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Direct photomodulation of peptide backbone conformations†

Jianzhang Zhao‡,* Dirk Wildemann, Mario Jakob, Carolyn Vargas and Cordelia Schiene-Fischer*
Max Planck Research Unit for Enzymology of Protein Folding, Weinbergweg 22, 06120 Halle/Saale, Germany. E-mail: j.zhao@bath.ac.uk

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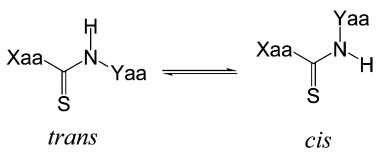
Significant photoswitching ability is found for secondary thioxopeptide bonds and can be used for the photomodulation of the backbone conformation of peptides or proteins.

Modulation of the backbone conformation of peptides or proteins has attracted much attention because the recognition, stability and reactivity of these biopolymers are conformer specific.[1,2] Photoswitching of the backbone conformation of peptides is one of the representative methods that can be used to achieve this goal. However, the reported molecular motifs concerning this aspect usually involve a bulky non-amino acid organic moiety, which is photoresponsive, such as azobenzene or spiropyran groups.[3,4] Although some promising results were obtained, these covalently linked hybrid systems also have some intrinsic disadvantages, such as limited biocompatibility and the conformational strain exerted by long-range transmission from the isomerizing bond to the peptide backbone *via* a number of flexible bonds. This renders the final effect of the isomerization of the photoresponsive moiety on the peptide backbone conformation to be almost unpredictable. Therefore, the challenge for the photomodulation of a peptide backbone conformation is to find a photoresponsive constituent, in which the peptide bond at a specific site can be switched to the *cis* or *trans* conformation, and at the same time, to make the modified peptide behave like native ones. Unfortunately, the existing methods fail to meet this critical requirement.[3,4] In this study, however, the secondary thioxopeptide bond turns out to be the most probable candidate for this purpose.

The thioxopeptide bond –CS–NR– (R = H, alkyl) represents an isosteric replacement of the normal peptide bond with only a slight change in the electron distribution in the ground state. This single-atom O/S substitution in a biologically active oligopeptide is of considerable interest because of the effect on conformation restriction, enhanced proteolytic stability and modulated activity and selectivity.[5,6] The imidic thioxopeptide bonds has been shown to be photoswitchable.[7] Now we will show that this photoswitching occurs with secondary thioxopeptide bonds (Scheme 1) in five representative thioxopeptides, F-ψ[CSNH]-A (**1**), A-ψ[CSNH]-F (**2**), A-ψ[CSNH]-A (**3**), Ac-A-ψ[CSNH]-A-NH_2 (**4**) and H^{12}-thioxo S-peptide (**5**, with 20 amino acid residues).[8] In addition to the photoswitching ability, the rotational barriers of thioxopeptide bonds were also studied.



Scheme 1 Schematic representation of *cis–trans* conformers of a secondary thioxo amide peptide bond (Xaa and Yaa: amino acid residues).

Peptidyl prolyl bonds play an important role in protein folding. However, recent study shows that unfolded proteins can also be kinetically trapped by the *cis–trans* isomerization of secondary peptide bonds[9] and a family of enzymes have been found to catalyse this kind of isomerization. It is highly possible that this *cis–trans* isomerization can also be crucial for bioactivity and biorecognition, which was thought to be associated only with peptidyl prolyl bond *cis–trans* isomerisation. Therefore, our study on the photoswitching of secondary peptide bonds might be very interesting for probing the conformation-activity correlations of the secondary peptide bonds.

Spectral isomer-specificity has been found for β-thioxo peptides,[10] *N*-methylthioacetamide[11] and imidic thioxopeptide bonds.[7] For example, the UV absorption maxima of the *cis* conformers are bathochromically shifted compared to the *trans* conformers, and the CD spectral peaks of the *cis* and *trans* conformers show the opposite sign.[7,11] The UV/Vis spectrum of peptide **1** changed significantly upon irradiation (Fig. 1), indicating that the amount of *cis* conformers increased with irradiation. The well-anchored isosbestic point at 274 nm and the reversibility of the UV/Vis spectrum changes show that the photoswitching is reversible and only the *cis* and *trans* conformers were involved. Similar spectra were recorded for peptides **2–5**. These spectral changes are due to the geometric variation of the peptide bond and the reorganization of the solvent molecules around the peptide bonds. In contrast to *N*-methylthioacetamide, no photodecomposition was observed for these peptides. For the normal oxo secondary peptide bond, however, significant photodecomposition occurs[12] and it is meaningless to attempt to increase the *cis* conformer by photoswitching, from the point of view of an isomer–activity correlation study, because the intrinsic rotational barrier of these peptide bonds is too low to trap the *cis* conformers.[13]

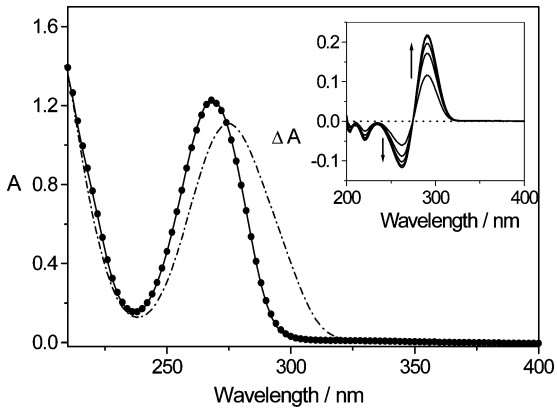


Fig. 1 UV/Vis absorption spectra of peptide **1** before and after irradiation. 1.4×10^{-4} mol dm^{-3} **1** in 5.0×10^{-2} mol dm^{-3} sodium phosphate buffer (pH 7.0), 16 °C. Before irradiation (solid line), after 3 min of irradiation at 254 nm (dot-dash line), re-equilibrated peptide after three cycles of irradiation-reequilibration (solid circles). Inset: evaluation time course of the difference UV spectrum during irradiation (*cis* isomer has a stronger absorbance in the 275–325 nm region, which was a maximum at 290 nm).

† Electronic Supplementary Information (ESI) available: details of the monochromatic light source, kinetic parameters, UV-vis spectroscopy and capillary electrophoresis. See http://www.rsc.org/suppdata/cc/b3/b309927j/

‡ Present address: Department of Chemistry, University of Bath, Bath, UK BA2 7AY

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The ability of capillary electrophoresis (CE) to separate *cis–trans* thioxo prolyl bond isomers has been established[14] and was used to prove the increase in the *cis* isomers in the photostationary state (PSS) (Fig. 2). By recording the CE curve at 274 nm (isosbestic point of the UV absorption of the *cis* and *trans* conformers), the *cis* : *trans* ratio can be determined from the CE peak areas. The separation mode of the *cis–trans* isomers was found to be pH-dependent. By extrapolating to the PSS, the *cis* concentration is corrected to 11.9% (pH 3.0, Fig. 2a), 20.0% (pH 4.0, Fig. 2b) and 19.0% (pH 7.0, Fig. 2c). Similar CE curves were observed for peptides **2**–**4**. This result is of great interest for the conformation–activity correlation study of the secondary peptide bonds, for which the *cis* conformers could only previously be obtained with the synthetically demanding conformation restriction method. But now with our results, the thermodynamically unfavourable *cis* conformers become readily available by only a one atom substitution of the normal peptides.

With a wavelength-continuously-tunable monochromatic light source, peptide **1** was found to be dual-directionally photoswitchable (Fig. 3). In one run, the peptide was irradiated at 270 nm until a PSS was established. Under our experiment conditions, this process is characterized by an apparent first-order rate constant of $k_{270}^* = (4.05 \pm 0.02) \times 10^{-3}\ s^{-1}$. Then, the light was switched off and the thermal relaxation gives a first-order rate constant of $k_{c/t} = (9.78 \pm 0.02) \times 10^{-4}\ s^{-1}$. In another run, after the PSS was achieved with 270 nm irradiation, however, the irradiation wavelength was switched to 291 nm, and the *cis* isomers switched back to *trans* isomers and a new PSS was established. Notably, the rate for this process ($k_{291}^* = (1.70 \pm 0.03) \times 10^{-2}\ s^1$) is accelerated by 17-fold compared to the free thermal relaxation. A further decrease of the *cis* population was achieved with 310 nm irradiation ($k_{310}^* = (9.23 \pm 0.74) \times 10^{-3}\ s^{-1}$). Similar results were also found for peptides **2–5**. For the imidic thioxopeptide bonds, however, it is impossible to practise such dual-directional photoswitching, due to their UV/Vis absorption behaviour.[7] For the bioactive peptides, modulation of the conformation means the regulation of bioactivity. Preliminary results show the activity of the modified RNase (from the S-protein and the thioxo S-peptide **5**) can be modulated by photoswitching (the *cis* conformer registered a 17.0% lower hydrolysis activity with cytidine-2′,3′-cyclic monophosphate).

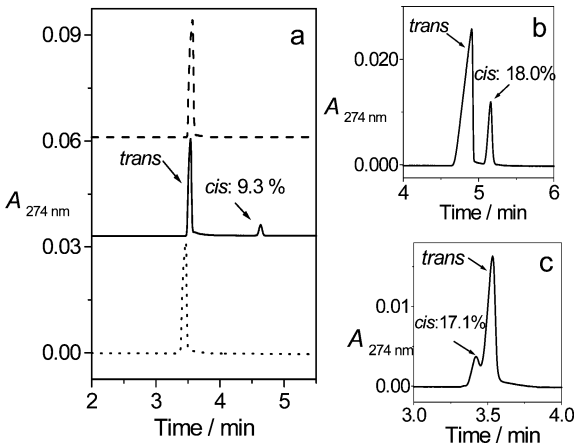

Fig. 2 Capillary electropherogram (CE) of peptide **1**. a) Before irradiation (dotted line), the peptide irradiated at 254 nm for 2 min (solid line) and the re-equilibrated peptide (dashed line). 1.4×10^{-2} mol dm^{-3} of **1** in 5.0×10^{-2} mol dm^{-3} sodium phosphate buffer, pH 3.0, 2 °C. CE analysis at pH 4.0 (b) and pH 7.0 (c). For clarity, only the CE of the irradiated peptide is shown in (b) and (c).

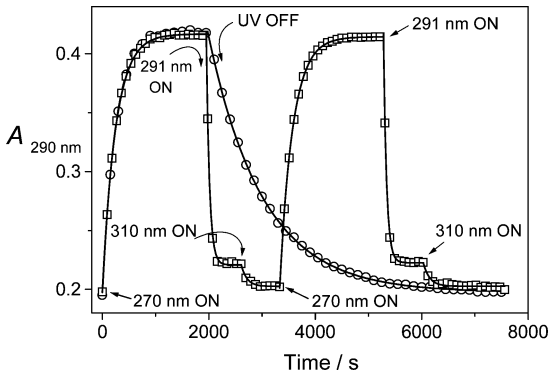

Fig. 3 Dual-directional photoswitch of peptide **1**, monitored by UV absorption at 290 nm. Two runs are presented (270 nm irradiation and UV switch off: circles; 270 nm irradiation, 291 nm irradiation, 310 nm irradiation: squares). Irradiation wavelength changes are marked on the curves. The solid lines represent nonlinear single exponential fits. 1.4×10^{-4} mol dm^{-3} Peptide **1** in 5.0×10^{-2} mol dm^{-3} sodium phosphate buffer, pH 7.0, 16 °C.

With the photoswitch method, an improved signal-to-noise ratio was obtained (Fig. 3) and as a result, reliable kinetic data for the *cis–trans* isomerization of the thioxo peptide bonds were accessible.†,[12,13] It was shown for the first time, with a facile yet accurate experimental approach, that the rotational barrier of the peptide bonds will be increased with thioxylation.[15]

In conclusion, significant photoswitching ability was found for secondary thioxo amide peptide bonds, which can readily be introduced to any interesting site within a polypeptide chain by chemical derivatization (as shown by peptide **5**), and was used to characterize their *cis–trans* isomerization. This approach can be used to establish a specific peptide bond with a *cis* or *trans* conformation. The isomer-specificity of the spectroscopies, large increase of the *cis* isomers in the PSS, slow thermal re-equilibration rates and the site-specificity of the photoswitching effect of the secondary thioxopeptide bonds should offer an unprecedented opportunity for studying the conformation–activity correlations of peptides or proteins.

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Notes and references

1 M. Brandsch, F. Thunecke, G. Kullertz, M. Schutkowski, G. Fischer and K. Neubert, *J. Biol. Chem.*, 1998, **273**, 3861.
2 G. Fischer, *Chem. Soc. Rev.*, 2000, **29**, 119.
3 I. Willner, *Acc. Chem. Res.*, 1997, **30**, 347.
4 R. Behrendt, C. Renner, M. Schenk, F. wang, J. Wachtveitl, D. Oesterhelt and L. Moroder, *Angew. Chem., Int. Ed.*, 1999, **38**, 2771.
5 S. Yao, R. Zutshi and J. Chmielewski, *Bioorg. Med. Chem. Lett.*, 1998, **8**, 699.
6 H.-T. Le, R. Michelot, M. Dumont, V. K. Shukla, M. Mayer, K. P.-P. Nguyen, H. Ruan and S. Lemaire, *Can. J. Physiol. Pharmacol.*, 1997, **75**, 9.
7 R. Frank, M. Jakob, F. Thunecke, G. Fischer and M. Schutkowski, *Angew. Chem., Int. Ed.*, 2000, **39**, 1120.
8 D. A James, D. C. Burns and G. A. Woolley, *Protein Eng.*, 2001, **14**, 983.
9 G. Pappenberger, H. Aygun, J. W. Engels, U. Reimer, G. Fischer and T. Kiefhaber, *Nat. Struct. Biol.*, 2001, **8**, 452.
10 T. Sifferlen, M. Rueping, K. Gademann, B. Jaun and D. F. Seebach, *Helv. Chim. Acta*, 1999, **83**, 2067.
11 C. Kato, H. Hamaguchi and M. Tasumi, *J. Phys. Chem.*, 1985, **89**, 407.
12 J. S. W. Holtz, P. Li and S. A. