Interaction of Ca^{2+} with uracil and its thio derivatives in the gas phase†

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The structures and relative stabilities of the complexes formed by uracil and its sulfur derivatives, namely, 2-thio-, 4-thio, and 2,4-dithio-uracil when interacting with Ca^{2+} in the gas phase have been analyzed by means of density functional theory (DFT) calculations carried out at the B3LYP/6-311++G(3df,2p)//B3LYP/6-31+G(d,p) level. For uracil and 2,4-dithiouracil, where the two basic sites are the same, Ca^{2+} attachment to the heteroatom at position 4 is preferred. However, for the systems where both types of basic centers, a carbonyl or a thiocarbonyl group, are present, Ca^{2+}–oxygen association is favored. The most stable complexes correspond to structures with Ca^{2+} bridging between the heteroatom at position 2 of the 4-enol (or the 4-enethiol) tautomer and the dehydrogenated ring nitrogen, N3. The enhanced stability of these enolic forms is two-fold, on the one hand Ca^{2+} interacts with two basic sites and on the other triggers a significant aromatization of the ring. Besides, Ca^{2+} association has a clear catalytic effect on the tautomerization processes which connect the oxo–thione forms with the enol–enethiol tautomers. Hence, although the enol–enethiol tautomers of uracil and its thio derivatives should not be observed in the gas phase, the corresponding Ca^{2+} complexes are the most stable species and should be accessible, because the tautomerization barriers are smaller than the Ca^{2+} binding energies.

Introduction

Metal dications, play a relevant role in a great variety of biological processes. However, little was known on the mechanisms, at the molecular level, involved in these processes until very recently,[1–10] and most of the information available is related to the interaction energies and the structure of the complexes.[11–22] One of the reasons behind this lack of knowledge on how a doubly charged metal interacts with neutral systems, is the difficulty of generating stable adducts in the gas-phase. As a matter of fact, in general, when a dication, M^{2+}, such as Cu^{2+}, Ni^{2+} or Pb^{2+} interacts in the gas phase with a molecular base, B, it triggers the deprotonation of the base, so that only [(B − H)M]$^{+}$ monocations are usually detected after the interaction.[23–25] This seems to be related to the high recombination energies of these doubly charged metal ions, which oxidize the base, and facilitate its deprotonation.[26,27] The situation is different when dealing with alkaline-earth metal dications such as Ca^{2+}, whose recombination energy is significantly lower than those of the metal dications mentioned above. As a matter of fact, the adducts between Ca^{2+} and different bases, such as urea,[2] glycine,[5] thiourea[8] and selenourea[10] could be produced and detected using gas phase electrospray ionization/mass spectrometry techniques.[28] But, what is more important, the unimolecular reactivity of these adducts could be investigated. This opened then, the possibility of analyzing the mechanisms that dictate the intrinsic reactivity of these molecules when associated with a metal dication, and in particular the role that coulomb explosions play in the observed reactivity.[2,5,8,10,29]

In our continued effort to investigate the reactions between small systems of biological relevance and Ca^{2+}, we have considered as good candidates uracil and its thio-derivatives. Uracil is an important component of nucleic acids, and 2-thiouracil and 4-thiouracil have been identified as minor components of t-RNA.[30] The latter are also constituents of anticancer and antithyroid drugs.[30] But perhaps one of the most important characteristics of this set of compounds is its possible implication in the mutations that can occur during DNA duplication. As a matter of fact, uracil and thiouracil derivatives present many different tautomeric forms, and although it is well established that, by far, the most stable forms in the gas phase are the oxo–thione tautomers,[31–36] their tautomerization seems to be essential in mutagenic processes. Many experimental and theoretical studies have investigated the interaction of uracil and its derivatives with different metal ions. In most of them the attention was focused on the structure and bonding of the complexes formed.[17,37–40] Very few have analyzed other questions such as the influence of the metal ion on the formation of different tetrads,[41] the influence of different substituents on the strength of the interaction with metal ions[42,43] or the possible induced proton transfers triggered by the metal ion.[44] However, not much information is available on the effect that these interactions may have on the tautomerization of these systems, or on the relative stability of the different tautomers, and to the best of our knowledge only two papers have pointed out the effect of doubly charged metals on the stability of the enolic forms of thymine.[45,46] Hence, the aim of this paper is to investigate the structure and stability of the complexes between Ca^{2+} and the different tautomers of uracil and its three thio-derivatives, in order to analyze the effect that the association with this divalent metal cation, which is ubiquitous in biological media,

