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Synthesis and optical behaviors of 6-seleno-deoxyguanosine

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Abstract

We have developed a simple method to synthesize 6-seleno-2'-deoxyguanosine (^{Se}dG) by selectively replacing the 6-oxygen atom with selenium. This selenium-atom-specific modification (SAM) alters the optical properties of the naturally occurring 2'-deoxyguanosine (dG). Unlike the native dG, the UVabsorption of ^{Se}dG is significantly influenced by the pH of the aqueous solution. Moreover, ^{Se}dG is fluorescent at the physiological pH and exhibits pH-dependent fluorescence in aqueous solutions. Furthermore, ^{Se}dG has noticeable fluorescence in non-aqueous solutions, indicating its sensitivity to environmental changes. This is the first time a fluorescent nucleoside by single-atom alteration has been observed. Fluorescent nucleosides modified by a single atom have great potential as molecular probes with minimal perturbations to investigate nucleoside interactions with proteins, such as membrane-transporter proteins.

Keywords

selenium derivatization and fluorescence; nucleoside with single-atom modification; fluorescent DNA; RNA; nucleic acid probes

1 Introduction

Selenium derivation of nucleic acids opens a new paradigm for nucleic acids and related research, especially probing the structures and biological functions of nucleic acids and their modifications [1–12]. Se-modifications of nucleic acids can lead to novel materials and therapeutics with unique combinations of biochemical and physiochemical properties [12–15]. Selenium replacement of oxygen in the nucleobases further stimulates the investigation of structure-and-function relationships such as fluorescence, nucleobase pairing, duplex stability, structure flexibility, replication efficiency and fidelity, and polymerase recognition of the Se-modified nucleobases.

Natural nucleic acids are colorless and non-fluorescent under physiological conditions [16]. However, strong acid solution (with a pH as low as 1.0) can induce weak fluorescence in native nucleobases, such as adenine and guanine [17–19]. Such acidic conditions are not suited for probing nucleic acids and their interactions with other molecules. Numerous efforts have been made to develop fluorescent nucleosides [20–28]. Various nucleoside analogs containing extended-fluorescent nucleobases and attached fluorescent labels have been designed and used to study the native nucleic acids and their interactions with proteins.

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Unfortunately, the modifications are normally bulky and often result in fluorescent nucleobases that are colorless. These modifications can cause perturbations and are not ideal for probing nucleic acid structure and function. However, it is very challenging to design and discover minimally modified nucleosides that are colored and exhibit significant fluorescence.

Our previous report suggests that the 6-Se-modification on guanosine causes minimal perturbation in the duplex structure [29]. The synthesis and antitumor activities of 6-Se-2'-deoxyguanosine and analogues have been reported earlier [30–33]. Herein we report a simple synthesis and optical studies of ^{Se}dG (3 in Scheme 1), achieved with our SAM strategy in which the exo-oxygen at C-6 of guanosine is replaced with selenium. The synthesized ^{Se}dG is bright yellow and exhibits a strong absorption at 357 nm in water (Figure 1(a)). We also found that its UV-absorption maximum is strongly dependent on the solution pH and the polarity of the solvent. Moreover, to our surprise, we observed that ^{Se}dG exhibits pH-dependent fluorescence; maximum fluorescence was observed in solution with pH 6 ($\lambda_{\text{ex}} = 305$ nm; $\lambda_{\text{em}} = 390$ nm). The fluorescent behavior is appreciably influenced by solvent polarity. A noticeable fluorescence was observed in isopropanol ($\lambda_{\text{em}} = 320$ nm; $\lambda_{\text{em}} = 395$ nm). With minimal perturbation, this Se-nucleoside may serve as a valuable visual and optical probe for exploring the structure and dynamics of nucleic acids and their complexes with proteins and small molecules.

In particular, this fluorescent Se-nucleoside would be very useful in studying the structure and dynamics of trans-membrane transporter proteins. The analysis of structure and function of transporter proteins has recently drawn widespread attention from multidisciplinary research teams [34–37]. This information on the nucleoside-protein interaction is crucial for understanding the molecular basis of the action of drugs that target the transporter proteins, which are important drug targets [38]. A variety of biochemical and biophysical techniques have been applied to elucidate the mechanisms of active transportation in biological membranes at the molecular level [39–42]. With a fluorescence emission at 390 nm, which is red-shifted by approximately 50 nm from the emission range of most proteins, the ^{Se}dG nucleoside and its nucleotides could also present a valuable chromophore or fluorescent ligand to investigate protein-nucleic acid interactions and further contribute to molecular understanding of the protein dynamics [43].

