamino-3-(methylamino)-3-oxopropyl]malonic acid (AMD-GLA), a model of γ-carboxyglutamic acid-containing peptides, and their magnesium, calcium and zinc salts. They are chosen to model the characteristic interaction of dicarboxylate group of gamma-carboxyglutamate residues with the magnesium, calcium and zinc cations as found in biopolymers. Density functional theory with the B3LYP and B97D functionals was applied for this investigation. Of particular interest is the overall shape of magnesium, calcium and zinc salts and how this shape changes

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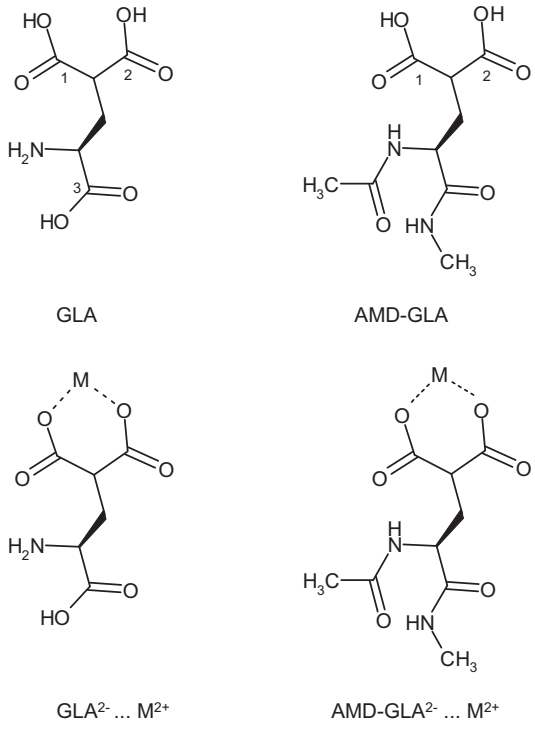

Figure 1. Structure of the systems studied.

upon dissociation and/or in aqueous solution. The gas-phase acidity and basicity of GLA and how these properties are changed in the presence of metal cations studied is also subject of our investigations. The results of this study are analyzed and compared with the available experimental data for structurally related systems.

2. Computational details

The geometry of the γ-carboxyglutamic acid (GLA), [2-acetylamino-3-(methylamino)-3-oxopropyl]malonic acid (AMD-GLA) and their M^{2+} complexes (M^{2+} = Mg, Ca, Zn) (see Figure 1), have been completely optimized with the GAUSSIAN 09 program [20], using density functional theory [21–23] with the B3LYP hybrid functional [24–26] and B97D Grimme's functional including dispersion [27] using the polarized triple-ζ 6–311++G(d,p) basis set [28].

The choice of the B3LYP functional was based by its reported good performance in the reproducing of thermodynamic quantities [18,29] of the cation-Lewis base complexes within the targeted accuracy of about 10 kJ/mol. The values of metal affinities computed using DFT are comparable to *ab initio* SCF results and mostly in good agreement with the corresponding experimental data [29,30].

The interaction enthalpy, ΔH^{298}, for the reaction of metal cations with Lewis bases (reaction 1) is given by Eq (2):

$$M^{2+}(g) + L^{2-}(g) \rightarrow ML(g) \quad (1)$$

$$\Delta H^{298} = \{E^{298}(L\cdots M) - [E^{298}(L) + E^{298}(n\,M)]\} + \Delta(pV) \quad (2)$$

$$M = Mg^{2+}, Ca^{2+}, Zn^{2+},$$

where E^{298}(M) and E^{298}(L) are the energies of the metal cation and ligand molecules, respectively, and E^{298}(L...M) is the energy of the complex corrected for thermal energy at T = 298.15 K. For the work term in Eq. (2) we substituted $\Delta(pV) = -RT$. The enthalpies and Gibbs energies of protonation and deprotonation of GLA were computed in the same way as in our previous publications [31,32]. Solvent effects on the species studied were evaluated using the conductor-like polarizable continuum model (CPCM) [33–36]. The structures of all gas-phase and condensed-phase (CPCM) species were fully optimized without any geometrical constraint.