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may have on the tautomerization processes. The gas-phase survey of the interactions of uracil and thiouracils with Ca^{2+} presented here can provide important clues for the understanding of the behavior of these systems in biological media, because, although solvation effects are absent many of the biological processes take place in essentially non-polar environments which are often better understood from the behavior of the systems in the gas-phase than in solution.[47] The N1 preferred glycosylation of uracil can be considered a paradigmatic example, where the differential N1 and N3 acidities in a non polar environment may be the reason of the observed selectivity.[48]

Computational details

The geometries of all possible complexes between uracil and thiouracils with Ca^{2+} have been optimized in the frame of the density functional theory (DFT). For this purpose we have chosen the hybrid functional B3LYP[49,50] as implemented in the Gaussian 03 suite of programs,[51] because, in a previous assessment,[52] it has been shown to provide reliable results when dealing with Ca^{2+} interactions. Due to the size of the systems investigated, the basis set used in ref. 39 is impractical here and we have used instead the 6-31+G(d,p) basis set expansion. Nevertheless, in order to ensure the reliability of the relative stability of the different tautomers, the final energy of each of the systems investigated was obtained in single-point calculations using a much larger and flexible 6-311++G(3df,2p) basis. Harmonic vibrational frequencies were computed at the same level used for the geometry optimizations in order to estimate the corresponding zero-point vibrational energy (ZPVE) corrections (scaled by 0.986)[53] and to classify stationary points of the potential energy surface either as local minima or transition states (TS). Ca^{2+} binding energies, D_0, were evaluated by subtracting from the energy of the most stable adduct the energy of the neutral and that of Ca^{2+}, after including the corresponding ZPVE corrections.

The binding characteristics were analyzed primarily by using the Becke and Edgecombe electron localization function (ELF)[54] topological approach.[55] ELF has been originally conceived as a local measure of the Fermi hole curvature around a reference point. A Lorentz transform allows ELF to be confined in the [0,1] interval, where 1 corresponds to regions dominated by an opposite spin pair or by a single electron. In this way the molecular space can be partitioned in basins, so that the valence shell of a molecule can be described in terms of two types of basins: polysynaptic basins (generally disynaptic), with the participation of two (or more) atomic valence shells and the monosynaptic ones, which correspond to electron lone-pairs. ELF calculations were carried out with the TopMod suite of programs.[56]

A second approach, the atoms in molecules (AIM) theory,[57,58] was also used in our bonding study. This theory is based on a topological analysis of the electron density, which permits the definition of a molecular graph as the ensemble of bond critical points (bcps), stationary points in which the electron density is minimum only in the direction of the bond, and bond paths. In general the electron density, as well as the energy density calculated at the bcps, give useful information on the strength and nature of the bond. For this purpose the AIMPAC series of programs was employed.[59] These analyses were complemented with natural bond orbital (NBO) and natural resonance theory (NRT) calculations.[60]

Table 1 Ca^{2+} binding energies (kJ mol^{-1}) of uracil and thiouracils for the adducts at basic sites X and Y

Compound	X	Y
Uracil	438	397
2-Thiouracil	439	356
4-Thiouracil	386	399
2,4-Dithiouracil	388	358

The former permits the bonding to be described in terms of localized hybrids and lone-pairs, and the second provides the weight of the different resonant structures that contribute to the stability of a given system. The Wiberg bond orders (BO) were also evaluated in the framework of the former approach. These calculations have been carried out with the NBO-5G series of programs.[61]

Results and discussion

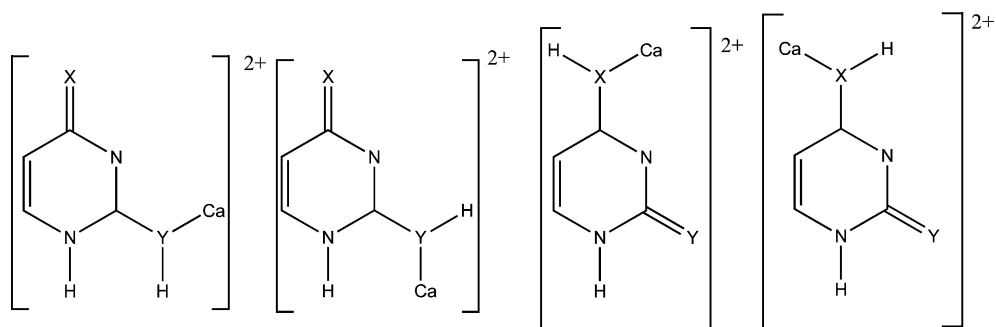The most stable tautomeric forms of uracil–Ca^{2+} and thiouracil–Ca^{2+} complexes are shown in Fig. 1.

