DNA Repair

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Selenium-Mediated Dehalogenation of Halogenated Nucleosides and its Relevance to the DNA Repair Pathway**

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Abstract: *Halogenated nucleosides can be incorporated into the newly synthesized DNA of replicating cells and therefore are commonly used in the detection of proliferating cells in living tissues. Dehalogenation of these modified nucleosides is one of the key pathways involved in DNA repair mediated by the uracil-DNA glycosylase. Herein, we report the first example of a selenium-mediated dehalogenation of halogenated nucleosides. We also show that the mechanism for the debromination is remarkably different from that of deiodination and that the presence of a ribose or deoxyribose moiety in the nucleosides facilitates the deiodination. The results described herein should help in understanding the metabolism of halogenated nucleosides in DNA and RNA.*

Halogenated nucleosides, such as 5-bromo-2′-deoxyuridine (BrdUd) and 5-iodo-2′-deoxyuridine (IdUd), are known to be incorporated into the DNA of dividing cells during the S phase of the cell cycle, essentially substituting for thymidine during DNA replication.[1] These compounds are widely used for birth-dating human cells and monitoring cell proliferation and, owing to their ability to cross the blood–brain barrier, they are combined with conventional chemotherapy and radiation treatment for cancer in several clinical trials.[2] However, halogenation of nucleobases and nucleotides is associated with DNA damage, mutagenesis, cytotoxicity, carcinogenesis, and loss of genome integrity.[3,4] Halogenated nucleosides are metabolized through dehalogenation in vivo, although the nature of biomolecules that can mediate the dehalogenation is still unknown. However, the dehalogenation of the halouracil moiety is a key process in DNA repair as the resulting uracil base can be removed from the DNA by uracil-DNA glycosylases by the base-excision repair (BER) pathway.[4a] It has been shown that thymidylate synthase (TSase), a key enzyme involved in the biosynthesis of 2′-deoxythymidine-5′-monophosphate (dTMP) from 2′-deoxyuridine-5′-monophosphate (dUMP; Figure 1), can mediate the dehalogenation of 5-bromo- and 5-iodo-2′-deoxyuridine-5′-monophosphate (BrdUMP and IdUMP) to form 2′-deoxyuridine-5′-monophosphate (dUMP) in the presence of thiols (Figure 1).[5] These reactions have direct relevance to human

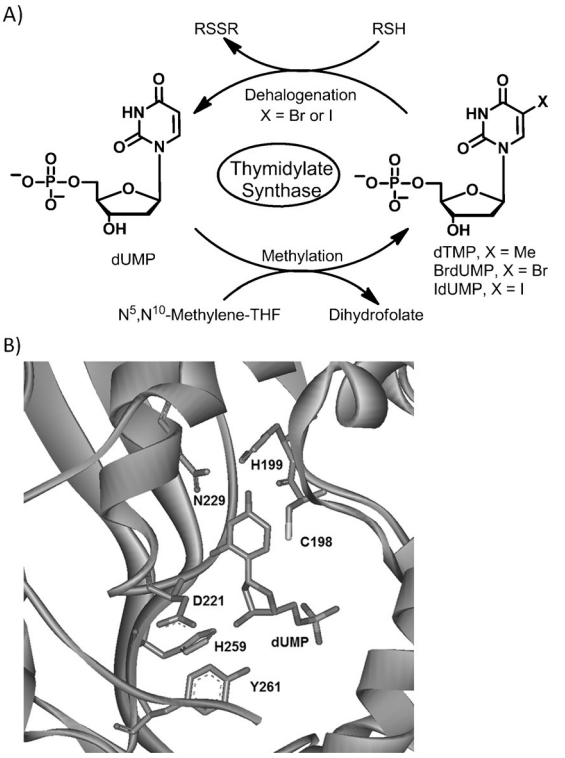

Figure 1. A) Biosynthesis of dTMP from dUMP and dehalogenation of 5-halo-2′-deoxyuridine-5′-monophosphate by TSase (THF = tetrahydrofolate). B) The active site of TSase from *L. Casei* showing the binding of dUMP and the presence of a cysteine residue that acts as a nucleophile (PDB accession number = 2TDM).

