Complexation of Ca^{2+} with selenocysteine and effects on its intrinsic acidity

Marcela Hurtado, Al Mokhtar Lamsabhi, Manuel Yáñez, and Otilia Mó*

Departamento de Química, Facultad de Ciencias, Módulo 13, Universidad Autónoma de Madrid. Campus de Excelencia UAM-CSIC. Cantoblanco, 28049 Madrid, Spain
E-mail: otilia.mo@uam.es

This paper is dedicated to Prof. Rosa Claramunt on the occasion of her 65th birthday

DOI: http://dx.doi.org/10.3998/ark.5550190.p008.217

Abstract

The interactions of Selenocysteine (***Sec***) with Ca^{2+} have been investigated through the use of B3LYP/6-311+G(3df,2p)//B3LYP/6-311+G(d,p) calculations. The global minimum of the [***Sec***-Ca]$^{2+}$ potential energy surface is a charge-solvated (CS) species in which the metal dication is bound to the SeH, to the amino groups and to the carbonyl oxygen. Within a gap of 5.0 kJ mol^{-1} in terms of free energies there are four more complexes three of which are salt-bridged (SB) structures. The interactions between Ca^{2+} and the amino acid are essentially electrostatic, in contrast with what happens when Ca^{2+} is replaced by Cu^{2+}, where the charge transfer from ***Sec*** to the metal cation is so large that in the [***Sec***-Cu]$^{2+}$ global minimum the amino acid moiety is oxidized. The significant electron density redistribution undergone by the amino acid when interacting with both doubly-charged metal ions is reflected in a significant enhancement of its intrinsic acidity, although this effect is much larger for Cu^{2+} than for Ca^{2+}. One of the direct consequences of the oxidation undergone by the amino acid when attached to Cu^{2+} is that the complexes formed are thermodynamically unstable with respect to a proton loss since the process ***Sec*** + $Cu^{2+} \rightarrow$ [(***Sec***-H)Cu]$^{+}$ + H^{+} is strongly exothermic. Conversely, a similar process when Cu^{2+} is replaced by Ca^{2+} is endothermic and therefore the [***Sec***-Ca]$^{2+}$ complexes should be stable with respect to a proton loss.

Keywords: Selenocysteine, calcium, acidity, DFT, amino acid

Introduction

Selenocysteine (Sec) is considered the 21st amino acid and it is found in all kind of living beings as a building block of selenoproteins,[1] which have been found to be essential in mammals.[2]

Indeed, the first selenoprotein identified in mammals, namely glutathione peroxidase, protect cells from oxidative damage.[3] Actually, a great majority of selenoproteins are enzymes involved in redox reactions.[1-2, 4-5] This is, for instance the case of formate dehydrogenase which catalyzes the oxidation of formate to carbon dioxide, and in which the presence of Sec seems to play a crucial role in the mechanism of the reaction.[6] It seems also well established that Sec-containing enzymes modulate and play some role in brain diseases, such as Parkinson's disease or epilepsy,[5, 7] and that the deficiency of some Sec-containing enzymes seems to be closely related with stroke risk,[8] whereas several others have been implicated in the risk or development of cancer.[9] Also importantly, 25 human genes encoding selenoproteins have been already identified,[10] although little is known, for the time being, about their biological functions.[11]

Calcium is also an essential element for living organisms controlling or participating in many cell physiological activities under the form of a doubly charged ion, Ca^{2+}. Actually it is well established that Ca^{2+} is the single most important information carrier at the cellular level[12] with different mechanisms that regulate the intracellular Ca^{2+} concentration.[13] Ca^{2+} also has important roles at nerve terminals as well as important postsynaptic,[14] and muscle contraction functions[15-17] stabilizes the folded proteins[18] and increase their thermal stability.[19]

At the molecular level, the interactions between a base and a doubly charged metal ion usually is accompanied by a dramatic electron density redistribution within the neutral molecule interacting with the ion, due to the strong coulombic field created by the latter,[20] which in the case of ions like Cu^{2+} may result in the oxidation of the base.

The specific interactions between ***Sec*** and Ca^{2+} are of particular relevance because, besides the different roles mentioned above for Ca^{2+}, it seems that the key proteins in cell protection against oxidative stress[21] and those which usually participate in the regulation of intracellular Ca^{2+} concentration[22] are ***Sec***-containing proteins. In this paper we aim at investigating the preferential way in which Ca^{2+} binds to Sec, and at analyzing the effects produced by the doubly-charged metal ion on the electron density of the amino acid and on its intrinsic properties, in particular on its propensity to get deprotonated.[23-25]

Computational Details

Geometry optimizations were carried out at B3LYP level using the 6-311+G(d,p) basis set. The B3LYP approach combines Becke's three parameter nonlocal hybrid exchange potential[26] with the nonlocal correlation functional of Lee et al.,[27] and it has been shown to provide reliable geometries for a wide variety of compounds,[28] with a rather good accuracy/cost ratio. The same functional has been shown to yield reliable gas-phase basicities and acidities,[29-31] provided that a flexible enough basis set is used. In all the cases, Gaussian09 series of programs[32] were used. The harmonic vibrational frequencies were used to classify the different stationary points as local minima or transition states. The connectivity between local minima and transition states was verified by means of intrinsic reaction coordinate (IRC) calculations as implemented in the

Gaussian09 suite of programs. Final total energies were obtained by single-point calculations using a 6-311+G(3df,2p) basis sets. For the subsequent analysis of the acidity of the most stable complex, the deprotonated species were optimized and the corresponding harmonic frequencies were obtained at the same level used for the neutrals. From the frequency calculations, the thermal corrections to enthalpies and Gibbs free energies were evaluated at 298.2 K. The gas-phase acidity ($\Delta_{acid}H$) of a compound A is defined as the proton affinity of the corresponding anion (equation 1); hence, the lower the value of $\Delta_{acid}H$, the higher the acidity of the system.