In what follows the heteroatoms bound to C4 and to C2 will be named X and Y, respectively. When a given tautomer presents more than one conformer they were named by adding **a** or **b** to the number identifying the tautomer. Their relative energies are summarized in Table 1, whereas their total energies, and ZPVE corrections are given in Table S1 of the supplementary data. In our theoretical survey we have considered more structures than those compiled in Fig. 1, but structures like the ones shown in Scheme 1, either lie very high in energy or finally collapse to one of the local minima shown in Fig. 1.

Geometries, relative stabilities and bonding

The optimized geometries of all conformers considered in this work are given in Table S2 of the supplementary data. Although a detailed discussion of the optimized geometries is not one of the goals of this study, it is worth mentioning, that whereas the C=O–Ca angles are always equal or very close to 180°, the C=S–Ca bond angles are systematically close to 123°, and the S–Ca bond does not lie in the plane of the molecule. These dissimilarities between O–Ca^{2+} and S–Ca^{2+} interactions were also found for other metal monocations, such as Li$^+$ and clearly reflect, the ionic character of the interaction, which will be discussed later. Assuming that the primary interaction is electrostatic, a suitable explanation of these dissimilarities was provided in terms of the characteristics of the molecular electrostatic potential and the average distance of the metal ion to the basic center.[62] When the basic site is a carbonyl oxygen, the distance between the O nucleus and the electrostatic potential minima associated with the O lone-pairs is shorter than the typical dication–oxygen distance in the complex. This means that the metal dication moves along isopotential lines connecting the two minima, and finally it nests between them, because in this way it polarizes both lone-pairs simultaneously. For the sulfur atom, the distance between the minima of the electrostatic potentials and the S nucleus is similar to the S-metal ion distance, and therefore the metal cation is trapped in either of these minima.

X = Y = O: uracil

X = O ; Y = S : 2-thiouracil

X = S ; Y = O : 4-thiouracil

X = Y = S : 2,4-dithiouracil
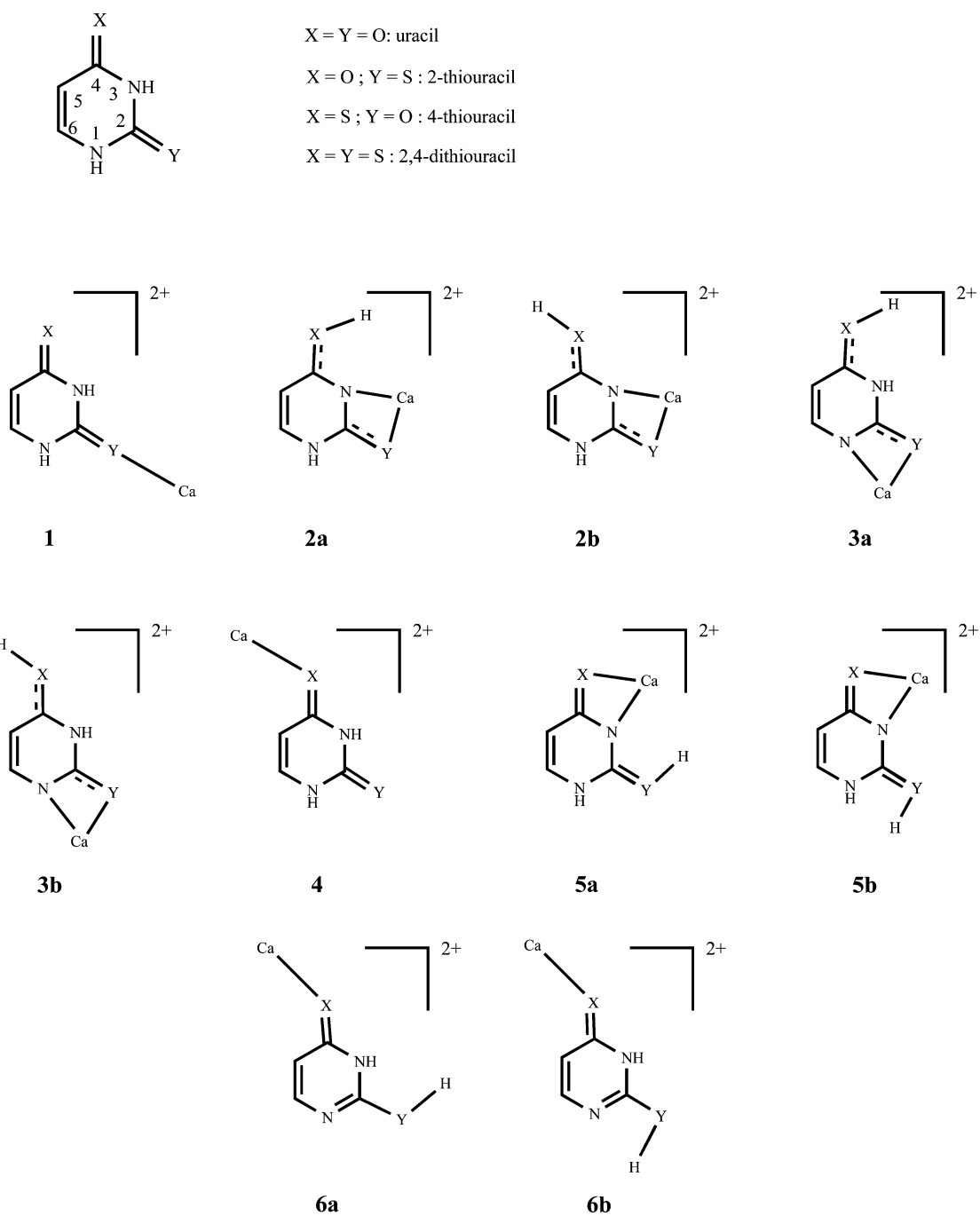
Fig. 1 Schematic representation of the different tautomeric forms of uracil– and thiouracil–Ca²⁺ complexes.