treatment given that dehalogenation is known to decrease the half-life of the halogenated nucleosides, which are used as radiosensitizers in cancer therapy.[2c,d] Furthermore, some biologically relevant thiols, such as cysteine and glutathione, were shown to mediate the dehalogenation reaction.[6]

In humans, two major enzymes, iodothyronine deiodinase (ID) and iodotyrosine deiodinase (IYD), mediate the deiodination of thyroid hormones and iodinated tyrosine, respectively. While the deiodination of thyroxine (T4) by three isoforms of ID regulate the thyroid hormone homeostasis in the body,[7] IYD is responsible for the recovery of iodide for subsequent reuse in T4 biosynthesis.[8] Recently, we reported that synthetic naphthalene-based compounds **1**–**5** bearing thiol/selenol groups in the 1,8-positions (Figure 2 A) mimic type 3 ID by mediating the deiodination of thyroid hormones and their metabolites.[9] As the reactivity of iodine in iodinated nucleosides is quite different from that of thyroid hormones, we studied the dehalogenation of nucleosides by deiodinase mimetics. Herein, we report, for the first time, that

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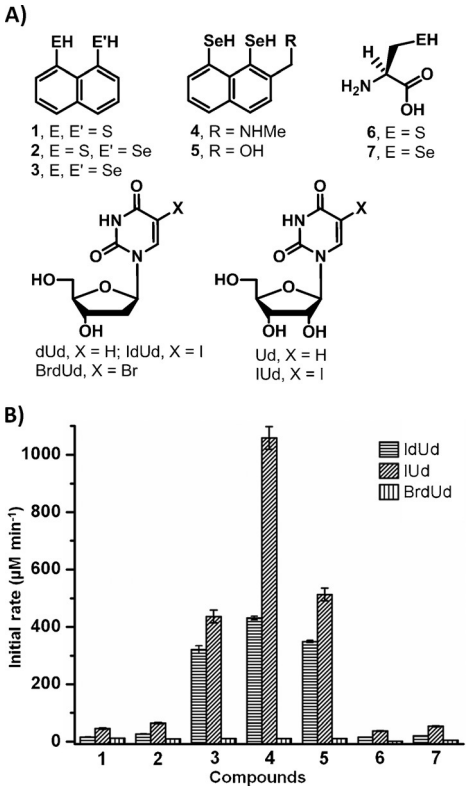

Figure 2. A) Structures of compounds **1**–**7** and different nucleosides used in this study. B) Comparison of initial rates for the dehalogenations of IdUd, IUd, and BrdUd by **1**–**7**.

selenium compounds can mediate the dehalogenation of halogenated nucleobases and nucleosides in aqueous media under physiological conditions. We also show that the mechanism of debromination by sulfur and selenium compounds is different from that of deiodination.

The dehalogenation reactions were carried out at 37 °C in phosphate buffer (100 mM; pH 7.0) and were monitored by HPLC and mass spectrometry. When IdUd was treated with **1** having two thiol moieties, the reaction afforded the expected deiodinated product 2′-deoxyuridine (dUd). The initial rate for this reaction was found to be $16.8 \pm 0.5 \times 10^{-2}$ μM min^{-1} (Figure 2B) and a significant enhancement in the reaction rate was observed when **2** with a thiol/selenol pair was used for the deiodination ($27.4 \pm 1.3 \times 10^{-2}$ μM min^{-1}). Similar to our earlier observations on the deiodination of T4,[9b,c] the activity of **3** having two selenol moieties ($321.0 \pm 13.6 \times 10^{-2}$ μM min^{-1}) was found to be an order of magnitude higher than that of **2** (Figure 2B). A further enhancement in the activity was observed when the substituted compounds **4** ($431.3 \pm 6.5 \times 10^{-2}$ μM min^{-1}) and **5** ($349.1 \pm 4.4 \times 10^{-2}$ μM min^{-1}) were employed. The activity of cysteine (**6**) and selenocysteine (**7**) was comparable to that of **1**.