2 Experimental

2.1 Materials and methods

The synthesis was carried out using commercially available reagents. The solid reagents were dried under vacuum and the reactions were performed under argon. The solvents were purged with argon before use. Water purified by ion exchange to a resistivity of 18.2 M Ω was used to prepare all buffers. Solvent mixtures are indicated as volume/volume ratios. Analytical thin layer chromatography (TLC, Dynamic Adsorbent) was performed using Merck Whatman 60 F₂₅₄ plates (0.25 mm thick), and visualized under UV light. A Ce-Mo staining solution (25 g phosphomolybdate, 10 g Ce(SO₄)₂ • 4H₂O, 60 mL conc. H₂SO₄, 940 mL H₂O) was used to monitor the detritylation reaction. Column chromatography was performed using Fluka silica gel (mesh size 0.040–0.063 mm). NMR spectra were recorded

on a Bruker 400 XWIN spectrometer. Chemical shift values are in ppm. High-resolution MS was determined by electrospray mass spectrometry.

2.2 Synthesis of 6-seleno-2'-deoxyguanosine, ^{Se}dG

N²-[2-(4-*tert*-Butylphenoxy)acetyl]-6-(2-cyanoethyl) seleno-2'-deoxyguanosine (2)—Compound **1** (160 mg, 0.183 mmol) synthesized by previously published procedures [29] was dissolved in acetic acid (80%, 1 mL) and stirred at 25 °C for 1 h to deprotect the 5'-DMTr group and yield **2**. Compound **2** was then purified by a silica gel column equilibrated with methylene chloride and eluted with a step-wise gradient of methanol/methylene chloride mixtures (1.0%, 2.0%, 3.0%, 4.0% MeOH in CH₂Cl₂, 200 mL each fraction) to afford **2** (95 mg, 91%; Figures S1–S3). HRMS (ESI-TOF): C₂₅H₃₀N₆O₅Se; [M + H]⁺: 575.1508 (calc. 575.1516), [M + Na]⁺: 597.1351 (calc. 597.1335); ¹H NMR (400 MHz, CD₃SOCD₃) δ: 1.25 (s, 9H, 3 × CH₃-*t*Bu), 2.30–2.35 and 2.69–2.76 (2 × m, *J*_{1'-2'} = 6.4 Hz, 2H, H-2'), 3.19 (t, *J* = 6.4 Hz, 2H, Se-CH₂-CH₂-CN), 3.53–3.62 (m, 2H, Se-CH₂-CH₂-CN), 3.53–3.62 (m, 2H) and 3.87–3.88 (br, 1H) (H-3' and H-5'), 4.43 (br, 1H, H-4'), 4.95 (s, 2H, CH₂-O), 6.35 (t, *J*_{1'-2'} = 6.4 Hz, 1H, H-1'), 6.86–6.88 (d, *J* = 8.64 Hz, 2H, CH-arom), 7.30–7.32 (d, *J* = 8.72 Hz, 2H, CH-arom), 8.59 (s, 1H, H-8), 10.82 (s, 1H, NH); ¹³C NMR (100 MHz, CD₃SOCD₃) δ: 18.37 and 18.89 (Se-CH₂-CH₂-CN), 31.22 (CH₃-*t*Bu), 33.68 (C-*t*Bu), 61.35 (C-5'), 67.12 (CH₂-O), 70.39 (C-3'), 83.31 (C-4'), 87.85 (C-1'), 113.83 and 125.99 (CH-arom), 119.84 (CN), 130.25 (C-5), 142.51 (C-8), 143.03 and 157.24 (C-arom), 148.46 (C-6), 151.57 (C-4), 155.53 (C-2), 167.23 (C=O).