$$A^- + H^+ \rightarrow \quad AH \qquad (\mathbf{1})$$

The acidity can also be measured in terms of the corresponding Gibbs free energy, $\Delta_{acid}G$. A NBO (Natural Bond Orbital) analysis[33] was used to study the most prominent interactions in each molecular structure. NBO analysis allows the separation of the molecular energy into two fundamental contributions: the total energy (where delocalization is included), and the energy of the hypothetical Lewis structure. Accordingly, the electrons are strictly located in bonds and lone pairs. The interactions between occupied and empty antibonding (or Rydberg) orbitals represent the deviation of the molecule from the ideal Lewis structure and can be used as a measure of delocalizations ($E_{deloc} = E_{total} - E_{Lewis}$). The formation of dative bonds, and the existence of back-donation effects are easily detected by the evaluation of second-order orbital interaction energies involving the occupied orbitals of the amino acid and the empty orbitals of the metal cation and between occupied *d*-orbitals of the metal and the empty antibonding orbitals of the amino acid, respectively. In the framework of the NBO approach it is also possible to calculate the Wiberg bond orders,[34] which provide additional quantitative information on the bonding between the metal and the base. All the NBO calculations were carried out at B3LYP/6-311+G(d,p). The bonding of the more stable complexes was also analyzed by means of the atoms in molecules (AIM),[35] and the electron localization function (ELF)[36-38] theories. By means of AIM theory we have obtained the molecular graphs showing the bond paths connecting the different bonded atoms and containing the bond critical points (BCPs), which correspond to stationary points in which the electron density is minimum along the line that connect two maxima and maximum in the other two directions. ELF is a function, which, conveniently scaled between [0,1],[37, 39] permits division of the physical space in regions where electron pairs, either bonding or lone pairs, are localized. These regions or basins are usually classified as monosynaptic (core or lone pairs) and disynaptic (involving two atomic valence shells), and their electron population provides useful insights into the bonding pattern of the molecule. ELF grids and basin integrations have been evaluated with the TopMod package.[39] For the three-dimensional plots, an ELF value of 0.8 is normally used.

Results and Discussion

One of the major problems associated with the theoretical study of the amino acids is the abundance of different conformers. Indeed, for L-homoselenocysteine at B3LYP level we have localized a total of seventy-seven conformers[40]. For ***Sec*** Vank et al.[41] have shown that 81 different conformers should be expected, although the potential energy surface (PES) only contains seven minima of the side chain.[42] Kaur et al.[43] located a total of 33 conformers of ***Sec*** within an energy gap of 10.4 kcal mol^{-1}, at B3LYP/6–311++G(d,p)// B3LYP /6–31+G(d,p) level of theory, while, Spezia et al.[44] have found seven structures within an energy gap of 3.83 kcal/mol. Since the main aim of our study is not to explore the different conformations of ***Sec*** but to look at the characteristics of its complexes with Ca^{2+}, for our survey, we selected the eight most stable ones, shown in Figure 1, from the five most stable conformers reported in ref. 43 and the seven conformations reported in ref. 44 after ruling out the common ones.

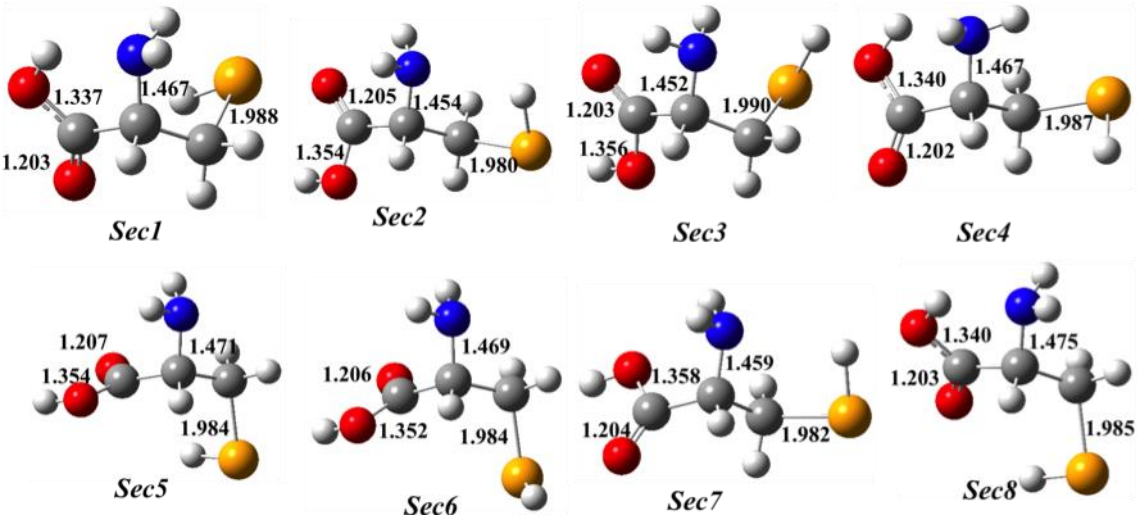

Figure 1. B3LYP/6–311+G(d,p) optimized geometries for the eight most stable conformers of ***Sec***. Bond distances are in Å.

If one assumes a Boltzmann distribution, and using the relative Gibbs free energies summarized in Table 1, ***Sec***, in the gas phase and at room temperature (298.2 K), should be an equilibrium mixture of the eight conformers summarized in Figure 1 and Table 1, with the relative abundances shown in the fourth column of that Table.

Table 1. B3LYP/6-311+G(3df,2p)// B3LYP/6-311+G(d,p) relative energies (Δ**E**), enthalpies (Δ**H**) and free energies (Δ**G**^{0}). All values in kJ mol^{-1} evaluated at 289.2 K

	Neutral *Sec*					[*Sec*-Ca]$^{2+}$						
	ΔE	ΔH	ΔG^{0}	Abundance (%)		ΔE	ΔH	ΔG^{0}		ΔE	ΔH	ΔG^{0}
					a	0.0	0.0	0.0	***o***	71.5	72.5	67.7
					b(z)	5.6	7.1	0.9	***p***	71.0	71.6	70.1
Sec1	0.0	0.0	0	38.7	***c***	1.5	1.4	1.4	***q***	74.9	76.2	71.4
Sec2	4.0	5.6	1.6	19.8	***d(z)***	9.1	10.9	3.7	***r***	73.3	73.9	72.1
Sec3	5.0	6.3	2.7	13.2	***e(z)***	9.5	10.6	5.0	***s(z)***	110.1	112.9	99.9
Sec4	3.6	3.8	3.4	9.7	***f(z)***	17.0	18.3	14.7	***t***	122.9	124.4	118.2
Sec5	6.2	7.7	3.7	8.7	***g***	22.2	22.3	22.1	***u***	124.2	125.6	119.7
Sec6	7.5	9.1	4.2	7.2	***h***	24.0	24.2	23.7	***v***	141.6	142.6	137.9
Sec7	10.2	11.6	7.0	2.2	***i(z)***	35.3	37.8	28.6	***w***	147.9	149.2	143.7
Sec8	11.3	11.6	11.1	0.4	***j(z)***	38.4	39.9	34.6	***x***	164.2	165.8	159.8
					k(z)	41.5	43.2	37.0	***y***	193.1	196.4	185.4
					l(z)	51.6	53.7	43.6	***z***	196.1	199.1	189.7
					m(z)	52.2	53.7	48.7	***aa***	208.6	211.3	201.3
					n	55.2	56.4	52.0				

Sec-Ca^{2+} complexes. Structure and bonding

Taken into account the rather low abundance of ***Sec7*** and ***Sec8*** conformers they have been discarded from our scrutiny of the complexes which may be formed by association of Ca^{2+} to ***Sec***. The coordination of this doubly-charged metal ion to the remaining six conformers of ***Sec*** generate the twenty-seven complexes reported in Table 1, which are named in alphabetic order from the most to the least stable in terms of their free energy. Note that when the complex formed corresponds to salt-bridge structure, in which the metal dication interacts with the zwitterionic form of ***Sec***, a ***(z)*** has been added to the letter which designates the complex. Their relative energies, enthalpies and free energies are summarized in Table 1. The structures of the six more stable complexes are given in Figure 2. Thermodynamic data and the structures for the remaining complexes are shown in Table S1 and S2, respectively, of the supporting information.

