Heavy-Atom-Substituted Nucleobases in Photodynamic Applications: Substitution of Sulfur with Selenium in 6-Thioguanine Induces a Remarkable Increase in the Rate of Triplet Decay in 6-Selenoguanine

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Supporting Information

ABSTRACT: Sulfur substitution of carbonyl oxygen atoms of DNA/RNA nucleobases promotes ultrafast intersystem crossing and near-unity triplet yields that are being used for photodynamic therapy and structural-biology applications. Replacement of sulfur with selenium or tellurium should significantly red-shift the absorption spectra of the nucleobases without sacrificing the high triplet yields. Consequently, selenium/tellurium-substituted nucleobases are thought to facilitate treatment of deeper tissue carcinomas relative to the sulfur-substituted analogues, but their photodynamics are yet unexplored. In this contribution, the photochemical relaxation mechanism of 6-selenoguanine is elucidated and compared to that of the 6-thioguanine prodrug. Selenium substitution leads to a remarkable enhancement of the intersystem crossing lifetime both to and from the triplet manifold, resulting in an efficiently populated, yet short-lived triplet state. Surprisingly, the rate of triplet decay in 6-selenoguanine increases by 835-fold compared to that in 6-thioguanine. This appears to be an extreme manifestation of the classical heavy-atom effect in organic photochemistry, which challenges conventional wisdom.

The high triplet yield makes the thiobases prime sensitizers for targeting DNA/RNA damage; however, their absorption properties limit the applications to the treatment of skin cancer cells. Replacement of the sulfur atom with selenium or tellurium should red-shift the absorption spectra into the visible with the potential additional benefit of maximizing their triplet yields. Recent quantum-chemical calculations predict that selenium- and tellurium-substituted nucleobases are highly promising photosensitizers. By using femtosecond broadband transient absorption spectroscopy, we demonstrate that selenium substitution of guanine’s carbonyl oxygen atom leads to a remarkable enhancement of the intersystem crossing (ISC) lifetime both to and from the triplet manifold. In particular, the rate of ISC to the ground state in 6-selenoguanine increases by 835-fold, or more than 2 orders of magnitude, compared to that of the 6-thioguanine (6tGua) prodrug. A strategy for harvesting the use of 6SeGua for photodynamic therapy and structural-biology applications is presented, which might enable heavy-atom-substituted nucleobases to reach their full potential.

Figure 1 shows the absorption spectra for 6SeGua and 6tGua normalized to their lowest energy π→π* transition. 6SeGua exhibits maxima at 357 and 209 nm, whereas 6tGua has maxima at 341, 254, and 204 nm. Selenium substitution broadens and red-shifts the UVA transition by 16 nm (1314.3 cm⁻¹) relative to sulfur substitution. The combined shifting and broadening of 6SeGua’s absorption give rise to a tail absorbing in the visible region. The oscillator strength of the UVA vertical transition for 6SeGua, shown in Figure 1, has a very similar magnitude to that for 6tGua, suggesting that the molar absorptivity in the UVA region is similar for both molecules. We estimate that using 6SeGua as photosensitizer could facilitate up to 90% deeper tissue treatment by UVA radiation than 6tGua (see Figure S1 and the Supporting Information (SI) for details).

Quantum-chemical computations have shown that the N⁹ tautomer of 6SeGua is most stable in solution. We have performed ground-state optimizations and subsequent vertical excitation energy calculations for the N⁹ tautomer of 6SeGua.
to characterize the excited states available to UVA excitation. A complete summary of computational results is given in Tables S1 to S3 and Figure S2. UVA excitation of 6SeGua in aqueous solution excites the S2(ππ*) state. Four lower-lying states are also available for electronic relaxation, namely, a dark S1(nπ*) state and three triplet states. Both the S2(ππ*) and T1(ππ*) states, as well as the S1(nπ*) and T2(nπ*) states, are isoenergetic within computational uncertainty. The S1(nπ*) and T1(ππ*) states are separated by an energy gap of 0.64 eV.

