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Electron capture dissociation of the disulfide bond—a quantum chemical model study

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Dedicated to Alan Marshall

Abstract

Four possible mechanism for ECD of disulfide bonds have been assessed using quantum chemical methods (B3LYP, G2, CASSCF and QCISD). For protonated peptides and proteins interacting with zero-energy electrons, the two most likely mechanisms appear to be either spontaneous dissociation of the S–S bond upon impact of a slowly moving free hydrogen or induced by uptake of a nascent hydrogen radical initially in close contact through a hydrogen bond.

Keywords: ECD; Disulfide bond; Zero-energy electrons

1. Introduction

A few years ago, Zubarev and co-workers [1–5] introduced a new method for primary structure elucidation of peptides/proteins, electron capture dissociation (ECD). By interaction in the gas phase between thermal electrons and multiply protonated peptides/proteins (formed by electrospray ionization), efficient and specific backbone cleavage of the molecules is observed. It is noticeable that it is the N–Cα bonds, and not the peptide bonds, which break up in the ECD process. The mechanism has been investigated, and it now appears that the considerable energy deposition (recombination energy: 3–5 eV) at the –C(OH)-radical site combined with the significant weakening of the N–Cα bond next to it is the driving force of the reaction as illustrated in Scheme 1 [6–9]. Experimental evidence and theoretical model calculations point towards fast energy conversion and bond dissociation—at least for a fraction of the molecules.

Another feature of ECD is its capacity to break disulfide bonds. This is also a highly specific process, and very useful for protein analysis, since individual peptide chains linked by S–S bonds become separated. The mechanism is of yet not clearly understood. It has been pointed out that the sulphur atom of a disulfide bridge of a protein is not sufficiently basic to be protonated [2]. Instead protons will be associated with terminal amino groups with far higher proton affinities. This would not be compatible with a mechanism of the type depicted in Scheme 1, and alternative mechanisms have been suggested: both a "hot hydrogen" mechanism [2] and direct dissociative electron attachment [10].

In this paper, we will look closer at the suggested mechanisms, including a situation analogous to that illustrated in Scheme 1. In order to provide substance to our discussion, we have carried out quantum chemical calculations of suitable model systems (see Table 1).

2. Methods

All quantum chemical calculations were done using Gaussian 98 [11]. The hybrid 3-parameter density functional theory method according to Becke, Lee, Yang and Parr (B3LYP) [12] with the 6-31G(d) atomic basis [13] set was used. All stationary points were subject to complete geometry optimization. The optimized structures were checked for the correct number of negative eigenvalues of the Hessian (the second derivative matrix). Analytical force constants were computed at this stage and the vibrational frequencies were obtained together with the rotational constants. These molecular parameters were used within the