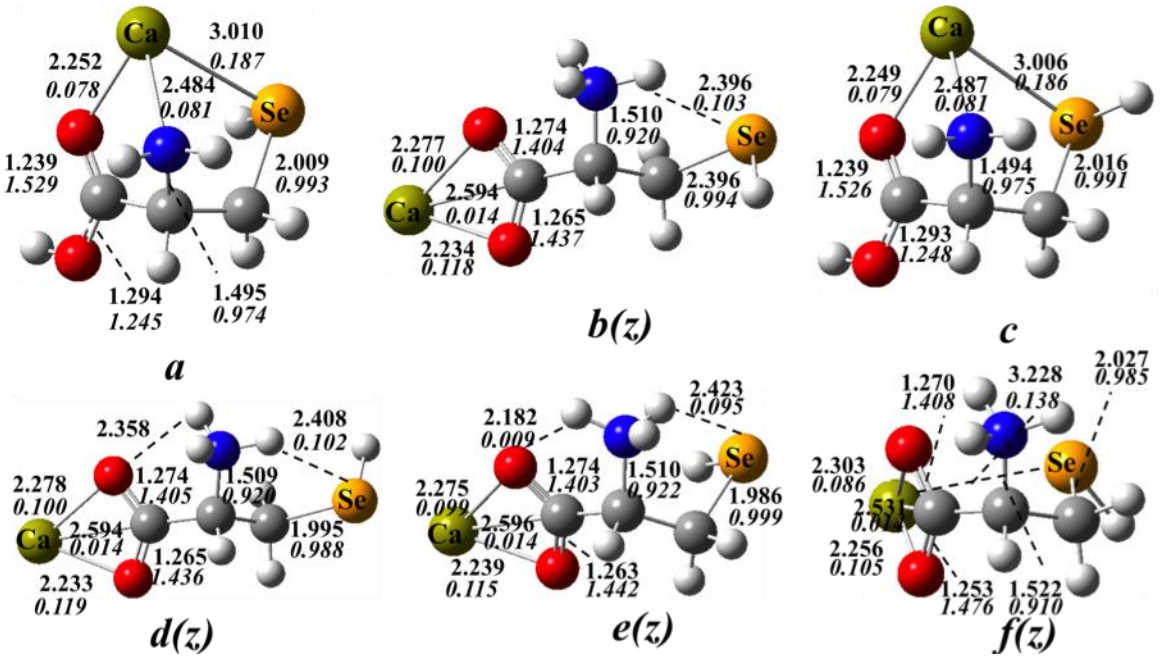

Figure 2. B3LYP/6–311+G(d,p) optimized geometries for the six most stable [***Sec*** –Ca]$^{2+}$ complexes. Bond distances are in Å. Values in italic corresponds to the Wiberg bond orders.

The global minimum of the potential energy surface for [***Sec***–Ca]$^{2+}$, ***a***, is a charge-solvated (CS) structure in which the metal dication is bound to the carbonyl group of the acidic function, the amino group and the SeH group. Similar tricoordinated CS structures have been found for other amino acids, as serine and cysteine, when interacting with Ca^{2+}.[45] The tricoordination of Ca^{2+} is nicely reflected in the corresponding molecular graph (See Figure 3a) which shows the existence of BCPs between Ca and the aforementioned three basic sites as well as the existence of a cage (green dot) critical point. Besides, the electron localization function (ELF) theory shows the existence of disynaptic basins for Ca-O, Ca-N and Ca-Se bonds (See Figure 3b). These findings are consistent with a second-order NBO analysis which shows interactions between the lone-pairs of oxygen, nitrogen and selenium with the empty *sd* hybrids of Ca, with second order interaction energies of 22, 25 and 92 kJ mol^{-1}, respectively. Accordingly, the corresponding Wiberg bond orders of the O-Ca, N-Ca and Se-Ca bonds (0.078, 0.081 and 0.187, respectively, Figure 2) follow a similar trend.

The internal rotation of the Se-H group implies a destabilization of the complex by 1.4 kJ mol^{-1} yielding the third local minima, ***c***, with a bonding pattern almost identical to that of the global minimum.

