Computational insights into the formation and nature of the sulfilimine bond in collagen-IV†

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Collagen IV is essential component of basement membrane in the tissues. It provides proper cellular structure by the formation of sulfilimine bond (S= N) between methionine and lysine or hydroxylysine (cross-links) residues which can be formed with or without post-translational modification. The sulfilimine bond has critical roles in tissue development and human diseases. Peroxidasin, a basement membrane peroxidase, generates reactive halogen species including hypobromous (HOBr) and hypochlorous (HOCl) acid which help to form halosulfonium or haloamine. The sulfilamine bond can be formed either by the formation of halosulfonium or by the formation of haloamine. The aim of the study is the investigation of the formation of sulfilimine bond and its nature in collagen IV using multi-scale approach that included MD, QM-cluster, systematic series of small models, and NBO analysis. These results suggest that sulfilimine bond can be formed either via brominated/chlorinated halosulfonium or haloamine pathway. The results of systematic series of small model indicate that the formation of sulfilimine complex from halosulfonium happens through the formation of positively charged halosulfonated sulfilimine complex. It also suggests that the formation of sulfilimine complex from haloamine occurs through the formation of positively charged sulfilimine complex where the S and N bond forms and halogen goes off at the same time. Furthermore, the NBO analysis suggest the S and N bond is strongly polarized toward nitrogen in both single protonated and neutral system, N\(^{+}\) \rightarrow S\(^{+}\) and also indicate the existence of a coordinate covalent (i.e. dative) bond.

1 Introduction

The basement membrane is a thin, highly specialized extracellular matrix that is essential for the proper structure and function of tissues. In particular, it provides structural support for epithelial cells and plays important roles in, for example, intracellular signal transduction and cell behavior regulation. Its importance is further underscored by the fact that its improper functioning, due to for instance genetic mutations, can result in a number of severe or even fatal diseases or conditions in organisms. However, despite its physiological importance much remains unclear or even unknown including its structure.

For instance, the most abundant molecular components of basement membranes are the collagen proteins of which there are least 28 sub-types. In particular, type IV Collagen (Collagen IV), discovered in 1966, is the most abundant molecular species of the basement membrane and is a critical component of the lamina densa, a sub-layer of the basement membrane. In particular, the collagen IV forms an essential and complex network that provides a supportive scaffold for other components of the basement membranes. Improper structures and/or defects in collagen IV are associated with several diseases including rheumatological and dermatological diseases. Furthermore, it is believed that the potentially fatal Goodpasture syndrome, a rare autoimmune disease, is thought to result from antibodies attacking certain alpha subunits within collagen IV in the basement membranes of the renal glomeruli and pulmonary alveoli. Another well-known collagen IV related pathology is the genetic disorder Alport's syndrome, caused by mutations within collagen IV, which is associated with proteinuria, progressive renal failure, ocular abnormalities, and hearing loss. In addition, it has been reported that collagen IV also has critical roles in wound healing and embryogenesis.

While the exact structure of collagen IV remains unclear, it is known to be composed of six highly homologous polypeptide chains; \(\alpha1(IV)\) to \(\alpha6(IV)\), with each comprising of an (i) amino-terminal; (ii) triple-helical, and (iii) carboxy-terminal domain. Three \(\alpha\)-chains bind together to form a triple helix, a protomer, which then aggregate end to end to form a hexamer and ultimately collagen fibrils. It is known that collagen IV can undergo several post-translational modifications and that protomers covalently cross-link through a variety of enzymatic and non-enzymatic processes. While these are often to help provide

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the structural integrity of the collagen IV network, some cross-links have also been associated with aging and disease. However, in 2009 it was discovered that covalent cross-links could also form between an hydroxysine (Hyl) or lysine (Lys) residue in one chain with a Methionine (Met) residue in another. It is noted that this also indicates that post-translational modifications are not essential for the formation of these cross-links. More specifically, this cross-linking involved the side-chain amine of Hyl/Lys and the sulfur of Met and led to the formation of a sulfilmine (S=\(\equiv\)N) crosslink. Furthermore, such inter-residue bonds are rare in biochemistry; so far the only known natural occurrence is in collagen IV. Furthermore, it is held that the sulfilmine bond of collagen IV was essential for evolution of tissues and eumetazoa.