3. Results and discussion

3.1. General considerations

In the crystal, GLA is known to exist as a zwitterion [37]. Based on this experimental solid-state structure we undertook extensive theoretical calculations with neutral, ionized, protonated and zwitterionic species of GLA and its amide analog AMD-GLA, respectively. The carboxyl moieties of the studied acids were considered in the more stable *syn* forms [38,39]. The molecular structures of the GLA and AMD-GLA in the gas phase and in water were investigated using two functionals of density functional theory. The hybrid Becke3LYP functional is, in combination with the triple-ζ basis set, one of the best DFT functionals for the accuracy of geometries [40]. The Grimme's B97D uses the empirical dispersion energy correction specifically designed for accurate evaluation of van der Waals interactions [27]. As an illustration of the overall shape of the GLA and AMD-GLA species studied in Figures A, B and C of the Electronic Supplemental Information molecular structures and geometries of these systems are presented. An analysis of the harmonic vibrational frequencies of the optimized species proved that all of them are minima (zero number of imaginary frequencies). The optimized geometry of the systems studied using B3LYP and B97D functionals is almost the same (Figure A of the Supplement) and only slight differences were observed in the computed lengths of metal coordination bonds of the salts investigated (Figure C of the Electronic Supplementary Information). The equilibrium distances M^{2+}...O decrease in the order $Ca^{2+} > Mg^{2+} \geq Zn^{2+}$. The hydration of the GLA·M^{2+} and AMD-GLA·M^{2+} (Figure 1) systems results in various changes of the optimum geometry of the parent metal complexes. The M^{2+}...O bonds in the solvated complexes are about 0.1–0.2 Å longer. Our high-level calculations have shown that in the gas phase GLA exists in a nonionized form, while the gas-phase zwitterion transforms without barrier into the neutral isomer. The zwitterions of GLA and its amide derivative were found to be the stable structures in solution.

Suitable starting geometries for GLA and AMD-GLA complexes with divalent cations were generated from dicoordinated complexes containing two malonate-like carboxylate moieties and a metal cation forming a bidentate (direct) bonding in which the metal cation is oriented symmetrically to two oxygen atoms of the functional groups (Figure 1). Such arrangement of metal coordination models the interaction of metal cations with negatively charged anionic sites of GLA domains of proteins [8,41]. To explicitly evaluate the effect of water on the reactivity of the complexes studied, we also examined dihydrated complexes of AMD-GLA. Dihydrated tetrahedral complexes AMD-GLA...M$(H_2O)_2$ model frequently occurring tetracoordinated complexes of metal cations in biological systems. Dihydration of the AMD-GLA^{2-}...M^{2+} complexes results in slight changes of the optimum geometry of the parent metal complexes (Figure C of the Supplementary Information). The equilibrium distances M...O slightly increase upon hydration.

3.2. Acidity and basicity

GLA is a polyprotic acid containing three acidic groups that can lose more than one proton. An amino group of GLA is a basic center,

which may undergo protonation [42]. Acidities (The Gibbs energies of deprotonation of individual carboxyl groups) of GLA computed by both functionals are (within the very reasonable ±3 kcal/mole) equivalent.

The gas-phase acidity of two side-chain carboxylic groups of GLA is almost the same and slightly greater than the acidity of the backbone COOH group. In the absence of experimental acidities for GLA, our computed acidities should be compared to such data available for the simpler glutamic acid (GLU) only. However, the experimental acidity ΔH_{acid} (1348 ± 21 kJ mol^{-1}, Jones et al. [42]) of the GLU backbone carboxylic acid group is by about 40 kJ mol^{-1} greater. GLA as a polyprotic acid can lose three protons. Possible forms of this acid, besides the neutral form, are also monoanions, dianions and trianions. The dissociation of the first proton of one of three carboxylic acid groups of GLA represents an intrinsic gas-phase acidity of an individual acidic group. The acidity of both side-chain carboxylic acid groups is almost the same and about 5–15 kJ mol^{-1} more acidic than those computed for the backbone deprotonated α-carboxylic acid of GLA. The dissociation of successive proton in the reaction GLA-COO$^{(-)}$ → GLA-COO$^{(2-)}$ + H$^+$ is more energy-consuming and results in a dramatic lowering of acidity by about 130–150 kJ mol^{-1} (B3LYP). The most stable dianion GLA-COO$^{(2-)}$ is stabilized by an intramolecular hydrogen bond, formed by the side-chain carboxylic acid OH group and the negatively charged backbone carboxylate moiety (Figure B of the Supplementary Information). The Gibbs energy of deprotonation of three carboxylic groups is about 900 kJ mol^{-1} larger than the sum of the corresponding Gibbs energies of deprotonation computed for individual acidic groups (Table 1). The resonance in the carboxylate groups results in the delocalization of the negative charge over several atoms, thus stabilizing the negative ion. The ionization of the carboxyl moiety in GLA in the gas phase involves a gain of entropy of about 120–130 J mol^{-1} K^{-1} (Table 1) and fits well to the experimentally estimated value of 94 and 88 J mol^{-1} K^{-1} for the acidic amino acids ASP and GLU, respectively [42]. The calculated gas-phase acidities correlate with the measured pK_a values of 1.7, 3.2, and 4.75 for dissociation of the first, second and third proton of GLA [43]. Because of the small differences between successive pK_a, the individual negatively charged species of GLA cannot be considered as an acid in their own right. The stepwise dissociation of the two carboxylic acid groups of the amide derivative of AMD-GLA follows the general trends in the acidity as observed for the parent GLA (Table 1).