Interestingly, the deiodination of 5-iodouridine (IUd) was found to be more facile than that of IdUd by **1**–**7** (Figure 2B; see also Table S1 in the Supporting Information), indicating that the nature of the sugar moiety plays an important role in the deiodination. The rate of deiodination of IUd by **4** ($1058.7 \pm 39.6 \times 10^{-2}$ μM min^{-1}) was found to be more than two times higher than that of IdUd. The enhanced deiodination of IUd by compound **4** can be attributed to the favored hydrogen bonding between the amine side chain of **4** and the ribose sugar moiety of IUd. The initial rates for the debromination of BrdUd by **1**–**7** were found to be remarkably lower than that of the deiodination under identical conditions. A comparison of the initial rates indicates that **1** exhibits the highest activity ($12.7 \pm 0.4 \times 10^{-2}$ μM min^{-1}) of the series and, quite unexpectedly, there is a decrease in the activity when the selenium compounds **2** ($9.9 \pm 0.1 \times 10^{-2}$ μM min^{-1}) and **3** ($10.9 \pm 0.2 \times 10^{-2}$ μM min^{-1}) are used for the debromination reactions (Figure 2B; Table S1). The initial rates observed for molecules **4** ($10.8 \pm 0.5 \times 10^{-2}$ μM min^{-1}) and **5** ($11.1 \pm 0.3 \times 10^{-2}$ μM min^{-1}) were also similar to or even lower than that of **1**. It should be noted that the deribosylation generally observable during the debromination of BrdUd with an enzyme nucleophile[10] was not observed with **1**–**7**.

To understand the effect of the deoxyribose and ribose moieties in the dehalogenation, we treated compounds **1**–**7** with 5-iodouracil (IU) and 5-bromouracil (BrU). Compared to IdUd or IUd, IU undergoes much slower deiodination by **1**–**7** (Figures 2B and 3A), suggesting that the presence of the deoxyribose or ribose moiety in IdUd and IUd facilitates the deiodination reactions. Similar to the debromination of BrdUd, debromination of BrU were found to be 3–24 times slower than the deiodination of IU (Figure 2B; Table S1). Furthermore, **4** was found to be about two times less active than **3**, indicating that the introduction of a basic amino group decreases the activity, which is in contrast to the deiodination activity of these compounds. Although the reactivity of BrU/BrdUd toward sulfur and selenium nucleophiles is expected to be different from that of IU/IdUd, the remarkable difference in the reaction rates suggests that the debromination and deiodination may follow different pathways. It should be noted that a common S_N2 mechanism has been proposed for both deiodination and debromination of 5-halo-2′-deoxyuridines by cysteine.[6] Dehalogenation of IdUd or BrdUd by TSase does not involve the cofactor N^5,N^{10}-methylene-THF (Figure 3B). Interestingly, the formation of 5-thioalkyl-dUMP (**8**) was only observed with BrdUMP, which is unusual as the intermediate INT-2 is expected to be a common intermediate for both BrdUMP and IdUMP. These observations also suggest that the deiodination and debromination may follow different mechanistic pathways.

Although the dehalogenation of BrU or IU or the corresponding nucleosides by cysteine and selenocysteine follows the mechanism proposed for the TSase, the mechanism by which **1**–**5** mediate the dehalogenation depends on the nature of halogen atom. When the reaction of **2** with BrU was followed by ^{77}Se NMR spectroscopy at different temperatures (Figure 3C), a new signal was observed at $\delta = 222$ ppm, which is shifted downfield relative to that of **2** (206 ppm), indicating the formation of **10** (Figure 3D). A similar reactivity was observed for **3**. For both **2** and **3**, the reactions led to the formation of uracil and the corresponding dichalcogenides (Figure 3D). Therefore, the mechanism of debromination of BrU by **1**–**3** follows an addition–elimination pathway (Figure 3D). In contrast, the deiodination of IU proceeds through a XB-mediated pathway (XB = halogen

