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Is Se–Se bond cleavage the most favourable process in electron attachment to diselenides? The importance of asymmetry†‡

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Accurate *ab initio* calculations reveal that, in contrast with what has been commonly assumed up to now, electron attachment to diselenides does not always result in a Se–Se bond fission and, in general, the mechanism behind the bond fission may involve the crossing of several states.

Selenium-containing proteins have lately received an increased interest as they have been identified in a large range of living beings.[1] They play important and diverse roles, like antioxidant activity, cancer prevention or inflammation protection.[2–4] Besides, selenoproteins appear as mutants of sulfur-containing proteins due to the close physicochemical properties of sulfur and selenium. Indeed, the substitution of cysteine (Cys) by selenocysteine (Sec) has been studied in a large variety of situations,[5–9] where little structural distortion and full biological activity of the variants were reported in all cases. Consequently, due to the importance of disulfide bridges for the structure[10–14] and enzymatic activity[14,15] of proteins, some investigation has been devoted to the study of diselenide bonds, spurred by the discovery of the natural occurrence of these unions in proteins.[16]

Nevertheless, diselenides are also important for their high antioxidant activity,[17,18] even higher than that of disulfides. Some studies[19] have been devoted to understand this enhancement and the mechanism of the reductive cleavage of the Se–Se bond, although they do not provide enough evidence to fully understand these questions. Although the electron capture dissociation (ECD) has been largely investigated for disulfides[20–25] it has been only recently that its general mechanism has been fully described.[26] Not surprisingly much less attention was devoted to Se–Se bond fission by electron capture to diselenides. Some studies carried out on dimethyldiselenide[27,28] indicate that electron attachment yields mainly the breaking of the Se–Se bond since the extra electron is accommodated in the σ^*(Se–Se) antibonding orbital, like for disulfides. However, the fact that both substituents attached to Se are identical prevents from

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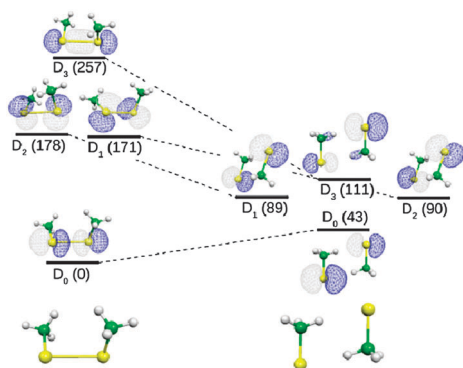
getting a general picture of the electron capture process in these systems. It seems obvious that the effect of asymmetry or the different electronegativity of the substituents attached to the Se–Se bridge, which have never been considered before, would distort the electronic density of the linkage and, consequently, influence the electron capture process and subsequent dissociation.[26] The aim of this communication is to show, precisely, that in most of the cases the Se–Se bond cleavage is neither adiabatic nor the most favourable process, in clear contrast with what was previously assumed.

To study the effect of asymmetry and the electronegativity of the substituents on the cleavage of the Se–Se bond, the $CH_3SeSeCH_3$, $CH_3SeSeNH_2$, CH_3SeSeF derivatives were investigated. To build up the necessary correlation diagrams to determine the non-adiabaticity of the reactions the CASSCF/MS-CASPT2 multireference approach was used. Final energies of the stationary points of the potential energy surfaces were obtained at the CCSD(T)/aug-cc-pVTZ level. Further computational details are available in the ESI.‡

Previous studies on electron capture of diselenides or their reactions with nucleophiles focused on the breaking of the Se–Se linkage. To investigate the effect of symmetry in this process we are going to compare two systems, $CH_3SeSeCH_3$ and $CH_3SeSeNH_2$. Adding an electron to these molecules causes a remarkable increase of the Se–Se distance, while the other bonds remain practically unperturbed[29] (see also Fig. S1, ESI‡). As a result, in the two molecules the cleavage of the Se–Se bond is expected to be the most favourable reaction.

In both anions, the extra electron occupies the σ^*(Se–Se) orbital, so it is fully delocalized between both selenium atoms at the equilibrium conformation. However, this picture has to gradually change as the Se–Se bond is stretched, because in the limit of the Se–Se dissociation the extra electron has to be necessarily localized on a particular fragment. This means that the dissociation of the Se–Se bond may require the interaction of at least two states, as later discussed. Fig. 1 represents a correlation diagram of the electronic states of the $[CH_3SeSeCH_3]^-$ anion (left-hand side of the figure) and the post-dissociation complex (right-hand side), formed by the weak interaction of the two SeCH$_3$ moieties produced by the Se–Se bond cleavage. For the $[CH_3SeSeCH_3]^-$ anion, the four D_0–D_3 states allocate the extra electron in orbitals arising from different combinations of two of the 4p orbitals of Se. In particular, the singly occupied molecular orbital (SOMO) for

Fig. 1 Correlation diagram of the electronic states of the [CH$_3$SeSeCH$_3$]$^-$ anion (left-hand side) and the post-dissociation minimum (right-hand side), where each state is represented by its single occupied molecular orbital (SOMO). Numbers in parenthesis correspond to MS-CASPT2/aug-cc-pVTZ relative energies (in kJ mol^{-1}).