GLA contains the basic amine, which at low pH exists as a positive ion [44]. The gas-phase basicity (negative Gibbs energy change for the reaction GLA-NH$_2$ + H$^+$ → GLA-NH$_3^+$) the calculated gas-phase basicity (−860.9 kJ mol^{-1}, B3LYP) is lower than the experimental value (−897.5 kJ mol^{-1}) measured by the kinetic method for the parent glutamic acid (GLU) [45]. For the energetically stable configurations of the metal salts GLA^{2-}...M^{2+} (Figure 1) the proton dissociation enthalpies, entropies and Gibbs energies of the backbone carboxylic acid were also computed (Table 2). Upon salt formation of the dicarboxylic acid side chain, the acidity of GLA^{2-}...M^{2+} complexes changes irregularly. In the presence of magnesium and zinc cations the acidity increases, however for calcium complex an appreciable decrease of acidity by about 30 kJ mol^{-1} was observed (Table 2).

3.3. Metal affinity

GLA, synthesized by the post-translational modification of glutamic acid residues, is an amino acid with a dicarboxylic acid side chain. The dicarboxylic acid moiety of GLA with its unique metal binding properties confers metal binding character to the proteins into which it is incorporated. The negatively charged dicarboxylic moiety is a principal chelation site of the GLA and AMD-GLA species, modeling bivalent metal cation binding sites occurring naturally in several biological molecules [4–15,41]. The calculated metal affinities (enthalpies) and Gibbs interaction energies of the

Table 1
Gas-phase acidities and basicities (enthalpies ΔH, entropies ΔS and Gibbs energies ΔG) of γ-carboxyglutamic acid (GLA) and [2-acetylamino-3-(methylamino)-3-oxopropyl]malonic acid (AMD-GLA) (at 298.15 K).

Ionization and/or protonation	Reaction		ΔH^{298} kJ mol^{-1}	ΔS^{298} J mol^{-1} K^{-1}	ΔG^{298} kJ mol^{-1}
	GLA, acidity				
Side-chain	1-COOH → COO$^{(-)}$ + H$^+$	A[a]	1375.9	126.3	1338.2
		B[b]	1380.6	128.4	1342.3
Side-chain	2-COOH → COO$^{(-)}$ + H$^+$	A[a]	1375.5	123.9	1338.6
		B[b]	1378.7	120.9	1342.6
Backbone	3-COOH → COO$^{(-)}$ + H$^+$	A[a]	1388.9	129.2	1350.4
		B[b]	1397.5	127.8	1359.4
Side-chain	GLA-COO$^{(-)}$ → GLA-COO$^{(2-)}$ + H$^+$	A[a]	1614.8	115.0	1580.5
		B[b]	1627.9	123.1	1591.2
Side-chain	2 COOH → 2 COO$^{(-)}$ + 2 H$^+$	A[a]	2990.3	238.9	2919.1
		B[b]	3006.6	244.1	2933.8
	3 COOH → 3 COO$^{(-)}$ + 3 H$^+$	A[a]	5035.8	374.5	4924.2
		B[b]	5090.1	390.6	4973.7
	AMD-GLA, acidity				
Side-chain	1-COOH → COO$^{(-)}$ + H$^+$	A[a]	1344.4	138.5	1308.1
		B[b]	1333.0	134.1	1293.0
Side-chain	2-COOH → COO$^{(-)}$ + H$^+$	A[a]	1371.3	125.2	1333.9
		B[b]	1362.5	104.8	1331.3
Side-chain	AMD–GLA-COO$^{(-)}$ → GLA-COO$^{(2-)}$ + H$^+$	A[a]	1723.6	105.0	1692.3
		B[b]	1731.5	122.5	1695.0
	2 COOH → 2 COO$^{(-)}$ + 2 H$^+$	A[a]	3068.0	227.1	3000.3
		B[b]	3064.5	256.6	2988.0
	GLA, basicity				
Backbone	−NH$_2$ + H$^+$ → −NH$_3^+$	A[a]	−900.1	−131.4	−860.9
		B[b]	−913.2	−126.9	−875.4
	GLU	C[c]	−897.5		−943.7