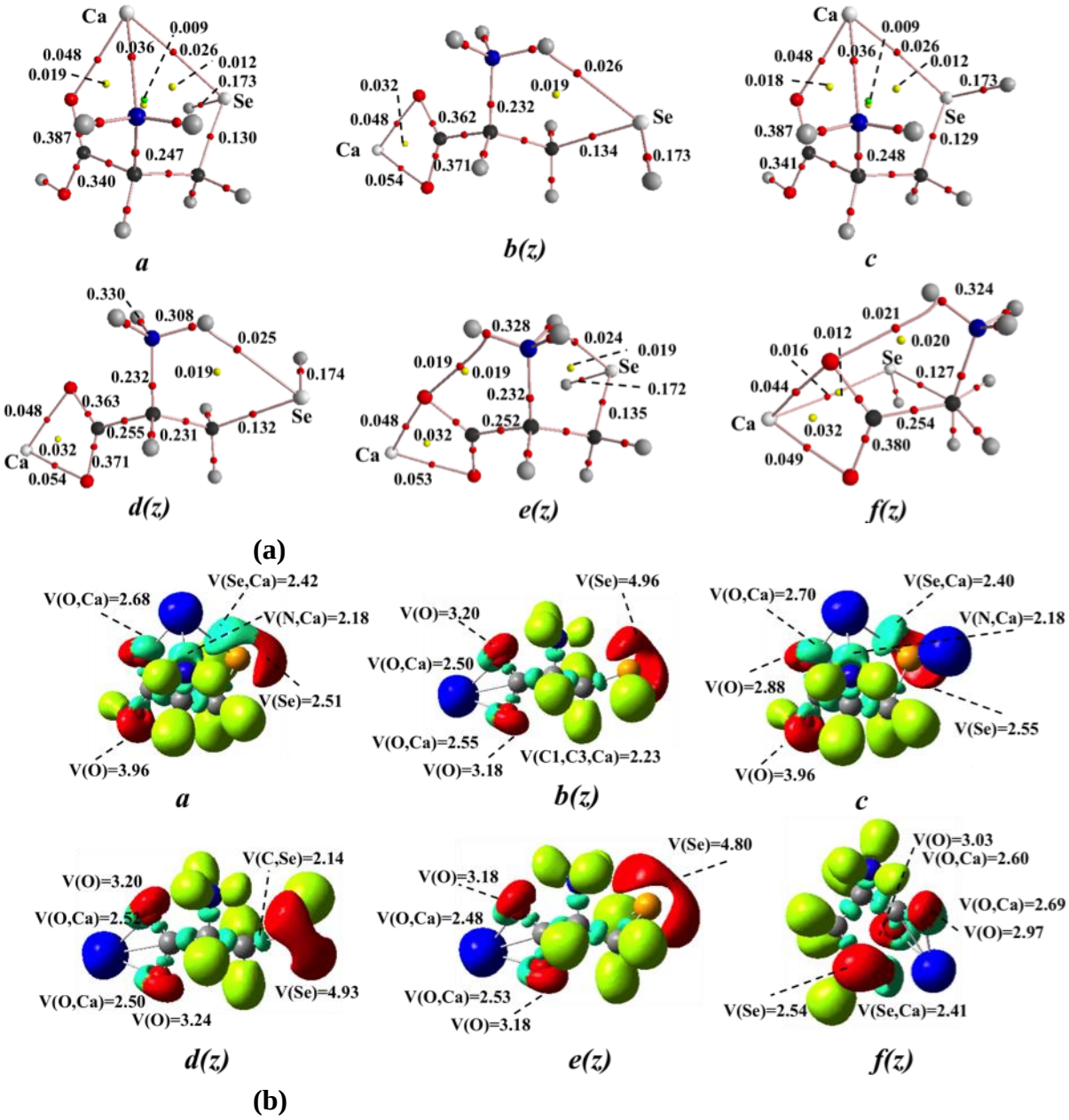

Figure 3. Bonding of the more stable [***Sec***-Ca]$^{2+}$ complexes (a) Molecular graphs. Red, yellow and green dots denote bond, ring and cage critical points, respectively. Electron densities are in a.u. (b) ELF plots. Blue and red lobes denote core and lone-pair monosynaptic basins. Green lobes correspond to disynaptic basins between heavy atoms. Yellow lobes denote disynaptic basins corresponding to bonds between heavy and hydrogen atoms. Populations are in e$^-$.

The second most stable adduct ***b(z)*** corresponds to a salt-bridge (SB) form, in which Ca^{2+} interacts simultaneously with the two oxygen atoms of the carboxylate group of the zwitterionic form of ***Sec***. It is worth noting that both AIM and ELF predict these two Ca-O bonds not to be strictly identical in strength. Indeed, the electron density at the BCP and the population of the O-

Ca disynaptic basin are slightly smaller for the linkage involving the oxygen atom closer to the amino group. This is due to the fact that, as illustrated in Figure 3b, the valence electron density of this oxygen atom is slightly polarized towards the nearby amino group. This SB structure is entropically stabilized. Note that in terms of enthalpy it is 7 kJ mol^{-1} higher in energy than the global minimum, whereas in terms of free energies the gap reduces to only 0.9 kJ mol^{-1}. As for the global minimum an internal rotation of the SeH group yields the ***d(z)*** structure, 2.3 kJ mol^{-1} higher in energy. A simultaneous internal rotation of the amino group and around the C-CSe bond leads to the fifth local minimum, ***e(z)***, 4.6 kJ mol^{-1} higher in energy. In this local minimum, the favorable orientation of the amino group with respect to the carboxylate one leads to the formation of an intramolecular hydrogen bond between one of the amino hydrogens and one of the carboxylate oxygens. A further rotation around the C-CSe bond yields the sixth local minimum, ***f(z)***. As shown in Figure 3a, in this structure Ca appears again tricoordinated, although the Ca-Se interaction is much weaker than that in structures ***a*** and ***c***, as reflected in the value of the electron density at the Ca-Se BCP.

It is worth noting that complexes ***a*** and ***c*** are the result of the association of the Ca^{2+} to the third more stable tautomer ***Sec3***, and differ only on the relative orientation of the SeH group. Conversely, complexes ***b(z)***, ***d(z)-f(z)*** arise from the association to the zwiterionic form of the global minimum ***Sec1***, the metal dication bridging between the two oxygen atoms of the carboxylate group. Also, in agreement with the behavior reported for serine,[25] the global minimum of the [***Sec***-Ca]$^{2+}$ PES is a CS structure, what is at variance with glycine,[46] where the global minimum of the PES is a SB structure.

The interconversion between local minima of the [***Sec***-Ca]$^{2+}$ PES is reported in Figure 4. Starting from the global minimum ***a***, the interconversion between the CS and the SB structures requires the OH group of the carboxylic acid situated close to the amino group in order to favor the necessary proton transfer. In the neutral system this could be achieved through an internal rotation around the C-COOH bond, but for the global minimum this possibility is energetically too demanding because the COOH group is attached to Ca^{2+}. An alternative mechanism maintaining the initial connectivity of Ca implies a H-shift from the OH towards the carbonyl group, which involves a rather high (136 kJ mol^{-1}) energy barrier yielding the local minimum ***p***.