Recently, experimental studies have suggested that the sulfilmine bond formation in collagen IV may be induced by the hypohalous acids, hypobromous (HOBr) and hypochlorous (HOCl) acid, though their exact roles remain unclear. Furthermore, the efficiency of HOBr facilitated sulfilmine bond formation is 50 000-fold greater than for HOCl. These acids are produced by peroxidasin, a multifunctional extracellular matrix-associated member of the heme peroxidase family.

Based on experimental observations it has been proposed that hypohalous acid (HOX; \(X = \text{Br}, \text{Cl}\)) mediated sulfilmine bond formation can proceed via either a halosulfonium or halomine intermediate as shown in Fig. 1. More specifically, the HOX acid may react with the side chain sulfur centre of Met93 to form a halosulfonium cation. This then reacts with the side chain amine nitrogen of Hyl, and with loss of HX forms a neutral sulfilmine crosslink between Met93 and Hyl211 (or Lys211). Alternatively, if the side chain amine of Hyl211 (or Lys211) is neutral, the HOX may react with to form a halomine. This then reacts with the sulfur of Met93 to form the neutral sulfilmine crosslink. Of these two possible reaction pathways, the halosulfonium pathway is the major pathway for the formation of sulfilmine bond. While the neutral sulfilmine bond is represented as a covalent double bond, a previous computational study concluded that it is more accurately described as a polarized coordinate-covalent (dative) single bond; \(N^{\delta-} \rightarrow S^{\delta+}\). Meanwhile, another computational study concluded that the sulfilmine bond is protonated at pH 7.

In this present study we have applied computational chemistry methods to examine the proposed mechanisms by which the sulfilmine bond formation in collagen IV is mediated by the hypohalous acids HOX (\(X = \text{Cl} \text{and Br}\)). More specifically, using molecular dynamics (MD) simulations, quantum mechanical (QM)-cluster methods to examine possible mechanisms for formation of possible sulfilmine crosslinks. In addition, we have applied Natural Bond Orbital (NBO) analyses to gain further insights into the nature of possible intermediates, as well as the nature of the product sulfilmine in the basement membrane.

2 Computational methods

2.1 Molecular dynamic simulations (MD)

A suitable X-ray crystal structure of collagen IV, containing the sulfilmine Met–Lys crosslink, was obtained from the Protein Data Bank (PDB ID: 1M3D). While the structure has 6 similar chains, only chains B and D were used in the subsequent MD simulations as these two are involved in the crosslink. The structure was prepared for MD simulations using the Molecular Operator Environment (MOE). All parameterization, solvation, minimization, heating, equilibration and MD production runs were performed using AmberTools 16 and the AMBER 16 program.

First, the prepared initial structure was solvated using TIP3P water model in which the structure was surrounded by a water layer of 10 Å deep with periodic boundary condition enabled. Then, the solvation was followed by minimization using the molecular mechanics (MM) force field AMBER ff14SB. The minimization was performed in two steps: (1) first hydrogen atoms on all heavy atoms were minimized, and then (2) the protein backbone and all heavy atoms using SHAKE algorithm. The resulting minimized system was gradually heated to 300 K using Anderson-like temperature coupling scheme (ntt = 2) at a constant pressure with periodic boundary conditions (ntp = 1) with a time step of 2 fs for 100 ps. Then, the heated system was submitted for equilibration and production run using NVT ensemble with constant temperature (300 K) and constant volume (ntb = 1) with a time step of 2 fs for 50 ns that includes 100 ps equilibration.

The Particle Mesh Ewald (PME) method was used for calculating coulombic interactions, with cutoffs for nonbonded long-range interactions set to 8 Å (the default). The generated conformations from the MD simulation were analyzed based on backbone atoms’ root mean square deviations (RMSD) using AmberTools 16. Then, the obtained RMSD values were clustered, and the structure with the shortest distance was chosen for the QM-cluster calculations. All parameterization, solvation, minimization, heating, equilibration and MD production runs were performed using AmberTools 16 and the AMBER 16 program.

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2.2 Quantum Mechanics (QM)-cluster and systematic series of small model studies

The Gaussian 09 and Gaussian 16 programs were used for all QM-cluster and small model calculations. The M06-2X/6-31G(d,p) level of theory was used to model the protein and solvent environment, which was utilized in the IEF-PCM method. The Gaussian 09 and Gaussian 16 programs were used for all QM-cluster calculations. QM-cluster optimization and harmonic frequency calculations were performed using M06-2X/6-31G(d,p) level of theory, which includes solvent effects and water molecules.