[a] A–B3LYP/6–311++G(d,p) method.
[b] B–B97D/6–311++G(d,p) method.
[c] Gas-phase experimental value for glutamic acid, reference [43].

Table 2
Gas-phase acidities (enthalpies ΔH, entropies ΔS and Gibbs energies ΔG) of γ-carboxyglutamic acid (GLA) in the presence of Mg^{2+}, Ca^{2+}, and Zn^{2+} ions (at 298.15 K).

Ionization	Reaction		ΔH^{298} kJ mol^{-1}	ΔS^{298} J mol^{-1} K^{-1}	ΔG^{298} kJ mol^{-1}
	GLA...Mg^{2+}				
Backbone	3-COOH → $COO^{(-)}$ + H^+	A[a]	1347.1	77.1	1324.1
		B[b]	1375.4	94.4	1347.3
	GLA...Ca^{2+}				
Backbone	3-COOH → $COO^{(-)}$ + H^+	A[a]	1411.1	82.2	1386.6
		B[b]	1423.9	109.2	1391.4
	GLA...Zn^{2+}				
Backbone	3-COOH → $COO^{(-)}$ + H^+	A[a]	1314.6	71.2	1293.4
		B[b]	1342.8	90.6	1315.8

[a] A – B3LYP/6–311++G(d,p) method.
[b] B – B97D/6–311++G(d,p) method.

systems studied are given in Table 3. The formation of the ionic bonds in the salts of $GLA^{2-}...M^{2+}$ and AMD-$GLA^{2-}...M^{2+}$ complexes leads to the formation of 'trifurcated' structures (Figure C of the Supplementary Information). The interaction enthalpies and Gibbs energies are without exception, larger for the Mg^{2+} than the Ca^{2+} salts. The stability of the alkaline-earth metal Mg^{2+} and Ca^{2+} complexes of GLA and AMD-GLA obeys the selection by ion size [46] (Ca^{2+} (1.0 Å), Mg^{2+} (0.65 Å)) (the magnesium salt of GLA is about 260 kJ mol^{-1} more stable than the calcium complex, and even a greater stability difference of about 320 kJ mol^{-1} was found with AMD-GLA salt). In another category of species are the GLA and AMD-GLA complexes with Zn^{2+} ion. The Gibbs interaction energies show an increasing binding affinity in the order $Ca^{2+} < Mg^{2+} < Zn^{2+}$ (Table 3). The transition metal Zn^{2+} is most effectively recognized by the dianions of GLA and AMD-GLA. Mg^{2+} and Zn^{2+} are of similar size [46] so that a comparison of their GLA and AMD-GLA salts can illustrate the difference of primary electrostatic bonding (Mg^{2+} systems) and bonding that involves significant charge transfer in addition to the electrostatic interaction (Zn^{2+} systems) [47]. Of the dianions studied the AMD-GLA dianion is the strongest Lewis base. In the case of the $GLA^{2-}...M^{2+}$ complexes, apart from complexes bearing a neutral backbone carboxylic acid group, also zwitterionic systems have been examined. However, in the gas-phase zwitterions of salts an intramolecular proton transfer occurs between the protonated N-terminus and the oxygen atom of the C-terminal carboxylate of GLA, stabilized by means of an intramolecular hydrogen bond O—H$^-$...N formed with the basic nitrogen atom of GLA and the carboxylic hydroxyl group in *anti* orientation (Figure C of the Supplementary Information). The computed gas-phase Gibbs interaction energies, ΔG^{298}, for zwitterionic complexes are close to those computed for neutral GLA (Table 3). Zwitterions of amino acids can also be stabilized through interactions with solvent molecules such as water [48]. The effect of solvent (water) on the geometry of the magnesium, calcium and zinc salts of the complexes investigated was examined using the CPCM solver of the GAUSSIAN 09 program. The zwitterionic form of the GLA salts studied was preserved in the solvated systems (Figure C of the Supplementary Information). Table 3 also contains the reaction Gibbs energies, ΔG^{298} (CPCM), for the $GLA^{2-}...M^{2+}$ and AMD-$GLA^{2-}...M^{2+}$ complexes in aqueous solution. Solvation has a dramatic effect on these interactions. In the gas phase, the binding reactions could occur with high Gibbs binding energies (Table 3). This is due to the strong attractive Coulombic interactions between the oppositely charged Mg^{2+}, Ca^{2+}, Zn^{2+} cations and