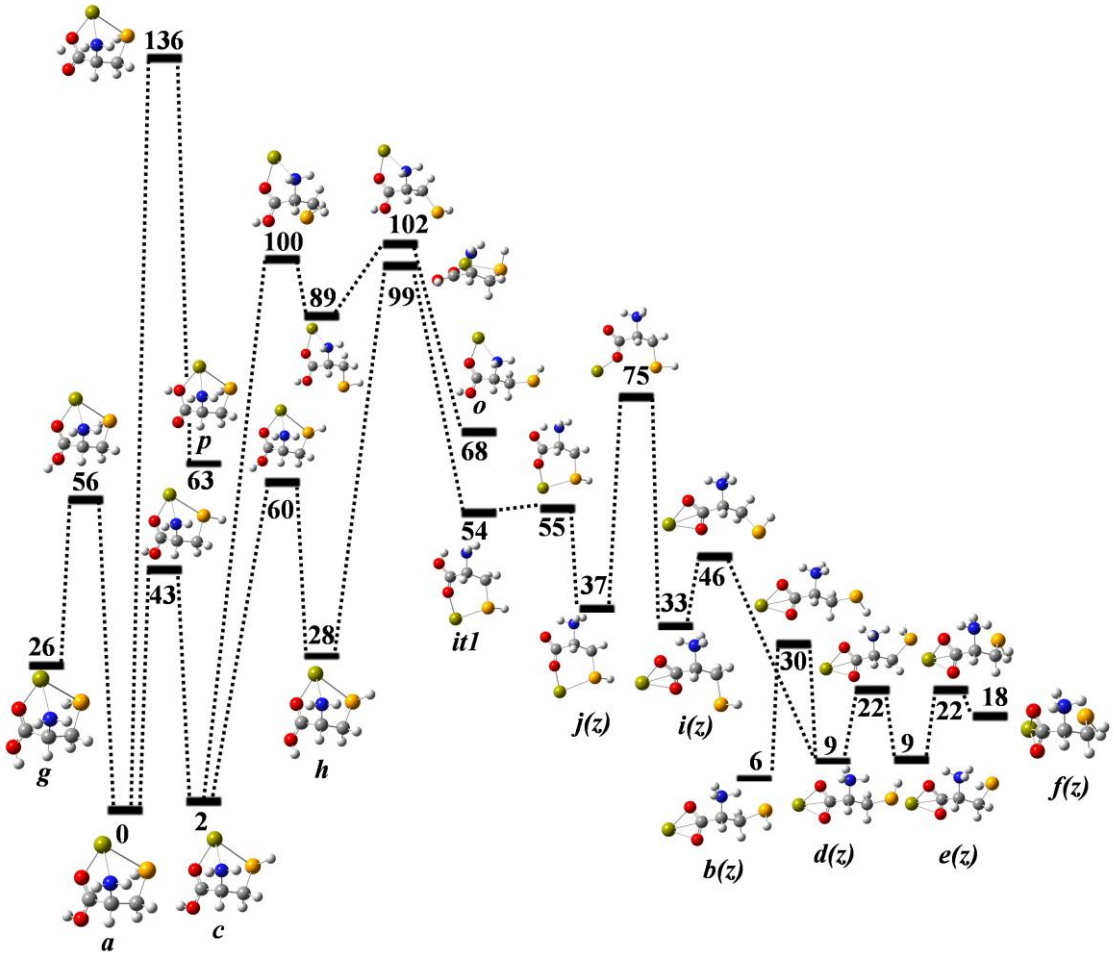

Figure 4. Energy profile of the [***Sec***-Ca]$^{2+}$ PES. B3LYP/6-311+G(d,p) relative free energies in kJ mol^{-1}.

There are other possibilities which require to break one of the three bonds in which Ca participates. The breaking of the Ca-Se linkage from the local minimum ***c*** requires an energy barrier of 100 kJ mol^{-1} to finally yield the local minimum ***o***. An alternative pathway, also with origin in structure ***c***, involves, passing by local minimum ***h***, a similar barrier of 99 kJ mol^{-1}. The advantage however is that the intermediate ***it1*** has the OH group with the appropriate orientation to favor the proton transfer towards the amino group. Indeed, this intermediate leads, through a practically barrierless process, to a SB structure ***j(z)*** in which Ca bridges between O and Se. Subsequently, a displacement of Ca would lead to a more stable SB structure ***i(z)*** in which the metal dication bridges between both carboxylate oxygen atoms. From here, different low-barrier internal rotations would lead to the more stable SB structures ***b(z)-f(z)***. It should be noted that, in general the internal rotations for the SB structures require lower activation barriers than similar process for CS complexes. For instance, while the activation barrier connecting ***a*** and ***c*** is 44 kJ mol^{-1} high, that connecting ***b(z)*** and ***d(z)*** is only 30 kJ mol^{-1}

Comparison between Ca^{2+} and Cu^{2+}

As we have indicated in the introduction one of the signatures of the interactions between neutral compounds and doubly charged metal ions is the significant distortion that the latter produces on the electron density distribution of the former. These effects normally reflect the polarization produced by the strong coulombic field created by the ion and ultimately may lead to a significant charge transfer from the base towards the metal ion. As indicated in previous sections, this is actually observed in ***Sec***-Ca^{2+} complexes, in which a nonzero second-order interaction energy between the lone-pairs of the ***Sec*** and the empty orbitals of Ca are found. The situation is quantitatively different however when the doubly-charged ion is a transition metal, such as Cu^{2+}. The global minimum is still a tricoordinated complex similar to structure ***a***, in which the metal interacts simultaneously with the carbonyl oxygen atom, the SeH and the amino groups. However, the interactions with these three groups are significantly stronger than those calculated for Ca^{2+}, so that the electron densities at the Cu-O, Cu-N and Cu-Se BCPs are higher (0.061, 0.088 and 0.063 a.u., respectively), as well as the corresponding Wiberg bond orders (0.169, 0.278 and 0.484, respectively). This bonding enhancement on going from Ca^{2+} to Cu^{2+} has a double origin. In the first place Cu^{2+} is a better electron acceptor because its 4s empty orbital lies much lower in energy with respect to the *3d* occupied than that of Ca^{2+} with respect to the *3p* occupied orbitals. In the second place, occupied 3d-orbitals of Cu(I) are much higher in energy than the 3p orbitals of Ca^{2+} and easily back-donate to the antibonding orbitals of Sec. The consequence is that whereas in $[Sec\text{-}Ca]^{2+}$ complexes there is only a polarization of the lone-pairs amino acid, with small orbital interaction energies, in $[Sec\text{-}Cu]^{2+}$ complexes, the interactions of the metal with the O lone-pairs are much stronger than those found for Ca (second order interaction energy 46 vs. 22 kJ mol^{-1}), and with Se and N are so strong that a Se-Cu covalent bond (89.4% of Se (12.1% s, 87.8% p) + 10.6% of Cu (67.7% s, 31.7% p), occupancy = 1.85) and a half-bond N-Cu (78.4% N (14.8%s, 85.2% p) + 21.6% Cu (41.3% s, 7.4% p, 51.3% d), occupancy = 0.96) are found. The obvious consequence is that while in complex ***a*** the natural charge on Ca is very close to 2 (1.81), in the $[\textbf{\textit{Sec}}–Cu]^{2+}$ global minimum is 1.04, indicating that, upon association with Cu^{2+}, ***Sec*** becomes oxidized. This is confirmed by the fact that the spin density, initially located in Cu^{2+}, which is a doublet in its ground state, is mainly located on the ***Sec*** moiety when the $[\textbf{\textit{Sec}}–Cu]^{2+}$ is formed.