3 Results and discussion

3.1 Molecular Dynamics (MD) results

As mentioned in the previous Computational methods section, a 50 ns MD simulation was first performed. This assisted examination of, for instance, key distances and interactions between residues, especially those involving the sidechains of Met93 and Lys211/Hyl211 in adjacent strands, as well as water availability around these side chains. It is noted that the side chain of Lys211/Hyl211 was protonated in the MD simulations.

The distances between the side chain S of Met93 (SMet93) and side chain N of Lys211/Hyl211 (NLys211/Hyl211) were determined over the course of the MD simulations. In agreement with the hypothesis that the hypohalous acids HOBr or HOCl, in the absence of the hypohalous acids HOBr or HOCl, are shown in Fig. 2. Over the course of the MD simulation, the average distance between SMet93 and NLys211/Hyl211 was 8 Å. However, during the MD simulation, the distances varied and notably, shortened to less than 4 Å, with the shortest distance of approximately being just 3.13 Å. In these short distances, the protonated side chain amine of Lys211/Hyl211 forms a weak electrostatic interaction with the side chain S of Met93. In addition, water molecules were also observed in the immediate vicinity of the Met93 and Lys211/Hyl211 side chains. Thus, these distances indicate the possibility for weak interactions and reasonable positioning of the two mechanistically key functional groups, as well as the availability of waters, for the required subsequent reactions.

To explore possible and proposed sulfinimine bond formation reactions involving HOBr and HOCl, as noted in the computational methods section, a suitable representative structure was then selected and hypobromous acid (HOBr) and hypochlorous acid (HOCl) manually inserted and positioned in proximity to the side chains of Met93 and Lys211/Hyl211. It is noted that except for the addition of HOBr or HOCl, and when needed a proton in the form of H3O+, no additional changes were made.

![Fig. 2](image-url)
3.2 QM-cluster studies on sulfilimine bond formation via the proposed halosulfonium or haloamine

Synthetically, the formation of sulphilmines can be achieved via reaction of a halosulfonium with a sulfide and several possible mechanisms, based at least in part on the results of kinetic studies, have been proposed. These include nucleophilic attack of the sulfide on the halosulfonium nitrogen, halogenation of the sulfide by the halosulfonium, or oxidative addition of the halosulfonium to the sulfide. It is noted that other possible synthetic mechanisms via other intermediates or related species (e.g., sulfoxides) have also been suggested. However, in the mechanisms proposed for collagen IV, it has been suggested that hypohalous acids produced by peroxidinase, HOCl and HOBr, could react with the sidechains of methionine (Met93) or the lysine/hydroxylysine (Lys211/Hyl211) residues to give the corresponding halosulfonium or haloamine derivatives, respectively. Subsequently, the halosulfonium could react with the sidechain amine of lysine/hydroxylysine (Lys211/Hyl211), or conversely the haloamine could react with the sidechain sulfide of Met93 to give a neutral sulfilmine crosslink. Thus, the reaction of HOCl and HOBr with the Met93 sulfur or Lys211/Hyl211 sidechain nitrogen was considered.

Gibbs free energies were obtained for the formation of the halosulfonium or haloamine, using the chemical models described in the Computational methods section, are provided in the Tables 1 and 2, and the energy diagrams are shown in the ESI (Fig. S2–S9†). Herein, only key features of the PESs obtained are described. It is noted that for formation of the halosulfonium and haloamine the sidechain of Glu214 was neutral (i.e., CH₂COOH). In both cases this resulted from addition of a H₂O which was needed to neutralize the hydroxide (HO⁻) formed during the reactions.

| Table 1 Gibbs free energies (kJ mol⁻¹) obtained using QM-cluster (see Methods) for the formation of the proposed sulfilmine bond and haloamine intermediates from hypobromous and hypochlorous acid |
|---|---|---|---|
| Reactions | Reactants | ΔG°‡ (kJ mol⁻¹) | ΔG° (kJ mol⁻¹) |
| Halosulfonium by HOBr | S(CH₃)₂ + CH₂NH₂ + CH₂COOH + H₂O + HOBr | 3.0 | -3.6 |
| Halosulfonium by HOCl | S(CH₃)₂ + CH₂NH₂ + CH₂COOH + H₂O + HOCl | 41.1 | 0.0 |
| Haloamine by HOBr | S(CH₃)₂ + CH₂NH₂ + CH₂COO⁻ + 2H₂O + HOBr | 52.0 | -51.2 |
| Haloamine by HOCl | S(CH₃)₂ + CH₂NH₂ + CH₂COO⁻ + 2H₂O + HOCl | 29.8 | -95.5 |

