Communication

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Complex formation of silver(I) ions with a glucosinolate derivative: structural and mechanistic insights into myrosinase-mimicking C−S bond cleavage†

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Glucosinolates are a class of plant compounds that are thought to play important protective roles, primarily as defense agents against herbivores and pathogens. Their core structure includes a β-D-glucopyranosyl residue linked to an aglycone, which is a sulfated thiohydroximate with a variable side chain derived from amino acids (Scheme 1). Upon tissue damage in the natural body, myrosinase (EC number 3.2.1.147), a hydrolytic enzyme always found in plants containing glucosinolates, cleaves the glucopyranosyl C−S bond, forming an unstable aglycone that rearranges to form isothiocyanates, nitriles and other products. The distinct aroma and flavor of glucosinolate-rich plants are attributed to their hydrolytic enzyme activity and structural analysis of complexation of the glucosinolate derivative: structural and mechanistic insights into myrosinase-mimicking C−S bond cleavage†

The crystal structure of an unprecedented silver complex of O-acetylsinigrin has shown chelating bonds of the sulfated thiohydroximate and an η1-bond of the ethylene moiety with Ag(I). Mechanistic studies on the formation and decomposition of the complex by the 1H NMR measurements and theoretical calculations with the DFT method indicated relevance to the glucosinolate degradation in biological systems.

Glucosinolates are a class of plant compounds that are thought to play important protective roles, primarily as defense agents against herbivores and pathogens.1 Their core structure includes a β-D-glucopyranosyl residue linked to an aglycone, which is a sulfated thiohydroximate with a variable side chain derived from amino acids (Scheme 1).2 Upon tissue damage in the natural body, myrosinase (EC number 3.2.1.147), a hydrolytic enzyme always found in plants containing glucosinolates, cleaves the glucopyranosyl C−S bond, forming an unstable aglycone that rearranges to form isothiocyanates, nitriles and other products. The distinct aroma and flavor of glucosinolate-rich plants are attributed to their hydrolytic products, particularly the isothiocyanates.2 Additionally, much of current interest in these metabolites is based on their health-promoting features and there is growing evidence to support claims that they are effective agents for the prevention of various types of cancer.3–5

Of the glucosinolates, sinigrin (Sin−) is one of the most studied and frequently encountered. X-ray crystallographic studies and binding simulations of Sinapis alba myrosinase6 revealed (i) hydrogen bonds for securing the sugar moiety, (ii) location of the allyl moiety in the hydrophobic pocket, (iii) a salt bridge formation of the sulfate group with the Arg259 residue, and (iv) possible S–π interaction7 of the glycosidic sulfur atom with the Trp142 residue. A proximal carboxylate residue of Glu409 intrinsically performs the nucleophilic attack on the C1 carbon of the glucose, releasing the aglycone as a leaving group. It spontaneously transforms into allyl isothiocyanate via the Lossen rearrangement (Scheme 1).6,8

Interestingly, the decomposition of Sin− analogous to the biological degradation can also be induced chemically, such as by acids, heat, or reactions with various metal ions.5,10 In the past over half a century, silver nitrate had been shown to rapidly decompose Sin− into glucose in an aqueous solution, with the formation of the silver–aglycone salt as a white powder precipitate.11,12 The resulting aglycone could then be liberated from the silver ion by the addition of NaCl to regenerate allyl isothiocyanate.13 This simple inorganic reaction has long been known as a precise analogue to the natural action of myrosinase on Sin−, but details of the rapid decomposition have still remained to be elucidated behind the development of biochemistry. Herein, we first succeeded in the synthesis and structural analysis of complexation of the glucosinolate with a silver ion. Further investigation of the simplified hydrolytic decomposition of glucosinolate with the inorganic metal salt clarified that the silver ion plays significant roles in the C−S bond activation upon its complexation with Sin−.

Scheme 1 Myrosinase-catalyzed degradation of sinigrin (Sin−) resulting in the formation of allyl isothiocyanate.
As the decomposition of Sin− was previously shown to be rapid in aqueous solution through silver nitrate, our experiments were carried out in methanol, and silver(i) triflate (AgOTf) was used for its high solubility in organic solvents. Monitoring 1H NMR spectral changes in MeOH-d₄ indicated that the addition of 1 eq. of AgOTf to Sin− caused downfield shifts of the proton signals at the anomeric C1 and vinylic positions, δΔ = ∼0.04 ppm for the anomeric proton, ∼0.13 ppm for the internal vinylic proton, and 0.13–0.20 ppm for the terminal vinylic protons14 (Fig. S1 in the ESI†). These downfield shifts reflect deshielding effects of Ag⁺ on the coordinating sulfated thiohydroximate and vinyl groups of Sin−. However, even in MeOH, Sin− is gradually decomposed to the silver-aglycone complex12 which forms a white powder precipitate in 30 min. The powder product was dissolved in DMSO-d₆ and identified by 1H NMR as the previously reported potassium silver aglycone,12 which shows only the allyl moiety signals at around δ = 5.80–5.95 ppm and 5.20–5.35 ppm for the internal and terminal vinylic protons (Fig. S2a in the ESI†).