Scheme 1

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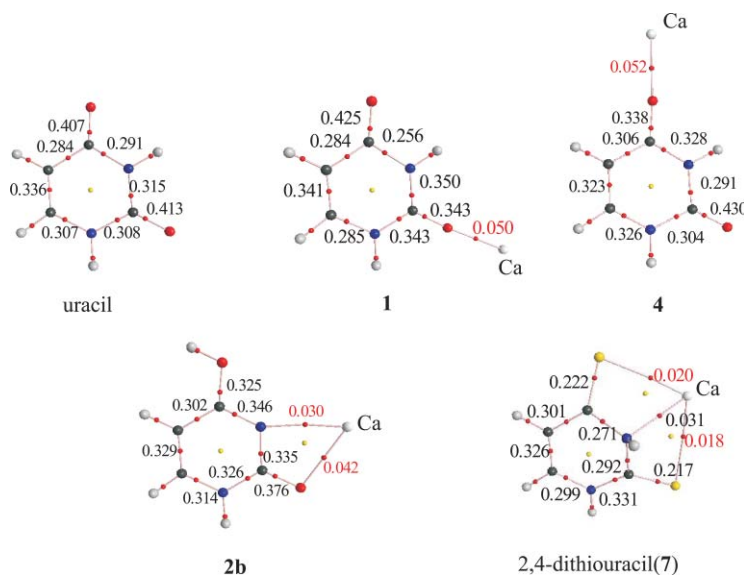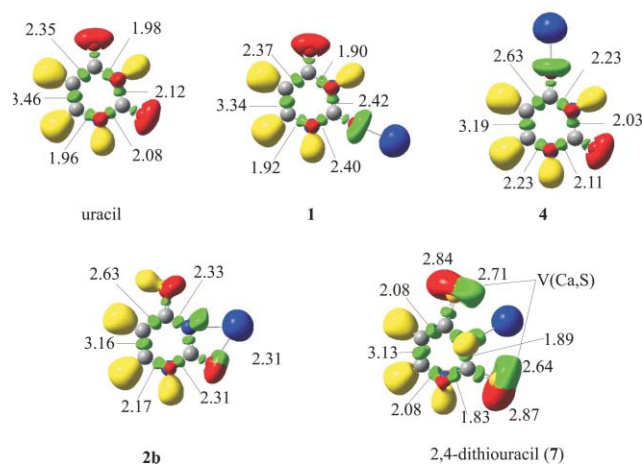
It is also important to note that all attempts to locate π-type complexes, in which Ca^{2+} interacts with the double bonds in the ring failed for the three thiouracils because all these structures finally collapsed to the most stable adduct in which the metal interacts with the heteroatom X. For uracil, as it was already reported in the literature,[17] a π-type complex actually exists as a local minimum, but it lies more than 250 kJ mol⁻¹ above the most stable adduct. The same happens when trying to attach the metal ion to the NH groups. Only in the case of 2,4-dithiouracil, structure **7** (see Fig. 2) was found to be a local minimum of the potential energy surface. Interestingly, these kinds of structures which are not stable for uracil were found to be also stable for all the selenouracil derivatives.[63] More importantly, a **7**-like structure was found to be the most stable adduct for 2,4-diselenouracil–Ca^{2+} complexes. As explained in ref. 63 two factors seem to be responsible for the enhanced stability of structures **7** when dealing with sulfur or selenium derivatives. On the one hand, when oxygen is replaced by sulfur or selenium, which are less electronegative than oxygen, an accumulation of electron density on the N3 lone-pair occurs. On the other hand, and more importantly sulfur and selenium are much more polarizable than oxygen and structure **7** is stabilized because the two highly polarizable centers are close to the metal dication. As a matter of fact an inspection of the topology of the electron density shows the existence of bcps between the metal dication and both sulfur atoms (see Fig. 2).