Fig. 2 Correlation diagram of the electronic states of the [CH$_3$SeSeNH$_2$]$^-$ anion (left-hand side), the post-dissociation minimum (right-hand side) and the MECP (middle part) connecting them. Each state is characterized by its SOMO and its relative energy (in kJ mol^{-1}) in parenthesis calculated at the MS-CASPT2/aug-cc-pVTZ level.

the ground state, D$_0$, is an antibonding σ*(Se–Se) combination, whereas for D$_3$ the SOMO is the bonding σ(Se–Se) combination. As the Se–Se bond is stretched to reach the post-dissociation minimum, the four states come closer in energy, because the four D$_0$–D$_3$ states should become strictly degenerate at infinite Se–Se distance, since both SeCH$_3$ fragments have two symmetry-equivalent 4p orbitals located on the selenium atoms, and by symmetry both moieties are identical. This implies that for [CH$_3$SeSeCH$_3$]$^-$ anion the Se–Se bond cleavage is a barrierless adiabatic process, where only the bond dissociation energy matters.

The picture of the Se–Se bond dissociation changes, however, when the system is no longer symmetric, like in CH$_3$SeSeNH$_2$. In this case, the most favourable dissociation yields [SeCH$_3$]$^-$ + [SeNH$_2$]$^\bullet$, while the [SeCH$_3$]$^\bullet$ + [SeNH$_2$]$^-$ products lie 40 kJ mol^{-1} higher in energy. This result seems to be somewhat counterintuitive because of the higher electronegativity of nitrogen with respect to carbon. It should be taken into account, however, that the electron affinity (EA) of [SeCH$_3$]$^\bullet$ is higher than that of [SeNH$_2$]$^\bullet$. In [SeCH$_3$]$^\bullet$ the LUMO is a non-bonding 4p AO of selenium, while for [SeNH$_2$]$^\bullet$, the LUMO is a π*(Se–N) antibonding MO arising by the resonance between the 4p AO of Se and the lone pair of N, which necessarily rises the energy of the LUMO, decreasing the EA of the system. As a result, the breaking of the diselenide bond in [CH$_3$SeSeNH$_2$]$^-$ localizes the extra charge in the SeCH$_3$ fragment. Importantly however, the [SeCH$_3$]$^-$ + [SeNH$_2$]$^\bullet$ products cannot be formed through an adiabatic process, because in D$_0$ the electron is delocalized between the two moieties. Hence, the dissociation occurs, as illustrated in the correlation diagram of Fig. 2, through a diabatic process where the D$_3$ state, whose SOMO is a σ(Se–Se) bonding MO, interacts with the ground state D$_0$ as the Se–Se distance increases. Upon this interaction the σ*(Se–Se) and σ(Se–Se) SOMOs combine and recover the two p orbitals on each selenium, allowing a charge localization on a particular fragment. This is indeed the situation found in the minimum energy crossing point (MECP) structure, shown in the middle part of Fig. 2, where the two couples of states (D$_0$/D$_1$ and D$_2$/D$_3$) locating the extra charge at different fragments are nearly degenerate. On their evolution towards to the products, these
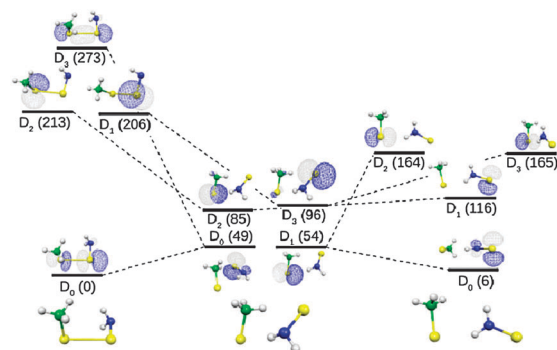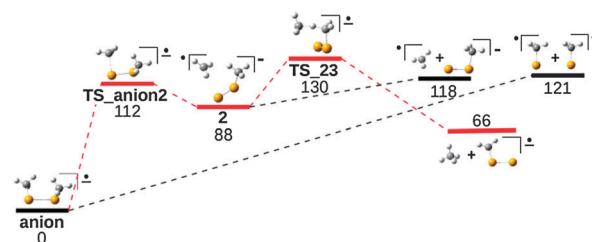
states split apart as a consequence of the enhanced stability of the [SeCH$_3$]$^-$ + [SeNH$_2$]$^\bullet$ products.

The most striking result of our theoretical study is, however, that for both anions, [CH$_3$SeSeCH$_3$]$^-$ and [CH$_3$SeSeNH$_2$]$^-$, the most stable products from the thermodynamic point of view are not the products arising from a Se–Se bond fission, but the release of neutral molecules: CH$_4$ and NH$_3$. However, as illustrated in Fig. 3 for X = CH$_3$ (Fig. S2, ESI‡ for X = NH$_2$), these products are obtained through multi-step hydrogen shifts involving high activation barriers, so that the fission of the Se–Se bond seems to be more favourable, as experimentally found.[27,28] Besides, since all transition states and final products are formed by the interaction of an anion and a neutral radical, the effect of solvation should not change this picture significantly.