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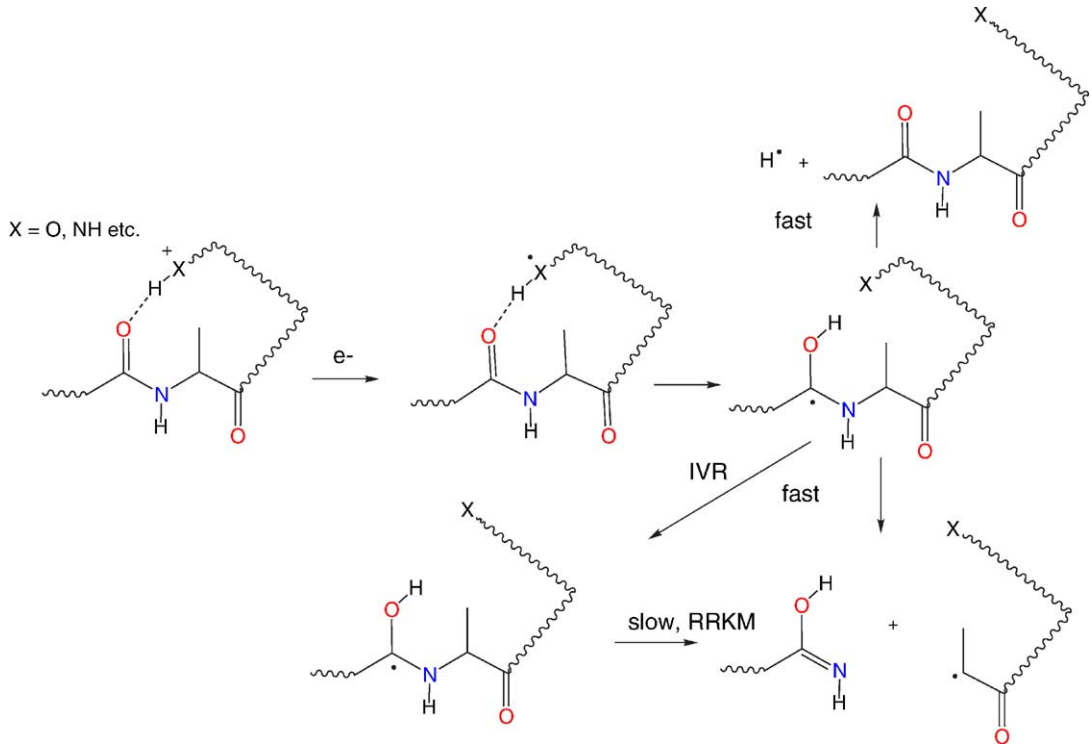

Scheme 1.

framework of the rigid-rotor/harmonic-oscillator approximation without correction factors to calculate the absolute zero-point vibrational energies (ZPVEs) and thermochemical quantities. In addition, the smaller molecules were also subject to G2 theory [14] calculations to obtain more accurate energy estimates. G2 theory is a compound technique, which involves initial geometry optimizations at the HF/6-31G(d) level and subsequent calculation of ZPVEs at the same level of theory. Then the geometry is re-optimized at the MP2(full)/6-31G(d) level, whereupon a number of single-point MP2, MP4 and QCISD(T) calculations are performed in order to obtain an energy estimate, which is effectively at the QCISD(T)/6-311+G(3df,2p) level. In one case, CASSCF and QCISD calculations were conducted in order to validate the use of a single determinant zeroth-order wave function.

Table 1
Energies from quantum chemical calculations

Molecule	B3LYP/6-31G(d) electronic energy (hartree)	B3LYP/6-31G(d) zero-point vibrational energy (hartree)	G2 (0 K) energy (hartree)
H (**1**)	−0.500273		−0.499810
HSSH (**2**)	−797.571776	0.018242	−796.671874
$HS^{\bullet}\cdots SH_2$ (**3**)	−798.131730	0.023799	−797.219424
$HS^{\bullet}$ (**4**)	−398.740028	0.006097	−398.286972
H_2S (**5**)	−399.385436	0.015172	−398.930707
$[HS^{\bullet}\cdots SH]^{-}$ (**6**)	−797.599511	0.014525	−796.683436
SH^{-} (**7**)	−398.806252	0.005865	−398.371583
CH_3SSCH_3 (**8**)	−876.207515	0.077942	−875.124551
$[CH_3S(SH)CH_3]^{+}$ (9)[a]	−876.516374	0.087953	−875.428652
$CH_3S\cdots HSCH_3$ (**10**)[a]	−876.762296	0.084737	−875.663093
10@**9**[a]	−876.687324		
CH_3S (**11**)	−438.059725	0.035850	−437.511253
CH_3SH (**12**)	−438.698347	0.046420	−438.148469
$CH_3NH_3^{+}\cdots(CH_3SSCH_3)$ (**13**)	972.450348	0.158656	–
$CH_3NH_3^{\bullet}\cdots(CH_3S\cdots SCH_3)$ (**14**)	−972.623694	0.153841	–
14@**13**	−972.542697		–
$(CH_3NH_2)\cdots(HS(CH_3)\cdots{}^{\bullet}SCH_3)$ (**15**)	−972.628030	0.151588	–
TS (**14**) → (**15**)	−972.621856	0.150028	–
CH_3NH_2 (**16**)	−95.853205	0.064430	–

[a] QCISD(T,E4T)/6-311G(d,p)//MP2(fu)/6-31G(d) energies are **9**: −875.2833551, **10**@**9**: −875.3983232, **10**: −875.5206172.