In an attempt to improve the stability upon reacting with Ag+, Sin− was acetylated into K-SinAc. Addition of AgOTf to a solution of K-SinAc in methanol usually keeps the solution homogeneous. Therefore, this reaction was successfully monitored by 1H NMR spectroscopy (Fig. 1 and Fig. S5 in the ESI†), where the progressive addition of Ag⁺ caused the gradual downfield shift of the vinylic protons as well as the anomeric proton in a mode similar to that of the Ag⁺-Sin− system. It is indicative of the rapid equilibrium between free and Ag⁺ coordinated species. The saturation behavior shown by the 1H NMR chemical shifts at around δ = 5.98–6.28 ppm and 5.19–5.52 ppm for the internal and terminal vinylic protons, respectively, indicates that a 1 : 1 ratio of Ag⁺-SinAc− complexation is dominant in solution (Fig. S5 in the ESI†, formation const. of the Ag⁺-Sin− complex: K = 2 × 10² M⁻¹). The synchronously observed downfield shift of the anomeric proton H1 (Δδ = ∼0.10 ppm) is highly proportional to it.

Very slow and careful evaporation of the concentrated methanolic solution containing K-SinAc and an excess of AgOTf (ca. 2 eq.) gave large colorless needle crystals of the silver(i) complex suitable for X-ray diffraction analysis. The crystal structure of the silver(i) complex with SinAc− (Ag-SinAc) revealed that an asymmetric unit consists of one SinAc− and a coordinated silver ion without any co-crystallized solvent molecules in the lattice (Fig. 2a). The chair conformation and equatorial arrangement of the acetyl groups in Ag-SinAc are typical in terms of bond distances and angles for an acetylated monosaccharide,15 while having the β configuration in the same manner as the starting material, K-SinAc. The acetyl moiety at the C6 position of the sugar is disordered (Fig. S6c in the ESI†). The molecules of Ag-SinAc are organized by a central core composed of the silver coordination and the sugar moieties projecting on either side (Fig. S6a and b in the ESI†), thus forming a 1D chain with this linkage.

The silver(i) ion in the lattice binds to three different molecules of SinAc−, to the ethylene moiety via an η²-bond, to the two sulfate O atoms, and to the one N atom (Fig. 2b). As for the lengths of the Ag-(η²-C=C) bonds, 2.465(7) Å (Ag1–C15) and 2.332(8) Å (Ag1–C16), the former for the internal C15 is typical and the latter for the terminal C16 lies on the shorter side in the range of other reported silver(i)-linear olefin com-

![Fig. 1](image-url) 1H NMR spectral changes of SinAc− with the addition of AgOTf. The anomeric proton H1 is marked ▼, the internal vinylic proton H15 is marked ◀, the terminal vinylic protons H16 are marked ■, 400 MHz, in MeOH-d₄.

![Fig. 2](image-url) (a) ORTEP view of part of the molecular structure of Ag SinAc with atom numbering. (b) The coordination structure around the Ag center. Atoms labeled “b” and “c” belong to distinct SinAc− molecules. Hydrogens and low occupancy disordered sites are omitted for clarity. Thermal ellipsoids are drawn at a 50% probability level.
plexes (2.35–2.58 Å). The C=C bond of Ag·SinAc is typically elongated to 1.34(1) Å compared to K·SinAc (1.302(6) and 1.306(9) Å). The Ag–O bonds, 2.365(5) Å (Ag1–O21 and 2.519 (6) Å (Ag1–O22), are also similar to those reported for the perchlorate oxygen bound to the silver(i) ion (2.29–2.75 Å). The Ag1–N17 bond, 2.412(5) Å, is also typical of silver(i)-coordinated imino N atoms (2.311–2.447 Å). The C1–S12 bond of Ag·SinAc is likely to be lengthened to 1.803(6) Å compared (1.800(4) and 1.802(3) Å). However, these lengths are slightly shorter than the value for non-acetylated SinAc (C1–S12 = 1.809 Å). However, these differences of the C1–S12 bond are not significant enough to be discussed at this point.