The effect of the electronegativity of X on the electron capture of diselenides is dramatic, as easily illustrated by the behaviour of CH$_3$SeSeF as a suitable benchmark case. Contrary to what has been previously assumed, when X is highly electronegative, the electron capture does not cause the activation of the Se–Se bond, but that of the Se–X linkage. In fact, for the anion [CH$_3$SeSeF]$^-$ the Se–F distance is extremely large (2.065 Å), while the other bonds are rather unperturbed (Fig. S1, ESI‡). This is so because highly electronegative substituents significantly stabilise the σ*(Se–X) antibonding orbital (orbital energy 0.1185 E_h) with respect to the σ*(Se–Se) (orbital energy 0.4428 E_h), strongly favouring the population of the former. This causes the [SeCH$_3$]$^\bullet$ + [SeF]$^-$ dissociation limit to be higher in energy (25 kJ mol^{-1}) than the [CH$_3$SeSe]$^\bullet$ + F$^-$ dissociation limit. More importantly, the energy barrier

Fig. 3 Energy profiles of the main fragmentations of [CH$_3$SeSeCH$_3$]$^-$. The loss of CH$_4$ is highlighted in red. Relative ΔH (in kJ mol^{-1}) calculated at the CCSD(T)/aug-cc-pVTZ level are also displayed.
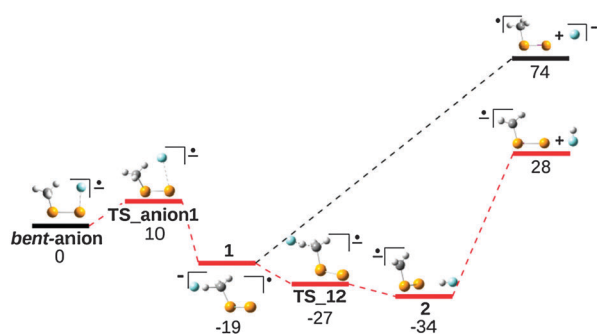
Fig. 4 Energy profiles of the main fragmentations of $[CH_3SeSeF]^-$. The loss of HF is highlighted in red. Relative ΔH (in kJ mol^{-1}) calculated at the CCSD(T)/aug-cc-pVTZ level are displayed under each structure.

associated to the cleavage of the Se–F bond, which connects the *bent-anion* with structure **1** (see Fig. 4), is quite low. The interaction between the F$^-$ and one of the hydrogen atoms of the methyl group in **1** leads to the formation of a HF molecule and to its eventual loss in a practically barrierless process.§ Hence, in this particular case the loss of HF is not only the most favourable process from the thermodynamic point of view, but also the most favourable process from a kinetic viewpoint.

A comparison of diselenides with disulfides[26] shows that the mechanisms associated with the A–A bond cleavage in ECD processes for $[CH_3AACH_3]^-$ and $[CH_3AANH_2]^-$ (A = S, Se) are alike, although the energy barriers and bond dissociation energies are higher for diselenides (Table 1). Experimentally, diselenides are better reductants than disulfides,[17,18,30] suggesting that the ejection of the extra electron must compete with ECD.

As a matter of fact, Table 1 also shows that the EAs of the neutral molecules are smaller than the energy required to break the A–A bond, and therefore the electron detachment process clearly competes with the ECD process. Consequently, a complete picture of the reductive cleavage of these linkages should take into account the electron detachment reaction, which is significantly less favorable for diselenides than for disulfides, what would be coherent with the enhanced reductant ability of the former.

In summary, the Se–Se bond cleavage, which was commonly assumed to be the only reactive process in electron attachment to XSeSeX′ diselenides, is only the most favourable process when the substituents X and X′ are not very electronegative, and the mechanism behind is rarely adiabatic. Hence, although in proteins presenting Se–Se bridges the most favorable

process, like in disulfide bridges, would be the Se–Se bond cleavage, the process should involve at least two states because the environment of the diselenide bridge is rarely symmetric.

Notes and references

§ The transition state associated with the hydrogen transfer, **TS_12**, has a lower enthalpy than the reactants, **1**, due to the smaller ZPE correction for transition states. Indeed, the electronic energy of **TS_12** is slightly larger than that of **1**, pointing anyway to a barrierless reaction. Once the proton is transferred, complex **2** is formed, where the $[CH_2SeSe]^-$ and HF moieties interact by means of charge–dipole interactions.

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Table 1 Reaction barriers and dissociation energies (DE) for the ECD of A–A bond in $CH_3AAX'H_n$ (A = S, Se; X′ = C, N). The adiabatic electron affinity (EA) of the neutral is also displayed. All values in kJ mol^{-1}.

		Barriers	DE	EA
C	S		83[a]	23[b]
	Se		121	53[c]
N	S	33[a]	35[a]	0[b]
	Se	48	93	49[c]

[a] Theoretical values taken from ref. 26. [b] Theoretical values taken from ref. 31. [c] Theoretical values taken from ref. 29.