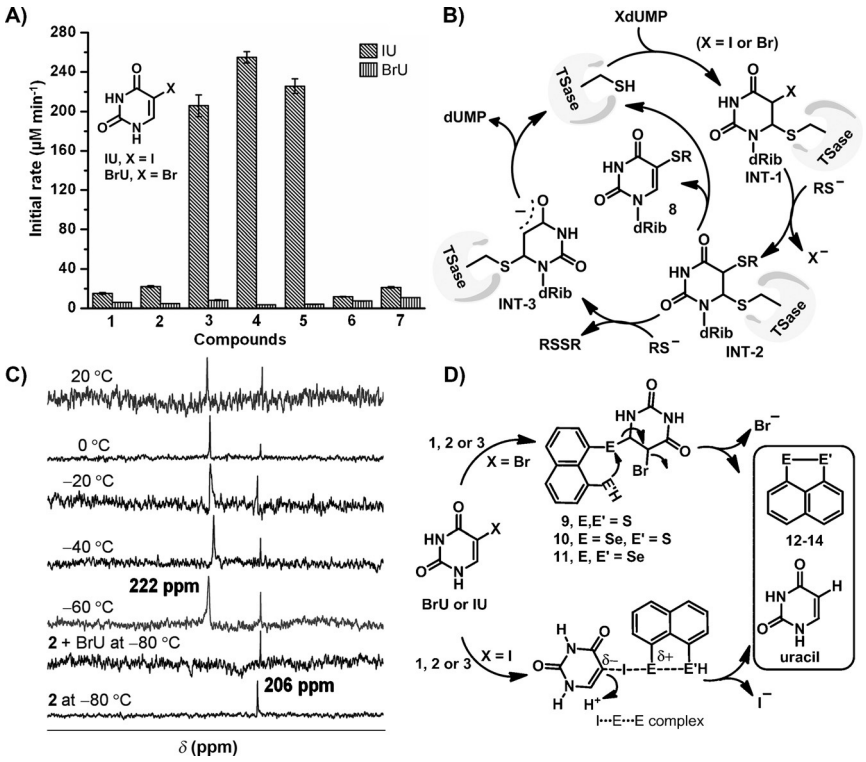

Figure 3. A) Comparison of initial rates for the dehalogenation of IU and BrU by **1**–**7**. B) A common catalytic mechanism proposed for the dehalogenation of both 5-iodo- and 5-bromo-2′-deoxyuridine-5′-monophosphate (XdUMP) by TSase to give dUMP in the presence of an exogenous thiol (RSH).[5] dRib = 2′-deoxyribose-5′-monophosphate. C) ^{77}Se NMR spectra of **2** at −80 °C (bottom) and of a 1:2 mixture of **2** and BrU at various temperatures. D) Proposed reaction mechanism for the dehalogenation of BrU and IU by **1**–**3**.

bond) involving the I···E···E complex (Figure 3D; see below), similar to the deiodination of T4.[9c] Cooperative interactions between the iodine and a chalcogen atom (XB) and between two chalcogen atoms facilitate the cleavage of the C–I bond to produce uracil and dichalcogenides. When correlating the reactivity of compounds **1**–**5** with that of TSase, it is possible that the highly reactive cysteine residue in the enzyme (Cys198, Figure 1B) can mediate the deiodination reaction by a halogen-bonding mechanism. Under such circumstances, both TSase and iodothyronine deiodinase (ID) may follow a similar mechanism for the deiodination. However, further studies are required to demonstrate the formation of a halogen bond between TSase and IdUMP.