Table 2 Gibbs free energies (kJ mol⁻¹) obtained using QM-cluster (see Methods) for the formation of the proposed sulfilmine bond from a halosulfonium or haloamine intermediate

| Reactions | Reactants | ΔG°‡ (kJ mol⁻¹) | ΔG° (kJ mol⁻¹) |
|---|---|---|---|
| Sulfilmine via bromo halosulfonium | BrS(CH₃)₂ + CH₂NH₂ + HOBr + H₂O | 118.2 | -28.0 |
| Sulfilmine via chloro halosulfonium | ClS(CH₃)₂ + CH₂NH₂ + HOCl + 3H₂O | 15.9 | -29.9 |
| Sulfilmine via bromo haloamine | S(CH₃)₂ + CH₂N[H]Br + HOBr + H₂O | 108.9 | -96.8 |
| Sulfilmine via chloro haloamine | S(CH₃)₂ + CH₂N[H]Cl + H₂O | 62.4 | -163.0 |

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haloamine products are calculated to be 1.90 and 1.75 Å respectively, suggesting that they are reasonably strong bonds (Fig. S4 and S5†).

3.2.3 Sulfilimine bond formation. In the proposed15,19 mechanisms the next step is reaction of the resultant halosulfonium with the neutral sidechain amine of Lys211/Hyl211 or, alternatively, reaction of the resulting haloamine with the sidechain sulfide of Met93. For both, multiple pathways by which the sulfilimine bond could be formed, including varying the number of water molecules required, were considered and key pathways are summarized in Fig. 3 and 4 (and in S6–S9†).

3.2.4 Further reaction of the halosulfonium to form a sulfilimine bond. Notably, it was found that formation of a protonated sulfilimine bond-containing species could occur in one step. More specifically, the Lys211/Hyl211 sidechain nitrogen can attack the halosulfonium’s sulfur trans to the halo group which results in the alter group leaving as a halide. Concomitantly, however, a proton transfers from the attacking amine via at least one water molecule to the halosulfonium’s leaving halide to form HX (X = Br or Cl). When the leaving halide is bromide three water molecules were required to transfer the proton from the attacking amine to the leaving bromide. In contrast, when the leaving halide was chloride, only one water was required. This reflects at least in part the larger size of the bromide compared to chloride, and differences in the length of the breaking S···X (X = Br, Cl) bond in the transition structure. The calculated reaction barriers for formation of the protonated sulfilimine species via the bromo and chloro halosulfonium were 118.2 and 15.9 kJ mol⁻¹, respectively. Thus, like that noted above for halosulfonium formation, a marked halogen-dependence of the calculated reaction barriers for formation of the protonated sulfilimine bond was observed. Importantly, however, both reaction barriers are thermodynamically feasible. Furthermore, the resulting protonated sulfilimine products lie 28.0 and 29.9 kJ mol⁻¹ lower in energy than the initial bromo- and chloro-containing reactants, respectively. That is, formation of the protonated sulfilimine is exothermic via either the bromo- or chloro halosulfonium.

Building upon these results, we also examined the possible stepwise formation of the protonated sulfilimine product. It was found that it was possible to form an initial complex between the halosulfonium and amine, with the nitrogen complexed to the sulfur trans to the halo (Fig. 3). The resulting cationic bromo- and chloro-containing complexes were calculated to be only slightly higher in free energy than the initial reactants by 11.4 and 8.0 kJ mol⁻¹, respectively. Subsequent loss of a proton, that is formation of a neutral halo-containing species did not produce a stable species. Rather, loss of a halide to formally give the double protonated sulfilimine resulted in a species lying only 72.4 and 76.8 kJ mol⁻¹ higher in free energy than the initial bromo- and chloro-containing reactants, respectively (see Fig. 3). Subsequent loss of a proton to the aqueous solution to give the single protonated sulfilimine species could then occur.

Sulfilimine bond formation via the haloamine intermediate was also examined. Again, it was found that the single protonated sulfilimine bond containing species described above, could be formed in one step from the initial reactants. More specifically, the sulfur of Met93 and sidechain haloamine nitrogen of Lys211/Hyl211 react directly to form an S–N interaction. Importantly, this bond formation occurs concomitantly with loss of the halide as X⁻ from the haloamine component.
3.3 What is the likely protonated state of the sulfilmine bond-containing product?

In the feasible pathways for sulfilmine bond formation via either a halosulphonium reacting with a neutral alkyl amine, or a haloamine reacting with a disulfide, the resultant product was the protonated sulfilmine product (see Fig. 3 and 4). However, the sulfilmine crosslink in collagen IV is commonly represented and/or assumed to be neutral.\(^\dagger\)\(^\ddagger\)\(^\S\) Thus, we further examined whether the single protonated sulfilmine may lose a proton to the aqueous environment. It is noted that other studies have suggested that at least some sulfilmines could potentially be classed as superbases with calculated proton affinities of over 1000 kJ mol\(^{-1}\).\(^\ddagger\)\(^\S\) In this present study, at the IEFPCM(water)-M06-2X/6-311+G(2df,p)//IEFPCM(water)-M06-2X/6-311G(d,p) corrected for Gibb’s Free Energy, the proton affinity of the nitrogen centre in the sulfilmine bond is 1208.9 kJ mol\(^{-1}\). This is in general agreement with calculated values for other sulfilmines. Importantly, it is significantly higher than the proton affinity of water calculated at the same level of theory (989.1 kJ mol\(^{-1}\)). Meanwhile, the proton affinity of the nitrogen centre in the single protonated sulfilmine bonded species is calculated to be slightly lower than that of water at 975.0 kJ mol\(^{-1}\). Thus, the present results indicate that under expected biological conditions the sulfilmine crosslink is most likely single protonated, in agreement with a previous computational study,\(^2\)\(^1\) but has the potential to vary its protonation state; but it is unlikely neutral.

The protonation state of the sulfilmine bond’s nitrogen centre significantly influences the length of the sulfilmine bond. For the current chemical models, the length of the sulfilmine bond in the optimized structures of the neutral, single- and double protonated nitrogen species are 1.606, 1.661, and 1.788 Å, respectively. That is, while the above proton affinities indicate that the single protonated species is more common in vivo, the neutral sulfilmine has a markedly shorter and stronger bond. We also optimized the structures of all three sulfilmine species at the considerably higher QCISD/6-311+G(2df,p) level of theory and determined the proton affinity...
3.4 Natural Bond Orbital (NBO) analyses

To gain further insights into the nature of the sulfinilide bond in the neutral, single- and double protonated species, we were examined using NBO. Pichierri et al. has previously used NBO to characterize the nature of a neutral sulfinilide bond as proposed for collagen IV. For completeness, we considered all possible conformers and isomers of the neutral, single- and double protonated sulfinilide chemical species (Fig. 5). Specifically, there were two conformers for each species as the methyl group on the nitrogen can be on the same or opposite side as the sulfur’s methyl groups. It is noted that thermodynamically the difference in conformers was not significant within the computational models used. In addition, there are also two possible isomers for each single protonated conformer key NBO results are summarized in Table 1.

The NBO method analyzes the wavefunction in terms of localized electron pairs (i.e., natural orbitals) and provides Lewis-type descriptions for molecules in terms of two-electron two-center (σ, π) bonds and lone-pairs (n). The σ_NS (orbital hybridization) NBO of the conformers 1 and 2 are shown in Table 3.

3.4.1 NBO analyses of conformer 1. In the double protonated system, the σ_NS is characterized as being formed from the interaction of a sp^3.90d^0.01 hybrid (79.40% p-character) on nitrogen with a sp^6.90d^0.13 hybrid (88.65% p-character and 0.07% of d-character) orbital on sulfur (Table 3). The two single protonated isomers show very similar, but not the same, characteristics. More specifically, for isomers 1 and 2, the σ_NS is formed from a sp^{3.19}d^{0.03} hybrid (81.07% p-character and 0.01% d-character) and sp^{3.30}d^{0.02} hybrid (76.36% p-character and 0.02% d-character) orbitals on the nitrogen, respectively. These respectively interact with hybrid sp^{6.03}d^{0.07} (isomer 1: 84.87% p-character and 0.04% d-character) and sp^{6.49}d^{0.07} (isomer 2: 82.04% p-character and 0.04% d-character) orbitals on the sulfur. Meanwhile, in the neutral species the σ_NS is formed from the interaction of a hybrid sp^{3.94}d^{0.01} (79.50% p-character and 0.03% d-character) orbital on nitrogen with a sp^{3.62}d^{0.04} hybrid (77.65% p-character and 0.04% of d-character) orbital on sulfur. As can be seen in Table 3, as the protonation state of the sulfinilide bond systematically decreases from double to neutral, the contribution from the nitrogen decreases while that of the sulfur increases by a slightly greater amount.

3.4.2 NBO analyses of conformer 2. The alternate conformer 2 shows similar trends and relative contributions to those described above for conformer 1, thus are not described in detail again here. As for conformer 1, a decreasing the protonation state of the sulfinilide bond from double to single to neutral, concomitantly decreases and increases the contribution from the nitrogen and sulfur respectively. Unlike conformer 1, however, there is negligible difference between the nitrogen and sulfur contributions in isomer 1 and 2, and no difference in the hybridization within the orbitals contributing to form the σ_NS bond (Table 3). Furthermore, the results for all conformer 1 and 2 species indicate that the sigma bond between N and S arises from the interaction of sp^3 hybrid orbitals on sulfur and nitrogen, with negligible sulfur d-character contributions, except in the double protonated systems. In addition, there is only a small difference in the s- and p-character observed in the NBOs of the two conformers for all protonation states considered (Fig. 6).
Fig. 6  Natural bond orbitals obtained for conformers 1 and 2 showing $2e-2e$ $\sigma$-type covalent bond between N and S as well as the lone-pairs on sulfur ($n_S$) and nitrogen ($n^a_N$ and $n^b_N$). The $\sigma_{NS}$ and $\sigma^*_{NS}$ represent bonding and antibonding orbitals, respectively. P(N) indicates the protonation state of N in sulfilimine complex while $1^1$ and $1^2$ denote isomer 1 and 2 of the single-protonated sulfilimine species, respectively. (Atom colours: N = blue; S = rose; C = black; H = white).
The NBO analyses also provided descriptions of the electron lone pairs on nitrogen and sulfur, and additional bonding and antibonding orbitals which are illustrated in Fig. S6.† Notably for both conformers there is a lone pair on sulfur ($n_S$), which remained unprotonated in all possible species considered herein. Meanwhile, the nitrogen centre in both neutral conformers has two lone pairs ($n_N$ and $n_a$) which are the sites of the first and second protonations (described above).

NBO analyses also provide values for the stabilization energy ($E$) associated with interaction(s) between filled and empty NBOs.31 For both double protonated conformers the highest stabilization energies of 4.78 and 6.19 kcal mol$^{-1}$ were obtained for the CH(9) → $\sigma_{SN}$ of conformer 1 and CH(12) → $\sigma_{SN}$ of conformer 2 interactions, respectively. For single protonated conformers and isomers, for conformer 1 stabilization energies of 5.95 and 9.48 kcal mol$^{-1}$ were obtained for the $n_N^a$ → $\sigma_{CH(4)}^*$ and $n_N^b$ → $\sigma_{SC(11)}^*$ interactions in isomers 1 and 2, respectively. In contrast, for conformer 2 much closer stabilization energies of 9.35 and 9.48 kcal mol$^{-1}$ were obtained for the $n_N^a$ → $\sigma_{SC(2)}^*$ and $n_N^b$ → $\sigma_{CH(13)}^*$ interactions in isomers 1 and 2, respectively. However, for the neutral (unprotonated) sulfinilime conformers 1 and 2 differences were observed between the origins and size of the largest stabilizing interaction(s). For conformer 1, it arose from the interaction of a lone pair on the nitrogen ($n_N^a$) and an antibonding σ S–C orbital ($\sigma_{SC}^*$), $n_N^a$ → $\sigma_{SC(11)}^*$ with a value of 23.25 kcal mol$^{-1}$. In contrast, for unprotonated conformer 2 the largest combined stabilization energy of 109.82 kcal mol$^{-1}$ arose from interactions of both S–C bonding orbitals (SC) and the $\sigma_{SN}$ orbital; SC(2) → $\sigma_{SN}$, SC(7) → $\sigma_{SN}$.