3. Results

3.1. The "hot hydrogen" model

Addition of a free hydrogen atom to the oxygen atom of an amide functional group requires passage of a potential energy barrier of approximately 50 kJ mol^{-1} [6]. This implies that a hydrogen must have at least this amount of translational energy to overcome the barrier. However, dynamical calculations have revealed that fast hydrogen atoms (with energy in the range 50–500 kJ mol^{-1}) do not induce N–Cα dissociation, but instead bounce off [9]. The "hot hydrogen" model for ECD of the peptide backbone has therefore been disputed.

On the basis of a quantum chemical calculation of the H atom adduct of CH_3SSCH_3, McLafferty and co-workers concluded that disulfide bridges dissociate spontaneously upon impact with free hydrogens [2]. It is not clear if these workers investigated the possible existence of a barrier in forming the association complex. To find out if this is the case, we conducted some simple tests. Our first test system was H (**1**) + H–S–S–H (**2**), for which we performed a series of B3LYP calculations where the incoming hydrogen atom was placed at a given distance from one of the sulphur atoms. This S–H distance was varied in small steps, and geometry optimization was conducted for each fixed value of S–H. The result is depicted in Fig. 1. We observe that the potential energy drops monotonically upon approach of the hydrogen, demonstrating there is no barrier associated with addition of a hydrogen to an S–S bridge (Fig. 1a). We also notice that the S–S bond distance increases in concert with shortening of the S–H bond (Fig. 1b). The potential energy minimum of Fig. 1a corresponds to the molecule $HSSH_2$ (**3**), which has an S–S distance of 3.082 and 4.193 Å with B3LYP/6-31G(d) and MP2/6-31G(d), respectively (the latter method is used for geometries within G2). The corresponding figures for HSSH (**2**) are 2.098 and 2.069 Å. It is therefore more correct to denote the former structure by $HS^{\bullet}\cdots SH_2$ (**3**).

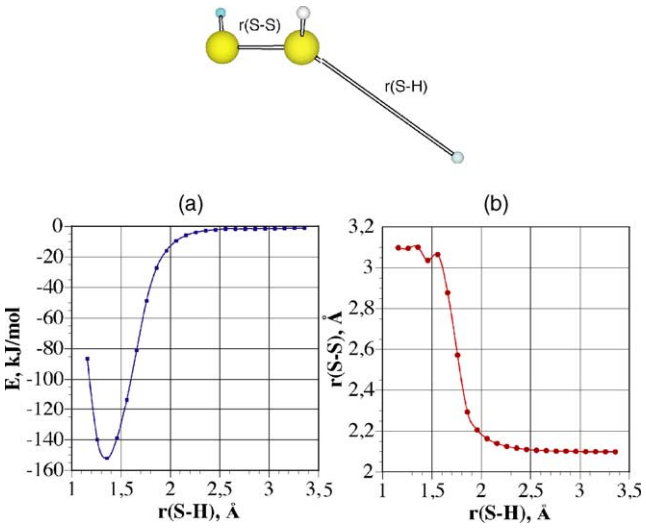

Fig. 1. Response on decreasing the S–H bond length upon (a) electron energy and (b) the length of the S–S bond. The data are from B3LYP/6-31G(d) calculations executed as explained in the text.