6-Seleno-2'-deoxyguanosine (3)—Compound **2** (10 mg, 0.017 mmol) was treated with 0.05 M K₂CO₃ (in methanol) at 25 °C for 2 h to remove the cyanoethyl and (4-*tert*-butyl-phenoxy)acetyl protecting groups. The crude product was neutralized with HCl and purified by reverse phase HPLC. **2** was eluted at 355 nm using a combination of two solvents at varying proportions: buffer A (2.5 mM TEAAc in water) and buffer B (2.5 mM TEAAc, 50% water and 50% acetonitrile). The fractions were lyophilized to afford bright yellow 6-seleno-2'-deoxyguanosine (**3**, 2.5 mg, 42%; Figures S4–S6). HRMS (ESI-TOF): C₁₀H₁₃N₅O₃Se; [M-H]⁻: 330.0103 (calc. 330.0111); ¹H NMR (400 MHz, CD₃OD) δ: 2.34–2.40 and 2.62–2.71 (2 × m, *J*_{1'-2'} = 6.4 Hz, 2H, H-2'), 3.70–3.80 (quat. of d, 2H) and 3.98 (br, 1H, H-3' and H-5'), 4.51 (br, 1H, H-4'), 6.26 (t, *J*_{1'-2'} = 6.4 Hz, 1H, H-1'), 8.23 (s, 1H, NH); ¹³C NMR (100 MHz, CD₃OD) δ: 41.38 (C-2'), 63.28 (C-5'), 72.67 (C-3'), 85.68 (C-4'), 89.39 (C-1'), 120.91 (C-5), 133.90 (C-8), 141.37 (C-4), 147.55 (C-2), 174.51 (C-6).

2.3 UV-Vis and fluorescence measurements

UV-Vis spectra were recorded on a Varian Cary-100 Bio (UV/Vis Model 240) spectrophotometer with respect to pure solvent/buffer reference. Fluorescence measurements were performed on a Perkin-Elmer LS55 fluorescence spectrometer. The excitation and emission slit widths were fixed at 10 nm. All the solution measurements were made in 10 mM sodium phosphate buffer. The pH of the buffer was adjusted using HCl or NaOH solutions. Sample concentration was fixed at 5 μM for all fluorescence measurements (unless otherwise specified). The baseline was subtracted at all times. Both dG and ^{Se}dG stock solutions were prepared in methanol for all measurements.

2.4 HPLC analysis and purification

The crude 6-seleno-2'-deoxyguanosine was purified and analyzed by reverse-phase high-performance liquid chromatography (RP-HPLC). The purification was performed on a Welchrom C18-XB, 10 μ m, 21.2 mm $\times$ 250 mm column using Shimadzu SPD-10A VP liquid chromatography with a flow-rate of 6 mL/min [buffer A: 2.5 mM triethylammoniumacetate (TEAAc, pH 7.1) in water; buffer B: 2.5 mM TEAAc (pH 7.1) in 50% acetonitrile] and a linear gradient from 100% buffer A to 100% buffer B in 15 min. The desired peak was collected and the buffers were removed by lyophilization. All samples were analyzed on a Welchrom C18-XB column (4.6 mm $\times$ 250 mm) and measured at a flow rate of 1.0 mL/min and a linear gradient of 0 to 25% buffer B in 20 min [buffer A: 20 mM TEAAc (pH 7.1) in water; buffer B: 20 mM TEAAc (pH 7.1) in 50% acetonitrile].

2.5 pH titration curve of 6-seleno-2'-deoxyguanosine

The measurements were made at room temperature in 10 mM sodium phosphate buffer. The pH of the solution was adjusted using HCl and NaOH solutions. The absorption was measured every 0.1 pH unit between pH 7–9 and at every 0.5–1.0 pH unit between pH 4–7 and pH 9–12. The pH of each solution was measured before and after spectrum collection and the error range was within ± 0.02 pH units.

3 Results and discussion

6-Se-2'-deoxyguanosine (Scheme 1) was synthesized using 5'-DMTr-2-(4-*tert*-butyl-phenoxy)acetyl-6-(2-cyanoethyl)-seleno-2'-deoxyguanosine (**1**). The synthesis of nucleoside **1** has been previously reported [29]. Nucleoside **1** was detritylated using acid solution. The cyanoethyl (CE) and (4-*tert*-butyl-phenoxy)-acetyl (*t*-BPAC) protecting groups were removed under ultra-mild conditions (0.05 M K₂CO₃ in methanol).