To understand the formation of XB between the iodine and the chalcogen atoms, compounds **15** and **16** (Figure 4A), which lack the second sulfur/selenium atom required for a facile deiodination, were crystallized with IU. Although the C=S and C=Se bonds in **15** and **16** are polarized and the compounds have significant thiolate and selenolate character, respectively, these compounds are quite stable against oxidation. The single-crystal X-ray structures indicate that the chalcogen atoms in **15** and **16** interact with iodine in IU to form the halogen-bonded (X-bonded) adducts **17** and **18**, respectively (Figure 4A–C).[11] Although theoretical investigations predicted possible XB between the nucleobases,[12] **17** and **18** provide clear experimental evidence for X-bonded systems involving a halogenated nucleobase. The C–I bond (2.097 Å) in **18** is elongated (Figure 4C) as compared to that in IU (2.052 Å, see Figure S10). As reported earlier for other X-bonded systems,[13] the elongation of the C–I bond is due to the donation of electron density by the selenium atom to the anti-bonding σ* orbital of the C–I bond. In agreement with this, the electrostatic potential maps obtained from DFT calculations[14] indicate that the iodine atom has a region of positive electrostatic potential (σ-hole)[15] for possible halogen bonding with sulfur or selenium (Figure 4D). As the strength of XB depends on the electrostatic potential, the deiodination of compounds with longer (or polarized) C–I bonds is expected to be more facile than that with shorter C–I bonds. As expected, the initial rate observed for the deiodination of IUd (average bond length = 2.092 Å) by **3** is significantly higher than that of IdUd (bond length = 2.075 Å), or IU (bond length = 2.052 Å; Figure 2B, Figure 3A, and Figure S11). In **17**, the sulfur atom interacts simultaneously with two iodine atoms and the strength of the S···I interaction is found to be weaker than that of the Se···I interaction in **18**. The formation of a XB between **16** and IU was also observed in solution. With the addition of an increasing amount of IU, the signal in the ^{77}Se NMR spectrum of **16** was gradually shifted downfield, whereas no such shift was observed in case of BrU (Figure S8). These results indicate that **16** does not form an X-bonded adduct with BrU. In agreement, compared to IU,

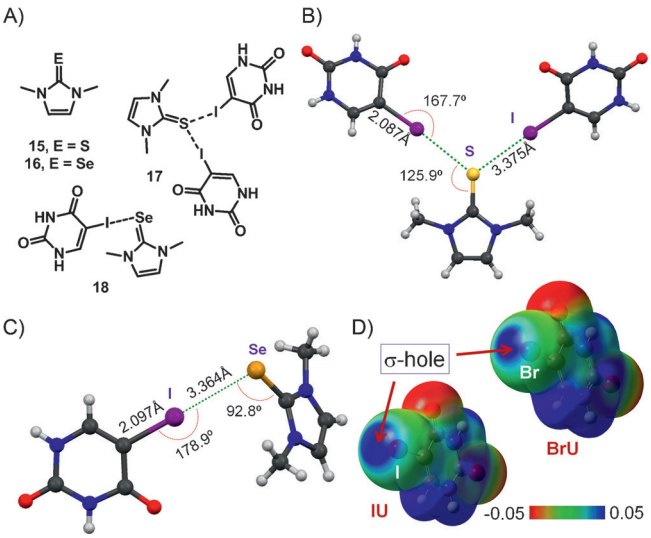

Figure 4. A) Structures of **15**–**18**. B, C) X-ray structures (ball-and-stick model) of B) **17** and C) **18**. D) Electrostatic potential map for IU and BrU.

a less pronounced positive potential was observed on the σ-hole of BrU (Figure 4D).

Interestingly, when the adduct **18** was left in the solution for a few hours, the signal in the ^{77}Se NMR spectrum of **18** at $\delta = 12.7$ ppm (Figure S8A) disappeared completely with a new signal appearing at 196 ppm, indicating the formation of compound **19** (Figure 5A), which was purified and