Despite the fact that the unrestricted spin procedures used showed negligible spin contamination of the final functional/function, we decided to conduct CASSCF calculations in order to find out if static electron correlation problems could induce artefacts in the computational results of this open shell molecule. Full geometry optimization of **3** starting with an S–S distance of 2.10 Å using a CASSCF(3,4)/6-31G(d) scheme ended up at 4.293 Å. The largest eigenvalue of the CI-matrix did not drop below 0.995 at any point along the geometry optimization path. It is also worth mentioning that B3LYP and G2 calculations showed that the quartet state of $HSSH_2$ is of no importance, since it is substantially higher in energy.

Dissociation of the complex **3** into its constituents $HS^{\bullet}$ (**4**) + H_2S (**5**) requires only 10 kJ mol^{-1} (B3LYP/6-31G(d)), alternatively 5 kJ mol^{-1} (G2).

The relevance of the these findings is clear, since they support the idea that hydrogen atom capture can lead directly to disulfide cleavage. It has been demonstrated both experimentally [15] and by quantum chemical model calculations [2,6,9] that ECD, in addition to giving the aforementioned cleavages, also gives rise to liberated hydrogen atoms. These atoms have translational energy probably ranging from near thermal to several hundreds kilo joules per mole [9]. Slow hydrogens are probably more effective in inducing S–S bond cleavage than the fast ones, since the latter are more likely to give non-reactive scattering.

3.2. Dissociative electron attachment

Electron-stimulated desorption from dimethyl disulfide adsorbed on a surface at 90 K has shown production of CH_3S^- down to a few electron volts electron energy [16]. Despite the fact that the cross-section for S–S cleavage approaches zero at 0 eV, and therefore should be of relatively little importance for "true" ECD, we think that this observation could explain observations of S–S cleavage where ECD has been conducted with electrons having energies of a few or some electron volts—working conditions intentionally or unintentionally probably used in many laboratories.

Geometry optimization of the anion of hydrogen disulfide starting at an S–S distance of 2.1 Å gave rise to equilibrium geometries with r(S–S, B3LYP/6-31G(d,p)) = 2.952 Å and r(S) = 2.952S, MP2/6-31G(d,p)) = 2.828 Å. The corresponding figure obtained for QCISD/6-311+G(2d,p) is 2.796 Å. The geometry is therefore best described as $[HS\cdots SH]^-$ (**6**). The dissociation energies for producing SH^- (**7**) + $HS^{\bullet}$ (**4**) are 133 kJ mol^{-1} (B3LYP/6-31G(d)) and 65 kJ mol^{-1} (G2), substantially higher than that of **3**. For comparison, the B3LYP electronic energy of $HSSH^-$ calculated at the geometry of HSSH is 190 kJ mol^{-1} above $[HS\cdots SH]^-$ (**6**). This shows that the thermochemistry allows for dissociation down to 0 eV. In addition, the LUMO

is at slightly negative energy, which could indicate dissociative electron attachment down to this threshold. However, as already mentioned, the experiment on the homologue CH_3SSCH_3 shows no signal for CH_3S^- down towards zero, so the cross-section must be extremely low at low electron energies.

3.3. Proton affinity of the disulfide bond

The proton affinity of dimethyl disulfide (**8**) has been determined to be $PA(CH_3SSCH_3) = 815\,kJ\,mol^{-1}$ by ICR bracketing [17,18]. Our calculated G2 value is PA = $803\,kJ\,mol^{-1}$. These values are considerably lower than that of an amide representative for a longer peptide chain, PA(*N*-methyl acetamide) = $889\,kJ\,mol^{-1}$. This shows that protonation is preferred at amide oxygens when compared to the sulphurs of disulfide bridges. It should, however, be realized that the most basic sites of most peptides are the nitrogens of basic side groups, for example reflected in PA(histidine) = $934\,kJ\,mol^{-1}$ [19]. In this situation, with protons attached to other basic groups, the role of the basic amido-oxygens is to act as hydrogen bond acceptors. Consequently, intramolecular hydrogen bonds of the type required for N–Cα bond cleavage (Scheme 1) are numerous in proteins and peptides. An analogous situation seems not to be typical for S–S bonds. Random inspection of crystallographic databases of proteins and peptides does not reveal many examples of contacts of this type. On the other hand, the chance for finding relatively stable $X–H^+\cdots S$ interactions should increase with multiple protonation.