Native 2'-deoxyguanosine (dG) reaches an absorption maximum at 254 nm. Introduction of selenium yields an interesting absorption profile to the nucleoside. The absorption spectrum of 2-(4-*tert*-butyl-phenoxy)acetyl-6-(2-cyanoethyl)-seleno-2'-deoxyguanosine (*N*²-*t*BPAC-6-CE-Se-dG, **2**) shows discreet bands at 256 nm and 308 nm (Figure 1(a)) in aqueous solution. When the protecting groups are completely removed, the resulting ^{Se}dG(**3**) shows absorption maximum at 357 nm and is bright yellow in color (inset: Figure 1(a)). Thus, a single atom replacement not only imparts a strong color to the otherwise colorless nucleoside, but also results in more than 100 nm red-shift in the UV absorption. This observation was confirmed by an HPLC analysis comparison of commercial dG with synthesized ^{Se}dG at 260 nm and 360 nm (Figure 1(b)). ^{Se}dG absorbs at both 260 nm (slightly) and 360 nm (strongly), whereas native dG has no absorbance at 360 nm.

Earlier reports, using X-ray crystallographic analysis and theoretical calculations, have confirmed the existence of 6-thioguanine in different tautomeric forms [44–46]. Tautomerism in 6-selenoguanine has also been investigated using high-level *ab initio* calculations. The 6-selenol form was found to be the most stable in gas phase, whereas the 6-selenone form was predicted to be the most stable in aqueous solution (Figure 2). The presence of selenium also influences the pK_a of N-1 imino proton in guanosine; the deprotonation generates the more stable 6-selenolic form [47, 48]. Interestingly, during the

pK_a measurements on ^{Se}dG , we observed that the 6-selenolic form has a distinct UV-absorption profile. The protonation and deprotonation of ^{Se}dG was monitored by UV spectrophotometry in solutions with pH values ranging from 1 to 12 (Figure 3; dG also shown for comparison). Under basic pH, the absorption maximum of ^{Se}dG shifts to 330 nm, whereas under neutral and acidic conditions the absorption maximum is 357 nm. Because the UV-absorption is strongly dependent on solution pH, we recorded the UV-Vis spectra of ^{Se}dG at different pH values and calculated the pK_a . The $pK_a(7.57 \pm 0.02)$ of ^{Se}dG was calculated from the fitted titration plot (Figure 4).

Guanine and its nucleosides and nucleotides are known to fluoresce under extreme pH conditions, such as pH 1 [16, 18]. This fluorescence is dictated by the electron distribution on the guanine base at low pH. Therefore, it is meaningful to investigate the impact of selenium modification on guanosine, especially ^{Se}dG fluorescence as a function of pH, because the electron-rich Se atom alters the dG electron distribution. For a direct comparison, we measured the fluorescence of both dG (Figure 5) and ^{Se}dG (Figure 6) in solutions with pH values ranging from 1 to 12. The pH-dependent fluorescence profile of native dG , with a fluorescence emission maximum at 395 nm, is consistent with the literature reports [16, 18]. However, the fluorescence profile of ^{Se}dG , as a function of pH, shows a different pattern (Figure 6). Unlike native dG , the excitation spectrum (excitation wavelength: 305 nm; emission maximum: 390 nm) of ^{Se}dG is significantly different than its UV-Vis spectrum (absorption maximum: 357 nm). Probably due to the fluorescence-quenching by the solvent (water) molecules, no fluorescence was observed by excitation of ^{Se}dG at 360 nm. We also found that ^{Se}dG is practically non-fluorescent under strong acid conditions (pH 1–2), probably due to the protonation of the ^{Se}dG 2-amino group (Figure 2). Higher pH (> 7.57), which causes deprotonation of the Se-nucleobase, will also decrease the fluorescence. Under other pH conditions, ^{Se}dG is fluorescent; here, the fluorescence reached a peak at pH 6.0. Thus, the ^{Se}dG fluorescence is sensitive to charges on the Se-nucleobase and such charges alter the electron delocalization on the nucleobase, which may explain why ^{Se}dG fluorescence reaches its maximum at pH 6.0. Moreover, our study indicated that ^{Se}dG is fluorescent under physiological pH 7.4. Concentration-dependent fluorescence profiles are presented in Figure 7 and S7. Due to the complicated conversion between the ^{Se}dG tautomers in protonated and deprotonated forms, it is challenging to figure out the effect of pH on the tautomerization. Currently, we are investigating how pH may affect both the tautomer formation and fluorescence intensity.