Table 3
Calculated enthalpies, ΔH, Entropies ΔS, and Gibbs energies ΔG of the ion–γ-carboxyglutamic acid (GLA) systems.

No.	System		ΔH^{298} kJ mol^{-1}	ΔS^{298} J mol^{-1} K^{-1}	ΔG^{298} kJ mol^{-1}	ΔG^{298}, CPCM kJ mol^{-1}
1	GLA(2-)...Mg^{2+}	A[a]	−2242.5	−134.9	−2202.3	−198.0
		B[b]	−2210.2	−137.8	−2169.1	−176.5
2	GLA(2-)...Ca^{2+}	A	−1982.5	−130.1	−1943.6	−160.3
		B	−1978.2	−134.5	−1938.1	−157.5
3	GLA(2-)...Zn^{2+}	A	−2432.4	−129.6	−2393.8	−262.9
		B	−2421.2	−133.3	−2381.5	−253.4
4	ZwittGLA(2-)...Mg^{2+}	A	−2244.2	−145.5	−2200.8	−204.0
		B	−2210.6	−145.1	−2167.4	−234.8
5	ZwittGLA(2-)...Ca^{2+}	A	−1982.6	−139.6	−1941.0	−142.7
		B	−1978.3	−141.1	−1936.2	−137.7
6	ZwittGLA(2-)...Zn^{2+}	A	−2434.3	−143.7	−2391.5	−258.6
		B	−2420.6	−138.0	−2378.8	−252.3
7	AMD-GLA(2-)...Mg^{2+}	A	−2297.3	−112.5	−2263.7	−188.8
		B	−2271.4	−144.9	−2228.2	−223.2
8	AMD–GLA(2-)...Ca^{2+}	A	−2038.1	−110.1	−2005.2	−151.8
		B	−2039.9	−141.6	−1997.7	−151.5
9	AMD-GLA(2-)...Zn^{2+}	A	−2486.8	−109.7	−2454.2	−258.4
		B	−2483.2	−142.1	−2440.8	−248.1
10	AMD-GLA(2-)...$Mg^{2+}(H_2O)_2$	A	−1918.1	−172.7	−1866.6	−207.3
		B	−1911.8	−209.1	−1849.5	−204.9
11	AMD-GLA(2-)...$Ca^{2+}(H_2O)_2$	A	−1767.8	−109.8	−1735.1	−150.2
		B	−1796.0	−237.2	−1725.3	−161.2
12	AMD-GLA(2-)...$Zn^{2+}(H_2O)_2$	A	−1929.2	−160.6	−1881.3	−192.6
		B	−1941.3	−191.3	−1884.3	−202.1

[a] A–B3LYP/6–311++G(d,p) method.
[b] B–B97D/6–311++G(d,p) method.