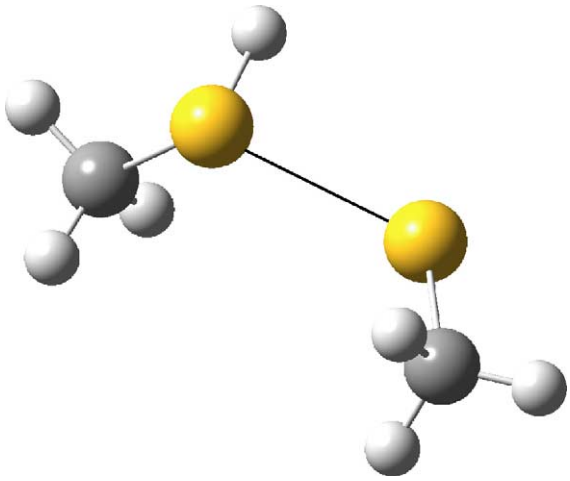

Fig. 2. Molecular structure of the intermediate species **11** formed upon electron capture by protonated dimethyl disulfide. The distance between the sulphur atoms increases by 1 Å by this, and the intermediate will dissociate swiftly into its components.

3.4. Recombination of protonated dimethyl disulfide

The vertical recombination energy computed for $CH_3SS(H)CH_3^+$ (**9**) + e^- → $CH_3SS(H)CH_3^\bullet$ (**10@9**) with QCISD(T,E4T)/6-311G(d,p)//MP2(fu)/6-31G(d) is $302\,kJ\,mol^{-1}$ (Fig. 2). Geometry optimization of $CH_3SS(H)CH_3^\bullet$ resulted in a weakly bonded complex $CH_3S^\bullet\cdots HSCH_3$ (**10**) with both B3LYP and MP2. This species dissociates extremely easily to give CH_3S (**11**) + CH_3SH (**12**), requiring $6\,kJ\,mol^{-1}$ (B3LYP/6-31G(d)) and $9\,kJ\,mol^{-1}$ (G2). The relaxation energy (QCISD) for **10@9** → **10**