Also the ELF shows the existence of disynaptic V(Ca,S) basins which actually reflect the strong polarization undergone by the sulfur lone-pairs in the presence of the metal dication (see Fig. 3). The fact that Se is more polarizable than sulfur explains why for the 2,4-diselenouracil structure **7** is the most stable adduct[63] whereas for 2,4-dithiouracil it is only the second most stable adduct. This difference in polarizability also explains that whereas for 2-selenouracil, structure **7** is a local minimum of the potential energy surface[63] for 2- and 4-thiouracil derivatives it is not.

The enhanced basicity of the heteroatom at position 4 (X) with respect to the heteroatom at position 2 (Y) has been well documented in the literature for uracil and thiouracils.[35,64–66]

Fig. 3 Three-dimensional representations of ELF isosurfaces with ELF = 0.80 for neutral uracil, uracil–Ca^{2+} complexes, **1**, **4** and **2b** and 2,4-dithiouracil–Ca^{2+} complex **7**. Yellow lobes correspond to V(N,H) and V(C,H) basins, red lobes correspond to V(N), V(O) and V(S) basins associated with N, O and S lone-pairs, respectively. Green lobes correspond to V(C,C), V(C,N), V(C,O), V(C,S) and V(Ca,S) basins. Blue lobes correspond to the Ca metal core. The populations of the different basins are also indicated.

Recently, it has been also concluded, based on density functional theory calculations that this position is the most basic one in selenouracils, both when the reference acid is a proton[67] or a Ca^{2+} dication.[63] Hence, it is not surprising to find that for uracil and 2,4-dithiouracil, where both heteroatoms are identical, the attachment of Ca^{2+} to X is preferred by 41 and 31 kJ mol⁻¹, respectively, with respect to attachment to Y (see Table 1). Note that the value of 438 kJ mol⁻¹ obtained for uracil is slightly below the binding energy recently estimated at the B3LYP/6-311+G(2df,2p) level (451 kJ mol⁻¹).[16]

This preference to bind to X was traditionally attributed to the contribution of zwitterionic mesomeric forms which accumulate negative charge in this position. However, similar mesomeric forms

Fig. 2 Molecular graphs of uracil, uracil–Ca^{2+} complexes, **1**, **4** and **2b** and 2,4-dithiouracil–Ca^{2+} complex **7**. Electron densities at the bcps are in a.u.

Table 2 Wiberg bond orders for uracil and its Ca^{2+} adducts **1** and **4**

Bond	Uracil	**1** (attached to Y)	**4** (attached to X)
N1–C2	1.05	1.23	1.03
C2–N3	1.10	1.29	1.00
N3–C4	1.04	0.98	1.22
C4–C5	1.09	1.09	1.28
C5–C6	1.70	1.74	1.50
C6–N1	1.14	1.06	1.28

accumulating negative charge at Y contribute also significantly to the stability of the neutral compound, so this factor alone cannot explain the preference of uracil and its derivatives to undergo the electrophilic attacks at X. A second important factor in favor of association to X is the electron density redistribution undergone by the system upon cation attachment, which is significantly different depending on the site where the cation enters. This is apparent when the ELF of complexes **1** and **4** are compared with that of the neutral compound (see Fig. 3). Let us take uracil as a suitable example. On going from the neutral to complex **1**, where the metal cation is bonded to Y, the electron density remains strongly localized, and the populations of the basins associated with the C5–C6, C5–C4, C4–N3 and C6–N1 hardly changed, the most significant changes affecting to the C2–N1 and C2–N3 bonds. Conversely, association of the metal dication to X to yield complex **4**, triggers a significant delocalization of the electron density, which involves a charge transfer from the C5–C6 basin, towards the C5–C4, C4–N3 and C6–N1 ones. This electron delocalization, which contributes to stabilize the molecular cation, is actually mirrored in a certain equalization of both the bond distances within the ring and the electron densities at the corresponding bcps (see Fig. 2). This picture is also consistent with the Wiberg BOs. As shown in Table 2, in neutral uracil, C5–C6 has a significant double bond character, whereas the remaining bonds within the ring are essentially single bonds. The situation changes very little upon Ca^{2+} attachment to Y, and only C2–N3 and C2–N1 slightly increase their double bond character. Conversely, when Ca^{2+} is attached to X, the changes in the BOs are significant. The double bond character of C5–C6 decreases significantly, whereas it increases for C5–C4, C6–C1, and C3–C4, ratifying the significant charge delocalization produced by the Ca^{2+} polarization. Similar trends are also observed for 2-, 4- and 2,4-thiouracils.