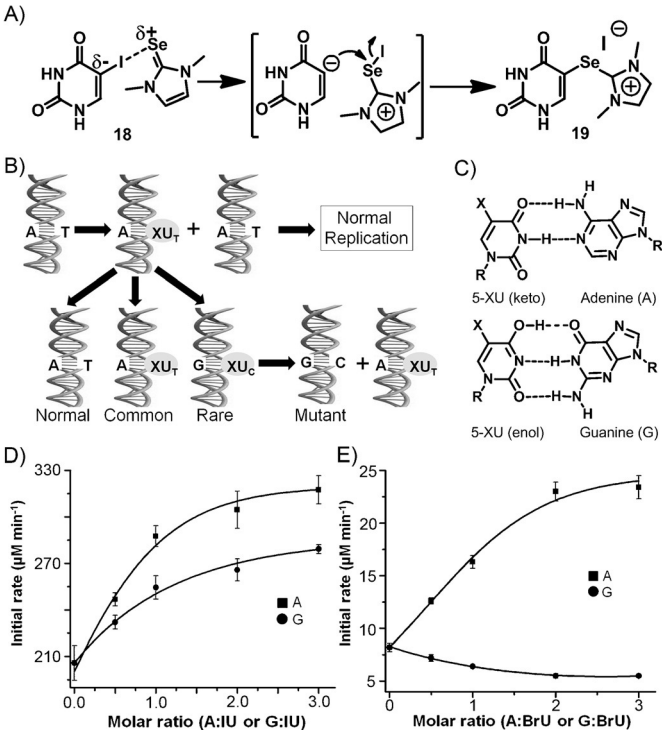

Figure 5. A) Mechanism for the formation of **19** from **18**. B) Mutation in DNA resulting from incorporation of 5-XU. The base pair between the enol form of 5-XU and guanine leads to the formation of a guanine–cytosine (G–C) base pair. C) Formation of base pairs between 5-XU and adenine or guanine. D, E) Effect of adenine and guanine on the deiodination of 5-IU (D) and debromination of 5-BrU (E) by **3**.

characterized by spectroscopic techniques (Figure S4–S5). A single-crystal X-ray structure shows that the selenium is covalently attached at the C5 position of the uracil moiety (Figure S12). However, no such monoselenide was produced even after 10 days when compound **16** was treated with BrU (Figure S7B). Similarly, the sulfur-based complex **17** does not undergo any further conversion into the corresponding monosulfide (Figure S9), probably because of a weaker S···I interaction.

It is known that 5-halouracil (5-XU) can be incorporated into DNA in place of thymine (T).[16] The rare base-pair formation between guanine (G) and 5-XU, which is stabilized by the enol tautomer of 5-XU, favors the formation of a guanine–cytosine pair (G–C) leading to a mutation in the gene (Figure 5B, C). To understand the effect of other bases on the dehalogenation reactions, we studied the removal of the halide in 5-XU by **3** in the presence of different concentrations of adenine and guanine (Figure 5D, E). For 5-IU, the base-pair formation with adenine as well as guanine enhances the rate of deiodination, indicating that the hydrogen bonding facilitates the halogen bonding between the selenium and iodine atoms. Although the addition of adenine to 5-BrU remarkably enhances the rate of debromination, no such enhancement was observed for guanine. In fact, the rate of debromination at a higher concentration of guanine was slightly lower than that of the control rate, indicating that the stabilization of the enol form of 5-BrU disfavors the addition–elimination reactions shown in Figure 3D.

In conclusion, we described the first example of a selenium-mediated dehalogenation of halogenated nucleobases and nucleosides. Although the mechanism for the debromination is different from that of deiodination, the XB-mediated deiodination provides a simple and efficient method for the removal of iodine from IdUd and IUd without affecting the deoxyribose or ribose moiety under physiologically relevant conditions. The results described herein are important not only for understanding the dehalogenation of halogenated nucleosides in DNA and RNA, but also for the development of novel compounds for DNA modification and repair. Furthermore, this study suggests that selenium compounds may play a broader role in the metabolism of halogenated organic compounds in biology, beyond thyroid hormone deiodination. As the reactive halogen species (RHS) such as HOX (X = Cl, Br and I) produced by heme peroxidases are known to modify nucleic acids by halogenation at sites of inflammation in vivo,[17] synthetic compounds with dehalogenase activity under physiological conditions can be considered potential candidates for the development of novel anti-inflammatory drugs.

Keywords: dehalogenation · DNA repair · halogen bonds · nucleosides · selenium

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