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Modulation of the Peptide Backbone Conformation by the Selenoxo Photoswitch

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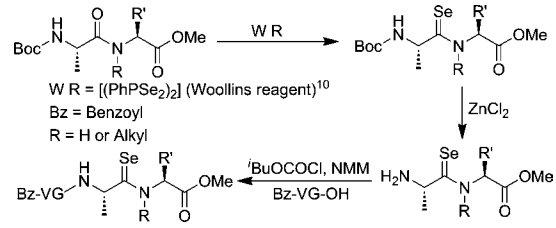
The conformation of the polypeptide backbone plays an important role in determining protein structure, stability, and dynamics.[1] External control of conformational changes in a backbone as defined by photoswitching at the one-bond level would provide a sound basis for structure−function studies of proteins. Therefore, incorporation of a photoresponsive element into the backbone, to control structure and bioactivity by a soft light switch, is of great interest. Herein, for the purpose of obtaining more stable peptide bond conformers characterized by a bathochromic shift of the $\pi-\pi^*$ band relative to the oxo or thioxo peptide UV/vis absorption, we introduced a single selenoxo peptide bond into the peptide backbone. Principally, the UV/vis spectral properties of amides allow us to trigger the *cis*/*trans* peptide bond photoswitch.[2] However, the repetitive character of the peptide bond in proteins and the low excitation wavelength of ∼200 nm required for producing excess *cis* isomer in the photostationary state (PSS) would considerably hamper the interpretation of photoisomerization experiments in oligopeptides and proteins. Azobenzene as a backbone constituent, for instance, was shown to trigger the folding and unfolding of α-helix and β-hairpin model peptides on the femtosecond time scale.[3] It should be noted, however, that the azobenzene moiety interrupts the regularly repeating pattern of a polypeptide chain, underlining the need for a chemically less perturbing backbone substitution.

Previous studies in our group have shown that irradiation of a thioxo peptide bond −C(═S)−NR− (R = H, alkyl) incorporated in peptides and proteins implements a one-bond *cis*/*trans* photoswitch, but the most effective wavelength of 254 nm is potentially destructive for sensitive protein constituents.[4] In addition, lifetime limitation in the thermal isomerization ($t_{1/2} \approx 10^2$ s at 10 °C for the −C(═S)−NH− moiety) for the photoproduced *cis* isomer is less suitable for biochemical investigations. Notably, the process of photoisomerization itself is completed within a few hundred picoseconds at high quantum yield and does not exhibit a prolonged reaction time for longer peptide chains.[5] A surprising result of the first photoswitch application of a thioxo peptide bond in a protein was that the conformation of the remote Ala^4-ψ[CS-NH]-Ala^5 moiety, which had previously not been considered important for catalysis, was implicated in the control of the catalytic machinery of RNase S. A similar approach gave clear evidence for the isomer specificity in the functional interaction of the ψ[CS-N]-Pro^3 kinin with the insect kinin receptor.[6]

The series of synthesized oligopeptides containing the seleno modification, namely Bz-Val-Gly-Ala-ψ[CSe-NH]Ala-OMe (**1**), Bz-Val-Gly-Ala-ψ[CSe-NH]Phe-OMe (**2**), and Bz-Val-Gly-Ala-ψ[CSe-N]Pro-OMe (**3**), comprised secondary selenoxo amides as well as an imidic selenoxo amide.[7] The thioxo tetrapeptide congeners were incorporated in this work for the sake of comparison.

Selenoxo amides are well-known intermediates in heterocycle synthesis[8] and were found to be unstable in solution.[9] However, the compatibility between selenoxo substitution and peptide synthesis protocols has never been investigated, and selenoxo peptides have not been described previously. Based on the strategy shown in Scheme 1, we synthesized the N-protected selenoxo tetrapeptide methyl esters in acceptable yields, indicating that the selenoxo peptide moiety is stable under conditions of modified N-Boc chemistry and mixed anhydride coupling (Table S1). Peptide **1** showed 7% contamination with the C_α epimer of the Se-containing residue, which was probably caused by the selenation procedure using Woollins' reagent but did not impede measurements presented here (Figure S1). Surprisingly, selenoxo peptide **1** proved to be stable at pH 4.8, 6.5, and 7.6 over a 4-day storage period. Only 5% selenium−oxygen exchange was observed after a 4-day period of incubation at pH 11, accompanied by partial hydrolysis of the C-terminal methyl ester group.