Femtosecond broadband transient absorption experiments were performed to unveil the electronic decay pathways following UVA excitation. The dynamics of selenium substitution at the C6 position were compared to sulfur substitution by performing successive experiments on both 6SeGua and 6tGua. Figure 2 shows the transient absorption spectra (TAS) of 6SeGua and 6tGua following 352 nm excitation. For 6SeGua, two bands appear concurrently on a sub-picosecond time scale: one with a maximum of ca. 490 nm and another with a tail apparent at 675 nm. A negative amplitude signal is observed at short wavelengths immediately after excitation. The signal overlaps with the ground-state absorption spectrum in Figure 1 and is assigned to ground-state depopulation. From a delay time of 0.6 to 104 ps, the 490 nm band blue-shifts 30 nm. Simultaneous to this shift, the

Figure 2. (a−c) TAS for 6SeGua following 352 nm excitation through a time delay of 3 ns. (b, inset) Ground-state recovery concurrent with blue-shift of the 490 nm band. (d−f) TAS for 6tGua obtained back-to-back with 6SeGua. The stimulated Raman emission band of water is observed around 400 nm within the cross correlation of the pump and probe beams. TAS were obtained in pH 7.4 PBS at room temperature under N2-purged conditions (see SI for details).
The TAS for 6tGua (Figure 2d–f) are similar to those of 6SeGua and consistent with those reported by Ashwood et al. A UV band at ca. 375 nm, a visible band at ca. 500 nm, and a ground-state depopulation signal are observed on a sub-picosecond time scale. The UV band increases in amplitude from a time delay of 0.77 to 68 ps, while the red edge of the visible band narrows. Like 6SeGua, a slight increase in negative amplitude signal occurs during this process. No apparent spectral changes occur from 68 ps through 3 ns.

Kinetics traces corresponding to the TAS in Figure 2 were analyzed globally. Figure 3 shows representative traces with fits obtained from three- and two-lifetime sums of exponential functions for 6SeGua and 6tGua, respectively. Lifetimes obtained from these fits are reported in Table 1. For both 6SeGua and 6tGua, τ1 reflects ultrafast ISC responsible for growth of the T1(ππ*) absorption bands, τ2 corresponds to spectral evolution shown in Figure 2b, and τ3 corresponds to the uniform triplet decay.

The TAS for 6SeGua and 6tGua are qualitatively similar through the second lifetime. Concurrent to ground-state depopulation, the 490 and 675 nm bands rise during τ1. We assign the rise of these two bands to T1(ππ*) absorption. This assignment is supported by the fact that 6SeGua undergoes an ultrafast ISC event following UVA excitation and similarly shows T1(ππ*) absorption with maxima at 375 and 500 nm. Heavy atom substitution enhances ISC dynamics in 6tGua relative to guanine, and selenium substitution should further enhance ISC relative to 6tGua. Indeed, the spin–orbit coupling between the S1(nπ*) and T1(ππ*) states increases nearly 5-fold for 6-selenoguanosine compared to 6-thioguanosine. 6SeGua’s ISC lifetime (τ1), which is 3 times faster than that in 6tGua, may increase the triplet yield (assuming that the lifetime of any other competitive relaxation pathway is similar in both molecules), arguably rendering 6SeGua a more effective photosensitizer when incorporated in DNA/RNA.

El-Sayed’s propensity rules suggest that ultrafast ISC should occur between singlet and triplet states of different character.27,28 Pathways that obey this rule for 6SeGua include S1(nπ*) → T2(nπ*) and S1(nπ*) → T1(ππ*) (Figure S2). Calculations for 6-selenoguanosine and 6-thioguanosine predict that ISC predominately occurs via the S1(nπ*) → T1(ππ*) pathway. However, nonadiabatic dynamics simulations indicate that ISC in 6tGua predominately occurs from the S1(nπ*) minimum to the T2(ππ*) state.29 Given the similarities between the theoretical and experimental results for 6SeGua and 6tGua in this study, the S1(nπ*) → T2(ππ*) ISC pathway cannot be excluded. Collectively, the theoretical results suggest that after excitation of 6SeGua to the S1(nπ*) state, electron population internally converts to the S1(nπ*) state before ISC to the T2(ππ*) and/or T1(ππ*) state occurs: S1 → S1 → T2 → T1.