Solvent polarity is another significant parameter when considering any environmental effect on the absorption and emission spectra [23, 49–51]. Therefore, we measured the UV-Vis absorption of dG in different solvents and observed that ^{Se}dG nucleoside exhibits interesting solvatochromicity (Figure 8(a)). In general, the UV-absorption maximum is red-shifted to approximately 370 nm in polar solvents. A detailed analysis on the fluorescence of ^{Se}dG , in solvents with different polarities, was also performed (Figure 8(b, c)). We were excited to observe that ^{Se}dG is noticeably emissive in *isopropanol* and has a little or no fluorescence in the other solvents used in this study, which suggests that the solvent polarity can significantly affect the emission profile. Such polarity-dependent emission is probably due to ground-state interactions between the nucleoside and the solvent molecules. The

excitation spectra of ^{Se}dG in different solvents were monitored at 405 nm. We found that the fluorescence excitation maxima were slightly red-shifted to 320 nm in the organic solvents. Concentration-dependent fluorescence profiles for ^{Se}dG in isopropanol (Figure 9) and ethanol (Figure S8) were also obtained.

4 Conclusions

We have developed a simple strategy for 6-seleno-2'-deoxyguanosine (^{Se}dG) synthesis. We have found that ^{Se}dG exhibits distinctive optical properties although it is structurally similar to the native dG. Unlike the corresponding native, ^{Se}dG is bright yellow and fluorescent under physiological pH. This is the first time that observations of a fluorescent nucleoside by single-atom alteration have been made. We also found that the UV absorption and fluorescent emission of ^{Se}dG are sensitive to pH and solvent polarity. Therefore, the Se-modified nucleoside has great potential as a useful visual and fluorescent probe for nucleoside, nucleotide, and nucleic acid structure-function studies such as nucleoside transportation, nucleic acid-protein interaction, and nucleic-acid folding.

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Figure 1.

(A) Absorption spectra of dG (dotted line), N^2 -*t*-BPAC-6-CE-Se-dG (broken line), and ^{65}Se -dG (solid line) in water at 25 °C; inset: ^{65}Se -dG or ^{65}Se -dG (left), yellow; dG (right), colorless. (B) RP-HPLC analysis of dG and ^{65}Se -dG [monitored at 260 nm (red) and 360 nm (black)], (a) and (b): commercial dG (retention time: 13.9 min); (c) and (d): synthesized ^{65}Se -dG (retention time: 14.8 min); (e) and (f): co-injection of commercial dG and synthesized ^{65}Se -dG (retention times: 13.9 and 14.8 min, respectively).

Figure 2.
The tautomers, protonated, and deprotonated forms of Se_dG .

Figure 3. UV-Vis spectra. Absorption profiles of (a) $S^{c,d}G$ and (b) dGas as a function of pH.

Figure 4. pK_a titration plot. pH versus wavelength (nm) plot for S^e_dG; the fitted curve yields the pK_a value $7.57 (\pm 0.02)$.

Figure 5.

Fluorescence spectra of dG in aqueous solutions. (a) Excitation spectra of dG as a function of pH at 25 °C; the emission wavelength was 395 nm; (b) emission spectra of dG as a function of pH at 25 °C, with excitation at 258 nm.

Figure 6.

Fluorescence spectra of SeDG in aqueous solutions. (a) Excitation spectra of SeDG as a function of pH at 25 °C; the emission wavelength was 390 nm; (b) emission spectra of SeDG as a function of pH at 25 °C, with excitation at 305 nm.

Figure 7. Concentration-dependent fluorescence spectra of $S^e dG$ at pH 7.4. (a) Excitation spectra of $S^e dG$ at pH 7.4 and 25 $^{\circ}C$. The emission wavelength was 390 nm; (b) emission spectra of $S^e dG$ at pH 7.4 and 25 $^{\circ}C$. The excitation wavelength was 305 nm.

Figure 8.

UV-Vis and fluorescence spectra of S^e_dG in different solvents. (a) Absorption spectra of S^e_dG in different solvents at 25 °C; (b) excitation spectra of S^e_dG in different solvents at 25 °C, emission wavelength 405 nm; (c) emission spectra of S^e_dG in different solvents at 25 °C, excitation wavelength 320 nm.

Figure 9.

Concentration-dependent fluorescence spectra of $S^e dG$ in iso-propanol. (a) Excitation spectra of $S^e dG$ in *iso*-propanol at 25 °C; emission wavelength 395 nm; (b) emission spectra of $S^e dG$ in *iso*-propanol at 25 °C; excitation wavelength 320 nm.

Scheme 1.

Synthesis of 6-seleno-2'-deoxyguanosine. Reaction conditions: (i) 80% acetic acid, 25 °C, 1 h, 91%; (ii) 0.05 M K₂CO₃ in methanol, 25 °C, 2 h, 42%.