Effects on the intrinsic acidity of *Sec*

The acidity of amino acids such as ***Sec*** is a useful information to understand the chemistry of peptides which they can formed.[47] It is quite obvious from the previous discussion that the association of ***Sec*** with doubly-charged metal ions should result in drastic changes in its intrinsic properties and, in particular, in its intrinsic acidity. To analyze this question we have evaluated the intrinsic acidity of the isolated amino acid, as well as that of the six most stable $[\textbf{\textit{Sec}}–Ca]^{2+}$ complexes.

Since as mentioned above in the gas phase and at 298.2 K ***Sec*** is an equilibrium mixture of eight conformers, we have evaluated the acidity of these eight forms considering that OH, SeH

and amino deprotonation would be possible. From this theoretical survey we can conclude that the deprotonation of the amino group never competes with OH or SeH deprotonation. However, there are conformers which behave as oxygen acids, whereas others behave as Se acids. Similar findings have been reported in the literature.[43] The results obtained through B3LYP/6-311+G(3df,2p)//B3LYP/6-31+G(d,p) calculations have been summarized in Table 2.

Table 2. B3LYP/6-311+G(3df,2p)//B3LYP/6-311+G(d,p) calculated acidity (Δ_{acid}H, kJ mol^{-1}) for the conformers of ***Sec*** present in the gas phase at 298.2 K

Conformer	Deprotonated site	Δ_{acid}H
Sec1	SeH	1394.1
Sec2	OH	1392.5
Sec3	OH	1404.6
Sec4	SeH	1381.0
Sec5	SeH	1361.2
Sec6	OH	1402.3
Sec7	OH	1386.4
Sec8	SeH	1357.2

It can be observed that whereas the global minimum, ***Sec1***, as well as ***Sec4***, ***Sec5*** and ***Sec8*** behave as a Se acids, the second local minimum and ***Sec3***, ***Sec6*** and ***Sec7*** are predicted to be oxygen acids, the deprotonation of the OH group being 12, 6, 23, and 15 kJ mol^{-1} more favorable than the deprotonation of the SeH group, respectively. Taking into account that ***Sec*** is an equilibrium mixture of these eight conformers with the relative abundance given in Table 1, the expected acidity of this amino acid should be 1390.9 kJ mol^{-1}. To the best of our knowledge the gas-phase acidity of ***Sec*** is not known, but our estimated value predict Sec to be less acidic than previous theoretical estimates.[43]

The association of ***Sec*** with Ca^{2+} leads to two important changes regarding the intrinsic acidity of the system. Firstly, there is an expected and huge acidity enhancement. Indeed the global minimum ***a*** is 815 kJ mol^{-1} more acidic than the isolated amino acid. Secondly, regardless of the kind of complex (CS or SB) which undergoes the deprotonation, the SeH group is the most acidic site. Among the complexes investigated ***f(z)*** is the strongest acid followed by complex ***c***, which is predicted to be 1.3 kJ mol^{-1} less acidic. The estimated acidity enhancement for Cu^{2+} complexes (993 kJ mol^{-1}) is larger than for Ca^{2+} as a consequence of the oxidation undergone by the amino acid when associated with the transition metal ion. However, the most important observation is that whereas the overall deprotonation process is strongly exothermic when the interaction involves Cu^{2+}, it is slightly endothermic if the metal ion is Ca^{2+}. As shown in Figure 5a for both the most stable CS and the most stable SB structures the [(***Sec***-H)Ca]$^{+}$ + H^{+} dissociation limit lies respectively 9 and 87 kJ mol^{-1} higher in energy than the ***Sec*** + Ca^{2+} entrance channel. Conversely, when Ca^{2+} is replaced by Cu^{2+}, the corresponding dissociation limits (See Figure 5b) lie 736 and 581 kJ mol^{-1} lower in energy than the ***Sec*** + Cu^{2+} entrance

channel. This implies that whereas the formation of the [***Sec***-Cu]$^{2+}$ is thermodynamically instable with respect to the loss of a proton and should undergo a spontaneous deprotonation, the [***Sec***-Ca]$^{2+}$ is stable with respect to same process.

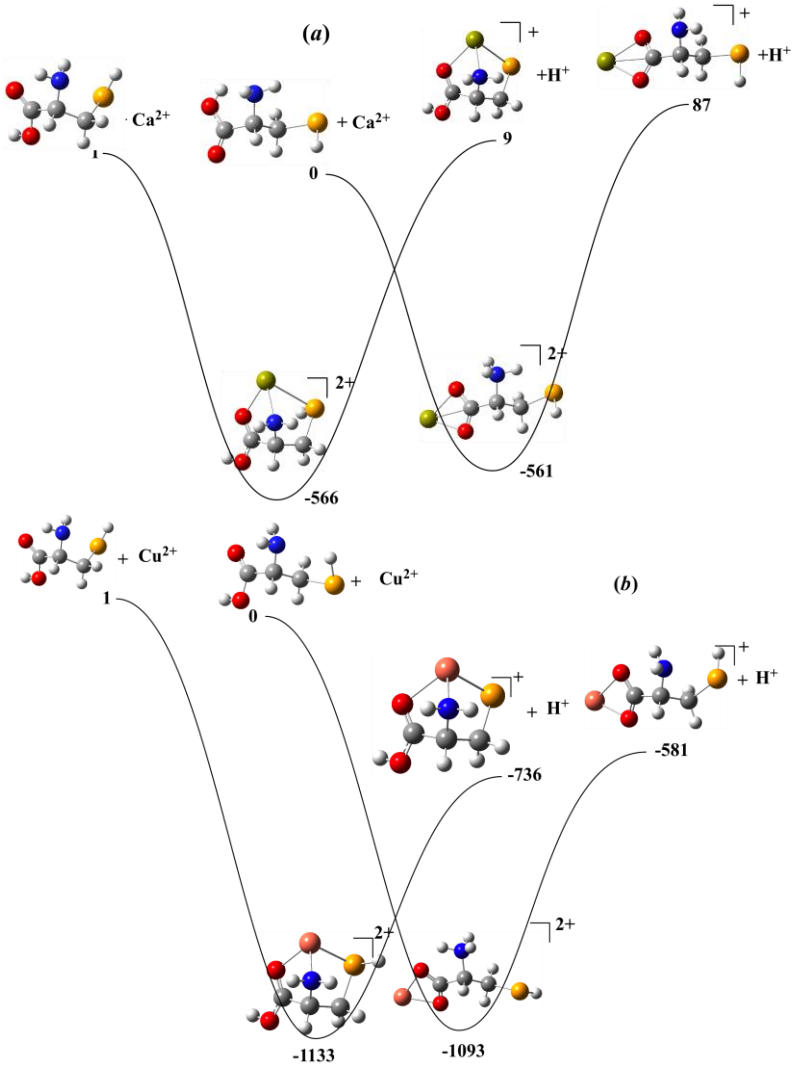

Figure 5. Potential energy curves corresponding to the formation of [***Sec***-M]$^{2+}$ complexes and their posterior deprotonation to yield [(***Sec***-H)M]$^{+}$ + H^{+}. (a) M = Ca; (b) M = Cu. Relative energies in kJ mol^{-1}.