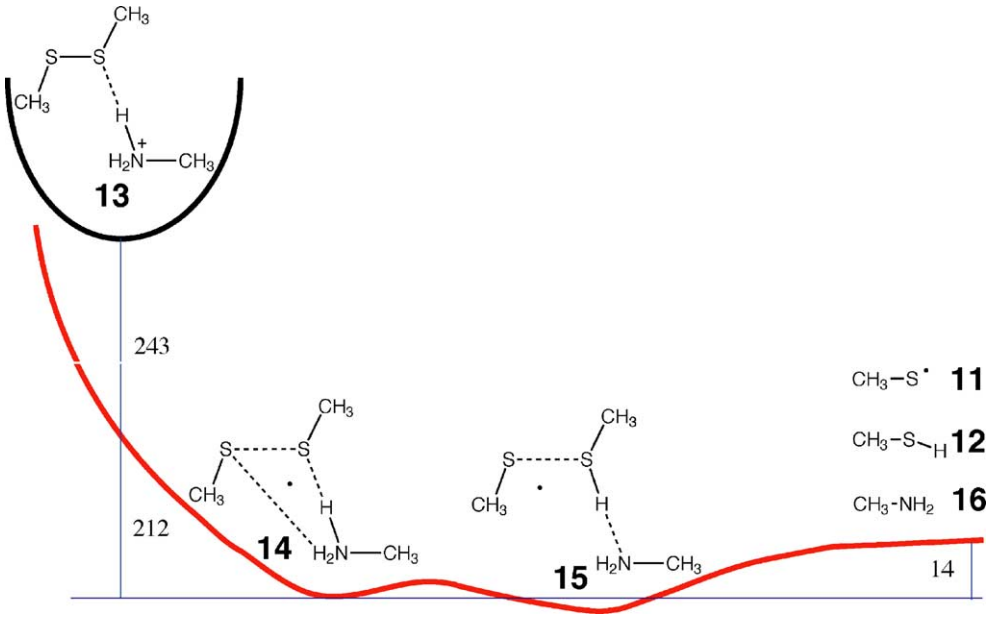

Fig. 3. Potential energy diagrams from B3LYP/6-31G(d) calculations. Of a model constructed to represent a hydrogen bonded disulfide bridge. The upper curve shows the initial situation, while the lower curve shows the anticipated behaviour upon ECD. As indicated the model predicts straightforward dissociation of the disulfide bridge.

is 321 kJ mol^{-1}. As we see, electron capture dissociation of protonated dihydrogensulfide and protonated dimethyl disulfide are very similar.

3.5. Recombination of intramolecular hydrogen bond model

As already described, direct protonation of the disulfide bond in a protein is unlikely. Instead, we will be interested in the more realistic situation where a sulphur acts as a hydrogen bond acceptor. In order to consider this situation, we have studied the complex (**13**) between protonated methyl amine (mimic of an ammonium site) and dimethyl disulfide (mimic of disulfide bridge). Geometry optimization shows that this complex is stabilized by a pertinent hydrogen bond, as anticipated (Fig. 3). Recombination with an electron has interesting consequences. Firstly, upon charge neutralization, the disulfide bond breaks open and a tricomplex $CH_3NH_3^{\bullet} \cdots (CH_3S \cdots SCH_3)$ (**14**) is formed. Secondly, although the proton affinity of methyl amine is higher than of dimethyl disulfide, the hydrogen atom affinity order is the opposite. The linking hydrogen moves from the nitrogen towards sulphur almost with no barrier to give $(CH_3NH_2) \cdots (HS(CH_3) \cdots {}^{\bullet}SCH_3)$ (**15**) via TS (**14**) → (**15**). In an analogous case studied by Turecek, there is a negligible barrier of 1 kJ mol^{-1} for spontaneous transfer of a hydrogen radical from an amino nitrogen to the oxygen of an amide within an intramolecular complex [7].

As is clear from Fig. 3, the result of ECD of **13** will be immediate formation of the three fragments CH_3S (**11**) + CH_3SH (**12**) + CH_3NH_2 (**14**) as soon as the ground state potential energy surface is hit. The shallow minima (**14**) and (**15**) are only transient intermediates.

4. Discussion and conclusion

This study shows simulations of four possible scenarios for reduction processes relevant to ECD of disulfide bonds, namely: (i) simultaneous addition of a proton and an electron in the form of a free hydrogen radical; (ii) addition of an electron; (iii) addition of a proton followed by addition of an electron; and (iv) addition of an electron to a disulfide bond, which has a hydrogen bonded contact to a proton. As we have seen, all scenarios are possible ways for breaking an S–S bond. Due to the aforementioned poor basicity, we regard mechanism (iii) to be of least relevance to ECD. We consider the related mechanism (iv) to be a more realistic alternative, as already discussed. Mechanism (ii) is a likely explanation of S–S cleavage upon 3–10 eV electron impact, but experimental evidence points to zero cross-section for 0 eV electrons. The electron attraction of a protonated molecule will be higher than that of a neutral. If this affects the cross-section for S–S cleavage in cases where the protons are remote is uncertain. Our over-all judgement is therefore that besides (iv), the most likely mechanism is (i). There is clear evidence for formation of free hydrogens upon ECD. The relative importance of (i) and (iv) will to a large degree depend on the detailed conformation of the macromolecule, in particular, the position of the S–S bond relative to acidic hydrogens, whether free or H-bonded.

Finally, we note the relevance of these calculations to solution red/ox chemistry of thiols and disulfides. A classical wet chemistry method of cleaving S–S bonds has been the use of a suitable reductant, e.g. Zn in acidic solution (nascent hydrogen).

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