the dianionic carboxylic groups of GLA and AMD-GLA. The binding Gibbs energy in the gas phase is substantially larger in magnitude than in a polar solvent (water). The computed binding energies in aqueous solution are low and negative, *i.e.* stabilizing. However, the entropy term is always high and negative, opposing association (Table 3). The computed Gibbs binding energies for the systems studied in aqueous solution range from −140 to −260 kJ mol^{-1} (B3LYP). Thus there is low tendency for the salts to associate by means M^{2+}-ligand ionic bonds, and in an aqueous environment the high hydration effect between cations and anionic species results in diminution of the direct electrostatic and ion–dipole interactions. Explicit consideration of two water molecules (systems **10**–**12**, Table 3) more explicitly models the hydrated properties of the AMD-GLA^{2-}...M^{2+} systems. Owing to the large positive charge of dications studied the best interaction sites for water molecules should be the end of the metal cation of these species (Figure C of Supplementary Information). Our calculations for the reactions AMD-GLA^{2-} + $M^{2+}(H_2O)_2$ → AMD-GLA$^{2-}M^{2+}(H_2O)$ indicate that the addition of two water molecules to the AMD-GLA$^{2-}M^{2+}$ system destabilizes by about 350–400 kJ mol^{-1} the AMD-GLA salts. An even larger destabilization results from the continuum CPCM calculations, however, the involvement of two explicit water molecules in complexes **10**–**12** does not have any significant effect on the solvent stability of parent systems **7**–**9** (Table 3).

The computed metal binding properties of the GLA^{2-}...M^{2+} and AMD-GLA^{2-}...M^{2+} systems, in which metal ions are arranged to the negatively charged carboxyl groups, correlate well with the binding properties of these cations in biological systems. In hemostasis, Ca^{2+} ions are one of the cofactors, which are essential in regulating the intricate balance between procoagulant and anticoagulant factors [8,41]. Magnesium, as one of the most plentiful intracellular divalent cations, plays a role in blood clotting, competing with Ca^{2+} ions [15]. As indicated by our calculations, because of the greater interaction energy Mg^{2+} is able to displace Ca^{2+} ions from their natural positions at GLA residues of GLA containing proteins. The replacement of Ca^{2+} sites in the GLA domains of Factor VIIa and the activated protein C by Mg^{2+} were recently proved also experimentally [14]. Thermodynamic analysis of the binding of Mg^{2+}, Ca^{2+} and Zn^{2+} ions to the side chain carboxylates of GLA in conantokin-G and conantokin-T indicated that the affinity of these divalent metal ions for the peptide is significantly greater for Mg^{2+} and Zn^{2+} ions than for Ca^{2+} [49,50], and this correlates well with the stability order $Zn^{2+} > Mg^{2+} > Ca^{2+}$ of model GLA^{2-}...M^{2+} and AMD-GLA^{2-}...M^{2+} complexes found by us (Table 3). However, more detail information about the role of bivalent metal cations in biopolymers containing γ-carboxyglutamic acid domains could be obtained by solving X-ray structures of appropriate metal complexes of proteins or by application of molecular dynamics simulations of complex systems [12,13,18].

4. Conclusions

The major conclusions of this study are the following.

1. The investigated polyprotic acids are in the gas-phase strongly acidic. The gas-phase acidity for monoionization of two side-chain carboxylic groups of GLA is almost the same and slightly greater than the acidity of backbone carboxylic acid. The dissociation of the successive protons in monoanions, dianions and trianions of GLA is more energy consuming and results in dramatic lowering of acidity.
2. In metal coordinated GLA^{2-}...M^{2+} and AMD-GLA^{2-}...M^{2+} (M = Mg, Ca, Zn) systems the binding Gibbs energies increase in the order $Zn^{2+} > Mg^{2+} > Ca^{2+}$. The computed gas-phase Gibbs interaction energies, ΔG^{298}, for zwitterionic complexes are close to those computed for neutral GLA and span a rather broad energy interval from −1735 to −2470 kJ mol^{-1} (B3LYP).
3. Solvation has dramatic effect on these interactions. The computed Gibbs binding energies for the systems studied in aqueous solution range from −140 to −260 kJ mol^{-1} (B3LYP). Thus there is low tendency for the salts to associate by means M^{2+}–ligand ionic bonds, and in an aqueous environment the high hydration effect between cations and anionic species results in a diminution of the direct electrostatic and ion-dipole interactions.
4. The computed metal binding properties of the GLA^{2-}...M^{2+} and AMD-GLA^{2-}...M^{2+} (M = Mg^{2+}, Ca^{2+}, Zn^{2+}) systems, in which metal ions are arranged around the negatively charged carboxyl groups, correlate well with the binding properties of these cations in biological systems.

This work yields quantities that may be inaccessible or complementary to experiments and represents the first quantum chemical approach in which both the gas-phase and solvated phase complexation between divalent metal cations and negatively charged carboxyl groups of γ-carboxyglutamic acid domains of proteins are modeled and absolute metal ion affinities were evaluated.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.cplett.2014.09.042.

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