Conclusions

The global minimum of the [***Sec***-Ca]$^{2+}$ potential energy surface is a CS species in which the metal dication is bound to the three basic groups of the amino acid, namely the carbonyl oxygen, the SeH and the amino groups, but within a gap of 5.0 kJ mol^{-1} in terms of free energies there are

other four complexes, three of which are SB structures. Actually, the second local minimum of the PES, is a SB complex entropically stabilized which lies only 0.9 kJ mol^{-1} higher in energy than the CS global minimum. The interactions between Ca^{2+} and the amino acid are essentially electrostatic, in contrast with what happens when Ca^{2+} is replaced by Cu^{2+} , where the charge transfer from ***Sec*** to the metal cation is so large that in the [***Sec***-Cu]$^{2+}$ global minimum the amino acid moiety is oxidized. The significant electron density redistribution undergone by the amino acid when interacting with both doubly-charged metal ions is reflected in a significant enhancement of its intrinsic acidity, although this effect is much larger for Cu^{2+} than for Ca^{2+}. Whereas the conformers of the isolated ***Sec*** are oxygen or selenium acids, the complexes with Ca^{2+} are always Se acids.

One of the direct consequences of the oxidation undergone by the amino acid when attached to Cu^{2+} is that the complexes formed are thermodynamically unstable with respect to a proton loss since the process ***Sec*** + $Cu^{2+} \rightarrow$ [(***Sec***-H)Cu]$^{+}$ + H^{+} is strongly exothermic. Conversely, a similar process when Cu^{2+} is replaced by Ca^{2+} is endothermic and therefore the [***Sec***-Ca]$^{2+}$ complexes should be stable with respect to a proton loss.

Acknowledgements

This work has been partially supported by the DGI Projects No. CTQ2012-35513-C02-01 and CTQ2010-17006, by the Project MADRISOLAR2, Ref.: S2009PPQ/1533 of the Comunidad Autonoma de Madrid, and by Consolider on Molecular Nanoscience CSC2007-00010. A generous allocation of computing time at the CCC of the UAM is also acknowledged.

References

1. Johansson, L.; Gafvelin, G.; Arner, E. S. J. *Biochim. Biophys. Acta*, **2005**, *1726*, 1-13. http://dx.doi.org/10.1016/j.bbagen.2005.05.010 PMid:15967579
2. Bosl, M. R.; Takaku, K.; Oshima, M.; Nishimura, S.; Taketo, M. M. *Proc. Nat. Acad. Sci. U. S. A.* **1997**, *94*, 5531-5534. http://dx.doi.org/10.1073/pnas.94.11.5531
3. Flohe, L.; Gunzler, W. A.; Schock, H. H. *Febs Lett.* **1973**, *32*, 132-134. http://dx.doi.org/10.1016/0014-5793(73)80755-0
4. Ottaviano, F. G.; Handy, D. E.; Loscalzo, J. *Circulation J.* **2008**, *72*, 1-16. http://dx.doi.org/10.1253/circj.72.1
5. Schweizer, U.; Brauer, A. U.; Kohrle, J.; Nitsch, R.; Savaskan, N. E. *Brain Res. Rev,*. **2004**, *45*, 164-178.

http://dx.doi.org/10.1016/j.brainresrev.2004.03.004
PMid:15210302
6. Leopoldini, M.; Chiodo, S. G.; Toscano, M.; Russo, N. *Chem. Eur. J.* **2008**, *14*, 8674-8681. http://dx.doi.org/10.1002/chem.200800906 PMid:18671310
7. Naziroglu, M. *Neurochem. Res.* **2009**, *34*, 2181-2191. http://dx.doi.org/10.1007/s11064-009-0015-8 PMid:19513830
8. Jin, R. C.; Mahoney, C. E.; Anderson, L.; Ottaviano, F.; Croce, K.; Leopold, J. A.; Zhang, Y. Y.; Tang, S. S.; Handy, D. E.; Loscalzo, J. *Circulation* **2011**, *123*, 1963-1973. http://dx.doi.org/10.1161/CIRCULATIONAHA.110.000034 PMid:21518981 PMCid:PMC3107543
9. Zhuo, P.; Diamond, A. M. *Biochim. Biophys. Acta* **2009**, *1790*, 1546-1554.
10. Kryukov, G. V.; Castellano, S.; Novoselov, S. V.; Lobanov, A. V.; Zehtab, O.; Guigo, R.; Gladyshev, V. N. *Science* **2003**, *300*, 1439-1443. http://dx.doi.org/10.1126/science.1083516 PMid:12775843
11. Thisse, C.; Degrave, A.; Kryukov, G. V.; Gladyshev, V. N.; Obrecht-Pflumio, S.; Krol, A.; Thisse, B.; Lescure, A. *Gene Expres. Patt.* **2003**, *3*, 525-532. http://dx.doi.org/10.1016/S1567-133X(03)00054-1
12. Berridge, M. J.; Bootman, M. D.; Lipp, P. *Nature* **1998**, *395*, 645-648. http://dx.doi.org/10.1038/27094 PMid:9790183
13. Soderling, T. R.; Stull, J. T. *Chem. Rev.* **2001**, *101*, 2341-2351. http://dx.doi.org/10.1021/cr0002386 PMid:11749376
14. Ellis-Davies, G. C. R. *Chem. Rev.* **2008**, *108*, 1603-1613. http://dx.doi.org/10.1021/cr078210i PMid:18447376
15. Dantzig, J. A.; Higuchi, H.; Goldman, Y. E. *Methods Enzymol.* **1998**, *291*, 307-48. http://dx.doi.org/10.1016/S0076-6879(98)91021-7
16. Niggli, E. *Annu. Rev. Physiol*. **1999**, *61*, 311-335. http://dx.doi.org/10.1146/annurev.physiol.61.1.311 PMid:10099691
17. Lescure, A.; Rederstorff, M.; Krol, A.; Guicheney, P.; Allamand, V. *Biochim. Biophys. Acta* **2009**, *1790*, 1569-1574. http://dx.doi.org/10.1016/j.bbagen.2009.03.002 PMid:19285112
18. Bryan, P. N. *Chem. Rev*. **2002**, *102*, 4805-4815.

http://dx.doi.org/10.1021/cr010190b
PMid:12475207
19. Forsen, S.; Kordel, J., Bioinorganic Chemistry. In Bertini, I.; Gray, H. B.; Lippard, S. J.; Valentine, J. S., Eds. University Science Books: Mill Valley, Ca., 1994.
20. Trujillo, C.; Lamsabhi, A. M.; Mó, O.; Yáñez, M. *Phys. Chem. Chem. Phys*. **2008**, *10*, 3229-3235.
http://dx.doi.org/10.1039/b802907e
PMid:18500399
21. Arbogast, S.; Ferreiro, A. *Antioxid. Redox Sign*. **2010**, *12*, 893-904.
http://dx.doi.org/10.1089/ars.2009.2890
PMid:19769461
22. Grumolato, L.; Ghzili, H.; Montero-Hadjadje, M.; Gasman, S.; Lesage, J.; Tanguy, Y.; Galas, L.; Ait-Ali, D.; Leprince, J.; Guerineau, N. C.; Elkahloun, A. G.; Fournier, A.; Vieau, D.; Vaudry, H.; Anouar, Y. *Faseb J*. **2008**, *22*, 1756-1768.
http://dx.doi.org/10.1096/fj.06-075820
PMid:18198219
23. Bertrán, J.; Rodríguez-Santiago, L.; Sodupe, M. *J. Phys. Chem. B* **1999**, *103*, 2310-2317.
http://dx.doi.org/10.1021/jp984534m
24. Lamsabhi, A. M.; Mó, O.; Yáñez, M.; Alcamí, M.; Tortajada, J. *Chem. Phys. Chem* **2004**, *5*, 1871.
http://dx.doi.org/10.1002/cphc.200400208
PMid:15648135
25. Lamsabhi, A.; Mó, O.; Yáñez, M. *Can. J. Chem*. **2010**, *88*, 759-768.
http://dx.doi.org/10.1139/V10-038
26. Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648-5652.
27. Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785-9.
http://dx.doi.org/10.1103/PhysRevB.37.785
28. Montgomery Jr., J. A.; Frisch, M. J.; Ochterski, J.; Petersson, G. A. *J. Chem. Phys.* **1999**, *110*, 2822-2827.
29. González, A. I.; Mó, O.; Yáñez, M.; Leon, E.; Tortajada, J.; Morizur, J. P.; Leito, I.; Maria, P. C.; Gal, J. F. *J. Phys. Chem.* **1996**, *100*, 10490-10496.
http://dx.doi.org/10.1021/jp953042w
30. Raab, V.; Gauchenova, E.; Merkoulov, A.; Harms, K.; Sundermeyer, J.; Kovacevic, B.; Maksic, Z. B. *J. Am. Chem. Soc.* **2005**, *127*, 15738-15743.
http://dx.doi.org/10.1021/ja052647v
PMid:16277515
31. Bouchoux, G.; Desaphy, S.; Bourcier, S.; Malosse, C.; Bimbong, R. N. B. *J. Phys. Chem. B* **2008**, *112*, 3410-3419.
http://dx.doi.org/10.1021/jp709677c
PMid:18288831

32. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J., J. A. ; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, O.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. Gaussian09, Revision A.02, Gaussian09, Revision A.02; Gaussian, Inc.: Wallingford CT, 2009.
33. Reed, A. E.; Curtiss, L. A.; Weinhold, F. *Chem. Rev.* **1988**, *88*, 899-926. http://dx.doi.org/10.1021/cr00088a005
34. Wiberg, K. B. *Tetrahedron* **1968**, *24*, 1083-1088. http://dx.doi.org/10.1016/0040-4020(68)88057-3
35. Bader, R. F. W., Atoms in Molecules. A Quantum Theory. Clarendon Press: Oxford, 1990.
36. Becke, A. D.; Edgecombe, K. E. *J. Chem. Phys*. **1990**, *92*, 5397-403.
37. Silvi, B.; Savin, A. *Nature* **1994**, *371*, 683-686. http://dx.doi.org/10.1038/371683a0
38. Savin, A.; Nesper, R.; Wengert, S.; Fäsler, T. F. *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 1808-1832. http://dx.doi.org/10.1002/anie.199718081
39. Noury, S.; Krokidis, X.; Fuster, F.; Silvi, B. *Comput. & Chem.* **1999**, *23*, 597-604. http://dx.doi.org/10.1016/S0097-8485(99)00039-X
40. Hurtado, M.; Mo, O.; Yanez, M. *Can. J. Chem.* **2010**, *88*, 744-753. http://dx.doi.org/10.1139/V10-034
41. Vank, J. C.; Sosa, C. P.; Perczel, A.; Csizmadia, I. G. *Can. J. Chem*. **2000**, *78*, 395-408. http://dx.doi.org/10.1139/v00-029
42. Hegedus, I. L.; Sahai, M. A.; Labadi, M.; Szori, M.; Paragi, G.; Viskolcz, B.; Bottoni, A. *J. Mol. Struct. Theochem* **2005**, *725*, 111-125. http://dx.doi.org/10.1016/j.theochem.2005.01.045
43. Kaur, D.; Sharma, P.; Bharatam, P. V.; Kaur, M. *Int. J. Quant. Chem*. 2008, *108*, 983-991. http://dx.doi.org/10.1002/qua.21556
44. Spezia, R.; Tournois, G.; Cartailler, T.; Tortajada, J.; Jeanvoine, Y. *J. Phys. Chem. A* **2006**, *110*, 9727-9735. http://dx.doi.org/10.1021/jp0614998 PMid:16884205
45. Corral, I.; Lamsabhi, A. M.; Mo, O.; Yanez, M. *Int. J. Quant. Chem.* **2012**, *112*, 2126-2134.

http://dx.doi.org/10.1002/qua.23169
46. Corral, I.; Mó, O.; Yáñez, M.; Salpin, J. Y.; Tortajada, J.; Moran, D.; Radom, L. *Chem. Eur. J.* **2006**, *12*, 6787-6796. http://dx.doi.org/10.1002/chem.200600127 PMid:16807970
47. Dudev, T.; Lim, C. *J. Phys. Chem. B* **2009**, *113*, 11754-11764. http://dx.doi.org/10.1021/jp904249s PMid:19642664

8217 Graphical Abstract

Complexation of Ca^{2+} with selenocysteine. Effects on its intrinsic acidity. *Marcela Hurtado, Al Mokhtar Lamsabhi, Manuel Yáñez and Otilia Mó.*