Scheme 1. Synthesis of Selenoxo Peptides



UV/vis spectroscopy of peptide **1** in phosphate buffer, pH 6.5 (Figure 1) showed a strong absorption band centered at 294 nm ($\pi-\pi^*$ transition, $\epsilon = 11280$ M^{-1} cm^{-1}) and a weak band centered at 366 nm (n−π^* transition, $\varepsilon = 177$ M^{-1} cm^{-1}), which is approximately 30 nm red-shifted for each band compared to corresponding thioxo peptides. ^{1}H NMR spectra of the secondary selenoxo peptides indicated a *cis* isomer content of <1% in the ground state (Figure S2). Excitation of peptide **1** at 286 nm served to achieve the PSS characterized by a *cis* content of 11.2%. The increased *cis*/*trans* ratio was accompanied by a small bathochromic shift of the $\pi-\pi^*$ transition that completely disappeared during thermal re-equilibration. Full recovery of the original UV/vis spectra even after four cycles of irradiation/equilibration indicated no tendency of the selenoxo peptides to experience photodecomposition during irradiation. The reconstructed UV spectrum of the pure *cis* isomer of peptide **1** showed an absorption maximum at 310 nm ($\epsilon = 12\,336$ M^{-1} cm^{-1}), indicating an isomer-specific shift of 16 nm (Figure S3). This is similar to the magnitude observed for a thioxo peptide (*trans*: $\lambda_{max} = 265$ nm, $\varepsilon_{265} = 12\,000$ M^{-1} cm^{-1}; *cis:* $\lambda_{max} = 281$ nm, $\varepsilon_{281} = 15\,215$ M^{-1} cm^{-1}).[6a] Peptide **2** shows a spectroscopic behavior similar to that of peptide **1**, with a first-order rate constant of $(4.4 \pm 0.1) \times 10^{-4}$ s^{-1} for thermal *cis*/*trans* isomerization. The resulting half time of ∼1600 s showed that selenoxo substitutions will make a suitable adjustment that satisfies the conditions for the evaluation of isomer specificities in biochemical reactions.

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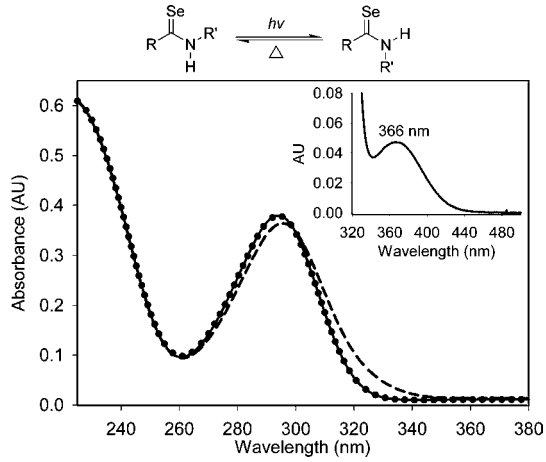

Figure 1. UV/vis characterization of photoisomerization for peptide **1** (3.4×10^{-5} mol L^{-1} peptide in 3.3×10^{-2} mol L^{-1} phosphate buffer, pH 6.5, 10 °C). Equilibrated peptide (solid line), peptide after 5 min of irradiation at 286 nm (dashed line), and peptide after four cycles of irradiation/equilibration (dotted line). Inset: n−π* transition.

Theoretical calculations have indicated that both the electron delocalization from N to chalcogen and the planarity of the chalcogen amide bonds increase in the order O < S < Se.[11] This translates into the heights of the rotational barriers, with a value of 20.5 kcal mol^{-1} for the selenoxo peptide **1** followed in decreasing order by the corresponding thioxo (19.4 kcal mol^{-1}) and oxo peptides (17.1 kcal mol^{-1}, Table S2). The decelerating influence of the selenium substitution on the *cis*/*trans* isomerization is driven entirely by a large unfavorable activation entropy term (Table S2).