Therefore, in order to confirm the C1–S12 bond activation for hydrolysis, we performed a reaction study on the silver(i) salt and SinAc. Heating a 1:1 mixed solution of AgOTf and K·SinAc in methanol to ca. 50° C readily formed a white precipitate, which contains only the Ag-aglycone compound previously reported upon 1H NMR analysis (Fig. S2b in the ESI†). Possible structures of the Ag3-aglycone compound are described in Fig. S7 in the ESI†. The thermal reactivity suggested that the crystal structure of Ag·SinAc represents a meta-stable intermediate coordination mode prior to the hydrolysis of SinAc by the silver(i) ion to generate its aglycone compound. The crystal structure of Ag·SinAc would also imply the plausible intermediate species in the corresponding pre-equilibrium complexation steps of the silver(i) ion and SinAc† reaction.

To gain further mechanistic insights into the decomposition of the SinAc complex upon the addition of Ag+, we carried out DFT calculations on the basis of the crystal structure of Ag·SinAc, resulting in three optimized structures 3a, 3b, and 3c (Fig. 3). In the first two structures, the Ag+ coordination is based on the crystallographic findings with the Ag+ bound to imino N17 and sulfate O22 in the sulfated thiohydroximate group (3a) or with the additional η2-binding of the ethylene moiety (3b). We also considered the well-known property that silver(i) binds to sulfur atoms with high affinity. The third structure (3c) places the Ag+ in contact with the glycosidic S12 and is chelated by several O atoms (sugar ring O10, sulfate O22, and acetyl O33). Although this coordination mode is not seen in the crystal structure, the proximity of Ag+ to the anomic proton through the Ag-S contact is supported by its downfield shift in the 1H NMR spectrum. These optimized structures form one, two, or three chelate rings for 3a, 3b, and 3c, respectively, which increasingly contribute to their stability. The selected bond distances in the calculated structures of Ag·SinAc complexes 3a, 3b, and 3c and their cartesian coordinates are listed in Tables S4 and S5 of the ESI†.

As shown in Table 1, these three silver-coordinated structures ordered from the highest to the lowest total energy are 3a > 3b > 3c. Relative to 3a as a standard, 3b is lower in energy by 6.1 kJ mol−1 and 3c is lower by 17.8 kJ mol−1. Thus, 3c is highly probable in solution and is even more stable than either 3a or 3b. It should be mentioned in this connection that the 1H NMR data of the Ag·SinAc and Ag·SinAc systems exhibited interesting downfield shifts of signals of the anomeric proton as well as the vinylic protons upon addition of AgOTf. It significantly indicates that the silver(i) ion would bind to the S12 atom close to the anomeric C1 atom in one of the rapidly equilibrated Ag·SinAc complex species in solution.

The C–S bond activation was estimated from the C1–S12 bond distances determined by DFT simulations of the optimized Ag-binding structures of SinAc. The calculated C1–S12 bond distances increased from 1.807 Å without metals to 1.808 Å when complexed with K+ (K·SinAc) and 1.810–1.812 Å when complexed with Ag+ (3a, 3b, and 3c in Table 1). As the C1–S12 bond distances indicate an increasing trend in the order SinAc† > K·SinAc > 3a > 3b > 3c, we performed further theoretical investigation to evaluate the following index parameters of bond activation. The calculated heterolytic bond dissociation enthalpy (BDEhet) for the C1–S12 bond was changed due to the complexation of the metal ions with SinAc†, resulting in the decrease in BDEhet from 275.5 kJ mol−1 without any metal (SinAc) to 228.3 kJ mol−1 for K·SinAc, 206.4 kJ mol−1 for 3b, 204.5 kJ mol−1 for 3a, and 203.1 kJ mol−1 for 3c as shown in Table 1. Thus, the calculated C1–S12 bond strengths for heterolytic cleavage, ordered from the strongest to weakest in terms of BDEhet, are SinAc† (no metal) > K·SinAc > 3b > 3a > 3c.

To understand further how the presence of the metal ions decreases the BDEhet, we carried out natural bonding orbital (NBO) population analysis, and it was observed that the presence of metal ions resulted in the electronic charge transfer (ΔQNBO) from the glucose unit to the thiocyanate group. The