Interestingly, the NBO analyses also indicate that in both double protonated conformers 1 and 2, the S⋯N bond is strongly polarized towards S (i.e., $N^d$ → $S^b$). However, upon loss of a proton to give the single protonated conformers the S⋯N bond is strongly polarized towards N (i.e., $N^d$ → $S^b$). This same polarization is observed in the neutral sulfinilime conformers as previously described by Pichierri.28 Indeed, these NBO results on all the neutral and protonated conformers support the previous conclusion of Pichierri28 that the S⋯N bond in collagen IV should be described as a coordinate covalent $N^b$ ← $S^a$ bond.

The charges obtained from the NBO analyses for the sulfur and nitrogen centre in each sulfinilime species (i.e., conformers 1 and 2, and for all the protonation states) are given in Table 2.

### Table 4 Natural atomic charges were obtained from the present NBO analyses for all conformer 1 and 2 sulfinilime species considered in this present study. $P(N)$ indicates the protonation state of N in sulfinilime complex while $1^*$ and $2^*$ denote isomer 1 and 2 of the single-protonated sulfinilime species, respectively

| $P(N)$ | Atoms & charges |
|--------|-----------------|
| Conformer 1 | |
| 2 | S = 1.118, N = –0.705 |
| $1^*$ | S = 1.065, N = –0.831 |
| $1^*$ | S = 1.096, N = –0.840 |
| $1^*$ | S = 1.048, N = –1.056 |
| Conformer 2 | |
| 2 | S = 1.119, N = –0.703 |
| $1^*$ | S = 1.100, N = –0.840 |
| $1^*$ | S = 1.100, N = –0.840 |
| 0 | S = 1.060, N = –1.026 |

4 Conclusions

We have performed a multi-scale computational investigation, using MD simulations and Quantum Mechanical (QM)-cluster methods to examine proposed mechanisms for formation of the sulfinilime bond in collagen IV between Met93 and Lys/Hyl211 residues. In addition, we have used NBO analysis to examine the nature of the possible neutral or protonated (single or double) sulfinilime bond-containing products formed.

4.1 Formation of a sulfinilime product

The present results suggest that formation of the sulfinilime crosslink via the proposed halosulfinilime or haloamine intermediate is thermodynamically feasible. In reaction of a halosulfinilime with a neutral alkyl amine, the first step involves formation of a complex in which the sulfur of the cationic halosulfinilime (Met93-X; X = Cl, Br) interacts with the lone pair on the amine’s nitrogen (Lys/Hyl211). The halogen is then lost as a halide to give a double protonated sulfinilime species. In contrast, the first step in the formation of an S⋯N bond via reaction of a haloamine with the Met93 sulfur requires concomitant loss of the halogen as a halide. This directly gives the most likely product, the single protonated sulfinilime species.

4.2 Protonation of the sulfinilime bond

Proton affinities of the nitrogen ($P(A_N)$) in the neutral and single protonated sulfinilime species were calculated. In the neutral sulfinilime bond $P(A_N)$ is >1200 kJ mol$^{-1}$, which is markedly higher than that of H$_2$O (~990 kJ mol$^{-1}$). In contrast, $P(A_N)$ in the single protonated sulfinilime bond is slightly lower (~15 kJ mol$^{-1}$) than that calculated for H$_2$O (Table S1†). Thus, single protonated is the most likely state of the sulfinilime species due to the changes of natural charges in $S$ and $N$. This phenomenon has previously35 been said to be a characteristic of dative bonding such as suggested for the sulfinilime bond in collagen IV (Table 4).
crosslink in vivo, but conditions may be able to influence its protonation state (e.g., single or double).

4.3 Nature of the sulfilimine crosslink

NBO analyses indicate that, as suggested previously for the neutral sulfilimine bond,²⁸ the S and N bond is strongly polarized toward nitrogen in the single protonated and neutral systems, \(N^\delta- \rightleftharpoons S^\delta+\), and that it is also well describe as a coordinate covalent (i.e., dative) bond.

Author contributions

Anupom Roy: computational calculations and writing, Taqred H. Alnakhli: computational calculations of bromo halosulphonium and bromo haloamine formation, James W. Gauld: supervision, review, and editing.

Conflicts of interest

There are no conflicts to declare.

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