It is also worth noting that the Ca^{2+} binding energy is 50 kJ mol^{-1} larger for uracil than for 2,4-dithiouracil, in spite of the fact that thiocarbonyl derivatives exhibit greater gas-phase proton affinities than their carbonyl counterparts. This confirms, as it has been shown previously in the literature,[63] the clear preference for Ca^{2+} to attach to oxygen, rather than to sulfur or selenium. As a matter of fact, the calculated Ca^{2+} binding energy of urea (449 kJ mol^{-1}) is larger than that of thiourea (404 kJ mol^{-1}) or selenourea (408 kJ mol^{-1}). Similarly, acetamide also binds Ca^{2+} stronger than thioacetamide or selenoacetamide.[63] This preference for Ca^{2+} to bind to oxygen explains the significant increase in the gap between adducts **4** and **1** on going from uracil to 2-thiouracil, because both effects, the enhanced basicity of X and the preference to bind an oxygen atom, go in the same direction. Conversely, for 4-thiouracil both effects go in opposite direction, although the preference to bind to oxygen slightly dominates, and structure **1** is found to be slightly more stable than **4**.

Also interestingly, the nature of the second heteroatom has an almost negligible influence on the Ca^{2+} affinity of both carbonyl and thiocarbonyl groups. Indeed, the Ca^{2+} binding energy to the oxygen of 2-thiouracil is 1 kJ mol^{-1} larger than that of uracil, and the Ca^{2+} binding energy to the sulfur atom is 3 kJ mol^{-1} smaller than that of X in 2,4-dithiouracil. Similarly, the sulfur Ca^{2+} affinity of 4-thiouracil is 3 kJ mol^{-1} smaller than that of 2,4-dithiouracil, and its oxygen Ca^{2+} binding energy 2 kJ mol^{-1} higher than that of Y in uracil.