![Fig. 3](#) DFT-optimized structures of the Ag·SinAc complexes. 3a (a), 3b (b) and 3c (c). Hydrogens are omitted for clarity.
electron charge transfer is determined by \( \Delta Q_{\text{NBO}} = Q_{\text{glu}}(\text{Ag}^-/\text{K}^-) - Q_{\text{glu}}(\text{SinAc}^-) \), where \( Q_{\text{glu}} \) is the NBO charge of the glucose unit. The order of the \( \Delta Q_{\text{NBO}} \) (e\(^{-}\)) values is as follows: 

\[
\text{K}^-\text{SinAc}^- \ll 3\text{b} < 3\text{a} \approx 3\text{c} \] (Table 1), which shows that in the presence of K\(^+\) and Ag\(^+\), the electron charge is transferred to the thiocyanate group from the glucose unit, resulting in more stable fragments (glu\(^+\) and thio\(^-\)) and lower bond dissociation energies. Thus, the BDE\(_{\text{het}}\) can be more directly related to \( \Delta Q_{\text{NBO}} \) rather than the C1–S12 bond lengths. It is likely that all three possible Ag\(^+\)–SinAc\(^-\) structures 3a, 3b, and 3c exist in rapid equilibrium in solution. Direct coordination by Ag\(^+\) to the sulfur atom results in a structure with the most activated C1–S12 bond as evidenced by its lengthening to 1.812 Å in the simulation and having the lowest BDE\(_{\text{het}}\) of 203.1 kJ mol\(^{-1}\) (Table 1). Overall, the enhanced C–S bond activation is attributed to the effect of the Lewis acidity of Ag\(^+\) causing a larger electron charge transfer. However, the greater stability of 3c is attributed to multidentate binding of the sulfated thiohydroximate with effective chelate rings, which lowers the Lewis acidity of Ag\(^+\) and permits the stable complexes to exist in solution without decomposition. In an attempt to explain the higher reactivity of non-acetylated Sin\(^-\) over SinAc\(^-\), DFT simulation of 3c without acetyl groups was carried out (Fig. S9 in the ES1†). The calculated structure of the Ag\(^+\)–Sin\(^-\) complex showed a coordination bond of the S12 atom of Sin\(^-\) upon formation of the chelate ring with the sulfate O atom (C1–S12 = 1.809 Å). The Ag–S12 bond formation is consistent with the significant downfield shift of the anomeric proton signal, caused by the addition of Ag\(^+\) to Sin\(^-\) as described above. In this case, solvation likely assists the C1–S12 bond activation. In an aqueous solution, solvation of the sulfate group may remove its interaction with the silver center and instead redirect its Lewis acidity to the glycosidic S atom, activating it further.

One can draw parallels between the silver-driven decomposition of Sin\(^-\) and SinAc\(^-\) and the action of myrosinase itself on Sin\(^-\) (Scheme 1 and Fig. 4). On the basis of our results, we conclude that the silver\((i)\) ion, by acting as a Lewis acid, serves to polarize and effectively weaken the C1–S12 bond, making the aglycone an even better leaving group. In the case of the myrosinase active site, several arginine residues, most importantly Arg259, form a salt bridge with the sulfate group of Sin\(^-\).\(^{21}\) The role of this positively charged arginine would be molecular recognition at the initial step. Indeed, insect myrosinase is remarkably similar to plant myrosinase in function, but a positively charged lysine interacts with the sulfate group instead of arginine.\(^{5}\) When the Sin\(^-\) is held in place in the active site, a glutamate residue essentially performs a nucleophilic attack on the anomeric C1 atom,\(^{6}\) displacing the allyl moiety and forming an \( \alpha \)-glucoside. In the case of the Ag\(^+\)–Sin\(^-\) complex, direct interaction between Ag\(^+\) and the S12 atom should also contribute to activation for nucleophilic attack, as evidenced by the \( ^1\)H NMR signal behavior of the anomeric proton. The eliminated leaving group is then stabilized by complexation with the silver\((i)\) ion in the inorganic system, while in the case of an enzymatic reaction, it is stabilized by the interaction with the amino acid residues of the protein surface at the active site.

In summary, we have synthesized and characterized the structure of a silver\((i)\) complex of SinAc\(^-\), which contains an \( \eta^1\)-linkage to the ethylene moiety and also the bonds with the sulfated thiohydroximate. From the further investigation of \(^1\)H NMR spectroscopic analyses and the DFT calculations, it was found that the silver\((i)\) ion binds to Sin\(^-\) in the several modes pre-equilibrated rapidly in solution, resulting in the direct coordination to the S12 atom (Fig. 4). The Ag–S bond formation causes the C1–S12 bond polarization to induce a nucleophilic attack on the anomeric carbon of the sugar moiety. The subsequent formation of the stable silver-aglycone complex facilitates the dissociation of the aglycone. In the simplified inorganic reaction, the noble metal ion performs these critical steps for the effective decomposition of the glucosinolate to best mimic the enzymatic reaction.

**Conflicts of interest**

The authors declare no conflict of interest.

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**Notes and references**

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<td>J. Gadamer</td>
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<td>12</td>
<td>H. E. Miller</td>
<td>The aglucone of sinigrin</td>
<td>MA thesis, Rice University</td>
<td>1965</td>
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