Table 1. Characterization of the Peptide Bond *Cis*/*Trans* Isomerization of Thioxo and Selenoxo Peptides

Peptides	$k_{c/t}$ $(s^{-1})^a$	% cis^b	pK_a^{c}
Bz-VGAψ[CS-NH]A-OMe	$(6.7 \pm 0.7) \times 10^{-3}$	−^{d}	11−13^e
Bz-VGAψ[CS-NH]F-OMe	$(2.6 \pm 0.2) \times 10^{-3}$	13.5	11−13^e
Bz-VGAψ[CSe-NH]A-OMe **1**	$(9.9 \pm 0.1) \times 10^{-4}$	11.2	9.5
Bz-VGAψ[CSe-NH]F-OMe **2**	$(4.4 \pm 0.1) \times 10^{-4}$	20.5	9.8
Bz-VGAψ[CSe-N]P-OMe **3**	1.3×10^{-5} ^{f}	37.4	−

^{a} Determined at 10 °C. ^{b} Extrapolated to PSS. ^{c} pK_a refers to NH dissociation. ^{d} Not observable by NMR. ^{e} Reference 12. ^{f} Extrapolated from the Eyring plot.

As peptidyl prolyl bonds play an important role in protein folding and signaling,[13] we characterized the photoisomerization of the imidic selenoxo peptide bond in peptide **3**. The UV/vis absorption bands at 302 nm (ε = 11 024 $M^{-1}cm^{-1}$) and 373 nm (ε = 272 $M^{-1}cm^{-1}$) were attributed to the $\pi-\pi$* and n−π* transitions, respectively (Figure S5). In the ground state, peptide **3** contained ~8.5% *cis* isomer. After irradiation at 296 nm for 10 min, the *cis* content increased to 37.4% (Table 1). However, unlike the case for peptides **1** and **2**, there was no obvious band shift for peptide **3** after photoswitching. It may result from the similar intramolecular environment (alkyl groups) of C═Se in both the *trans* and *cis* isomer, whereas the NH proton, in the case of secondary selenoxo peptides, makes an isomer-specific difference. It is noteworthy that the *cis* isomer was especially stable, with a half time of isomerization of 70 min at 40 °C. The linear Eyring plot extrapolated to 10 °C indicated a rotational barrier of 22.9 kcal mol^{-1}.

An additional feature peculiar to secondary amide selenoxo peptides was a decrease of the 294 nm absorbance at pH 11.3 while a new absorption band appeared near 260 nm, exhibiting an isosbestic point at 275 nm (Figures S6 and S7). Moreover, the original spectrum recovered when titrated from pH 11.3 to 6.3. No such spectral change was observed for peptide **3** (Figure S8). Presumably, the −C(═Se)−NH− form is converted to its selenolate tautomer −C(−Se^-)═N−, while peptide **3** is unable to undergo this reaction. The pH dependent −C(═Se)−NH− absorbances revealed pK_a values of 9.5 and 9.8 for peptides **1** and **2**, respectively.

In conclusion, we report the first synthesis of selenoxo peptides. Our biophysical studies demonstrate that they feature a surprisingly high stability in aqueous buffer, both in the dark and under the conditions of photoswitching by UV irradiation. The large Se-mediated bathochromic shifts of the $\pi-\pi$* transition permitted us to trigger a ≫10-fold increase of the *cis* peptide bond isomer population in the PSS using irradiation with soft UV light. The concentration transients of the isomers in the dark after switching off the light are stable enough to allow for isomer specific interactions at the one-bond level to be detected. Therefore, the selenoxo peptide bond may serve as a unique probe to study isomer-specific contributions in biochemical reactions such as protein folding, receptor−ligand interactions, and ion channel gating.

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Supporting Information Available: Full experimental details, including peptide synthesis and analysis, UV spectroscopic characterizations, and NMR data. This material is available free of charge via the Internet at http://pubs.acs.org.

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