There are however some dissimilarities in the nature of the O–Ca^{2+} and the S–Ca^{2+} interactions. Independently of the nature of the basic site, these interactions are essentially ionic, as indicated by the small value of the electron density at both the O–Ca and the S–Ca bcps (typically around 0.05 a.u. for O–Ca bonds and 0.03 for S–Ca bonds), and in the positive value of the energy density, as it corresponds to the interaction between two close-shell systems. Also consistently, the NBO analysis describes these complexes as the interaction between the polarized uracil or thiouracil moiety and Ca^{2+}. However, while in O–Ca^{2+} interactions the natural charge of the metal is close to +2.0, in S–Ca^{2+} interactions there is a small charge transfer from the sulfur lone-pairs towards the 4 s empty orbital in Ca^{2+}, reflecting the larger polarizability of sulfur. Consequently, in these cases the net natural charge on Ca is about +1.8. The strong polarization undergone by the base upon Ca^{2+} association is nicely reflected in the ELF, which shows a significant increase in the population of the V(O) (or the V(S)) basin of the basic site upon Ca^{2+} attachment. More importantly, when Ca^{2+} is bonded to a thiocarbonyl group, a disynaptic V(S,Ca) basin is located, showing that in this case the covalent character of the interaction is not negligible.
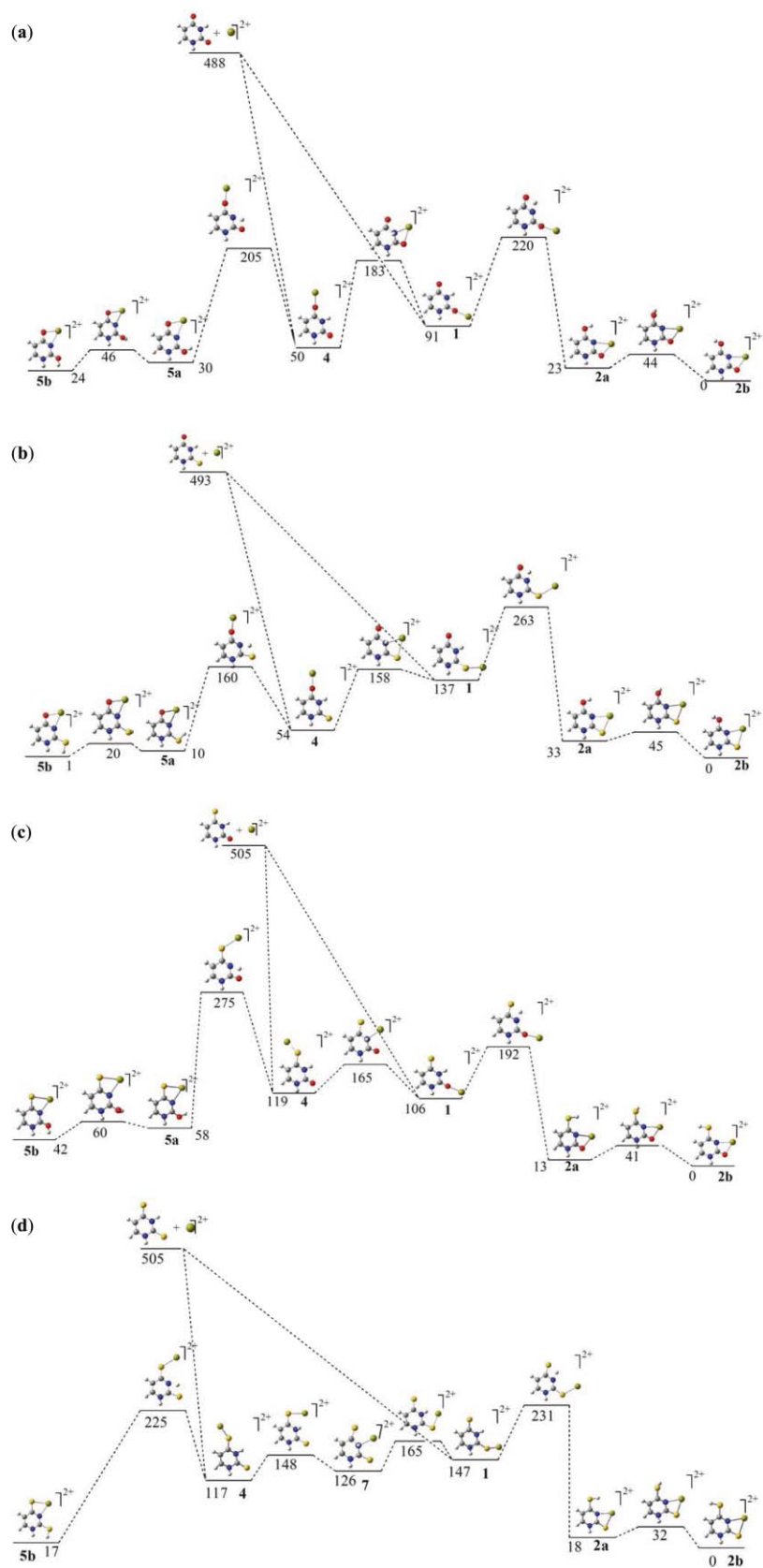
Tautomerization processes. Catalytic effect of Ca^{2+} association

Since uracil and its thioderivatives only exist as dioxo and oxo–thione forms in the gas phase, only complexes **1**, **4** and **7** can be formed by direct association of Ca^{2+} to the available basic sites. However, among all the possible tautomers included in Fig. 1, the aforementioned adducts are the least stable complexes (see Table 3 and Fig. 4), which means that Ca^{2+} association completely alters the stability trends observed for the isolated neutrals. This had been already pointed out for uracil by Russo *et al.*,[16] even though the most stable tautomeric form (**2b**) was not considered in that study.

Table 3 Relative energies (kJ mol^{-1}) of the different tautomers of uracil– and thiouracil–Ca^{2+} complexes

Tautomer	Uracil	2-Thiouracil	4-Thiouracil	2,4-Dithiouracil
1	91	137	106	147
2a	23	33	13	18
2b	0	0	0	0
3a	30	31	8	5
3b	14	16	4	2
4	50	54	119	117
5a	30	10	58	—
5b	30	0.4	42	17[a]
6a	118	66	137	131
6b	120	78	174	122
7	—	—	—	126

[a] For 2,4-dithiouracil structure **5a** is not a stationary point of the PES and collapses to structure **5b**.

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Fig. 4 Energy profiles for the isomerization process of: a) uracil–Ca²⁺ adducts; b) 2-thiouracil–Ca²⁺ adducts; c) 4-thiouracil–Ca²⁺ adducts; d) 2,4-dithiouracil–Ca²⁺ adducts. Relative energies are in kJ mol⁻¹.