The spectral dynamics that occur during τ2 are also similar for both 6SeGua and 6tGua. The blue-shift of the 495 nm band to 460 nm in 6SeGua and the narrowing of the visible band in 6tGua both suggest vibrational relaxation during τ2. Thus, the blue-shift is assigned to vibrational cooling of the hot T1(ππ*) state following population via ultrafast ISC (see Figures S2 and S3). This assignment is supported by the observation that the shifting process is completed prior to the uniform decay of both triplet bands, and excess vibrational energy is dissipated prior to the monotonic decay of the T1(ππ*) state.30,31

The negative amplitude signal at ca. 370 nm increases simultaneously to vibrational cooling of the T1(ππ*) state. This phenomenon is also observed for 6tGua, for which it was unclear whether the increase was due to ground-state recovery or overlap with a growing UV band (Figure 2e). For 6SeGua, this event corresponds to ground-state recovery, as the absorption change at 360 nm (ca. 2 mΔA) is an order of magnitude larger than the decline of the 495 nm band (ca. 0.2 mA). This event is therefore concurrent, yet unrelated to the vibrational cooling dynamics observed in the T1(ππ*) state. Ground-state recovery during τ3 may be achieved via internal conversion of a residual S1(nπ*) state population to the ground state. Triplet self quenching is an unlikely mechanism, as it was not supported by preliminary experiments in which the relative absorbance of the 6SeGua solution or pump excitation intensity was varied by 60 to 68%, respectively.
The most remarkable difference between 6SeGua and 6tGua is manifested in \( \tau_2 \). Kinetic traces for 6tGua reach an offset following \( \tau_2 \), indicating that further relaxation events occur on a nanosecond time scale. Previous work has shown that triplet decay occurs over 1420 ns under \( N_2 \)-saturated conditions.\(^{22}\) Due to its long-lived triplet state, photoexcited 6tGua sensitizes singlet oxygen with a 21% yield.\(^{22}\) 6SeGua instead exhibits immediate, uniform decay of both triplet bands following \( \tau_2 \). Simultaneous recovery of the ground-state bleaching signal confirms that this event represents triplet decay with a lifetime of 1.7 ns (Figure 3). In addition to the stronger T1/S0 spin–orbit coupling in 6SeGua than in 6tGua, we argue that there is competitive as the rate of triplet decay increases.

The incorporation of 6SeGua into DNA and RNA has been demonstrated\(^{35−37}\) and molecular dynamics simulations have shown that 6SeGua-substituted DNA duplexes are stable.\(^{38,39}\) Hence, regardless of the sub-2 ns triplet decay lifetime, the incorporation of 6SeGua into DNA/RNA duplexes should increase the probability that a reaction between the triplet state of 6SeGua and an adjacent nucleobase may occur. The close contact of 6SeGua to an adjacent \( \pi \)-stacked canonical nucleobase in a DNA/RNA duplex relaxes the requirement of diffusional encounter for a reaction to occur, enabling 6SeGua to photosensitize damage to DNA before triplet-state decay. Indeed, 6SeGua is a known chemotherapeutic agent.\(^{40}\)

The results presented in this study further show that it is prudent to investigate the photosensitization mechanism of 6SeGua-substituted DNA/RNA duplexes prior to studying tellurium-substituted nucleobases. The substitution with tellurium atoms is expected to further increase the rate of triplet decay to the ground state, which may further decrease the probability of a photosensitized reaction with an adjacent nucleobase compared to a selenium-substituted nucleobase.

\section*{ASSOCIATED CONTENT}

\subsection*{Supporting Information}

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.8b07665.

Materials and computational and experimental methods; data analysis and supporting results; single-electron transitions and character composition of computed excited states; Kohn−Sham orbitals corresponding to vertical electronic transitions; Jablonski diagrams; plot of depth of tissue penetration; decay-associated spectra obtained from sum of exponential model; procedure for synthesis of 6-selenoguanine; NMR spectra (PDF)

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\section*{Notes}

The authors declare no competing financial interest.

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\section*{REFERENCES}


(34) It is possible that the presence of a deprotonated anionic species of 6SeGua in equilibrium with the neutral form26 at pH 7.4 may contribute to the observed dynamics. Additional experiments in which the pH of the solution is systematically varied are necessary in order to examine the putative extent of participation of deprotonated species in the dynamics under physiological conditions. Such an investigation is currently underway in our group, but outside of the scope of the present communication.