The formation of enol or enethiol forms by 1,3H shifts from one of the NH groups toward X or Y, facilitates the interaction of the metal dication with the N-pyridine-like nitrogen atoms, which have a rather big intrinsic basicity, and with the neighbor X or Y heteroatom. The possibility of polarizing two basic sites simultaneously enhances the stability of these complexes which become more stable than adducts 1, 4 or 7. Actually, tautomers 6 in which this kind of arrangement is not possible are the least stable structures (see Table 3). Furthermore, as it has been found before for thymine-M^{2+} (M = Ni, Cu, Zn) complexes,[46] the aromatization of the enolic ring is an additional factor which contributes a lot to the stabilization of the enol or enethiol forms. This aromatization is clearly reflected in the equalization of the bond distances on going from adduct 1 to complex 2b, which is the global minimum of the Ca^{2+}–uracil and Ca^{2+}–thiouracils PESs. Coherently, there is a parallel equalization of the electron densities at the bcps, and of the bond orders. Furthermore, the nucleus independent chemical shift (NICS) evaluated in a point 1 Å above the ring center, which can be considered a good index to measure the aromaticity of a system, is for complex 2b twice as large, in absolute value (−4.85 ppm) than for complex 1 (−2.40 ppm). It is worth noting that in all cases the global minimum of the potential energy surface corresponds to structure 2b, in which Ca^{2+} interacts simultaneously with Y and N3, the second more stable being tautomer 3b, in which Ca^{2+} bridges between Y and N1. 2-Thiouracil is an exception to this general trend, because for this compound tautomer 5b, in which the metal interacts with N3 and X is almost degenerate with tautomer 2b. It is interesting to note, that conversely, for 4-thiouracil the energy gap between forms 2b and 5b is the largest of the whole set compounds considered. Both findings ratified nicely the preference of Ca^{2+} to bind oxygen rather than sulfur.

Tautomers 2 and 5 can be directly obtained from adducts 1 and 4 by a single 1,3H transfer, whereas a multi-step mechanism is required to connect 1 and 4 with tautomers 3. The energy profiles associated with the 1→2, 1→4 and 4→5 tautomerization processes have been plotted in Figs. 4a–d. The first important feature is that all tautomerization barriers are well below the entrance channel. This means that the complex formed by addition of Ca^{2+} to any of the oxo or thione forms of the compounds under scrutiny will have enough internal energy to overpass these barriers. The important consequence is that, although for the neutral systems only the oxo–thione forms are to be found in the gas-phase, for the Ca^{2+} complexes, all tautomeric forms are energetically accessible, the enol/enethiol forms being the most stable. Consequently, these tautomeric forms might be formed experimentally in the gas phase, notably during the electrospray ionization process preceding mass spectrometry analysis, as recently demonstrated for protonated uracil.[68] Also importantly, the activation barriers involved in these tautomerization processes are much lower than those calculated for the isolated neutral compounds.[35] For example, for 2-thiouracil the 1–2 tautomerization barrier decreases 28%, whereas the 4–5 decreases 39%. For 4- and 2,4-dithiouracil these decreases are 47% and 37%, respectively for the 1–2 tautomerization process and 39% and 35%, respectively for the 4–5 tautomerization process.

Conclusions

From our theoretical survey of the interaction between uracil and its thio-derivatives with Ca^{2+} in the gas phase, we can conclude that:

(a) For uracil and 2,4-dithiouracil, where the two basic sites are the same, Ca^{2+} attachment to the heteroatom at position 4 is preferred, due essentially to a significant electron delocalization within the six-membered ring when the metal binds to X, which is not observed when it is attached to Y.

(b) For those systems where both types of basic centers, a carbonyl or a thiocarbonyl group, are present, Ca^{2+} association to the oxygen is always favored with respect to association to sulfur.

(c) The most stable complex corresponds systematically to structure 2b, in which the metal dication bridges between Y of the 4-enol (or the 4-enethiol) tautomer and the dehydrogenated ring nitrogen, N3. The enhanced stability of these enol forms is two-fold. On the one hand, the structure of the enol (enethiol) facilitates the interaction of Ca^{2+} with the N-pyridine-like nitrogen atom and with the neighbor X or Y heteroatom, polarizing both basic sites simultaneously. On the other hand, a significant aromatization of the six-membered ring takes place.

(d) Ca^{2+} association has a clear catalytic effect on the tautomerization processes which connect the oxo–thione forms with the enol–enethiol tautomers. Hence, although the enol–enethiol tautomers of uracil and its thio derivatives should not be observed in the gas phase, the corresponding Ca^{2+} complexes are the most stable species and might be accessible experimentally, because the tautomerization barriers are smaller than the Ca^{2+} binding energies.

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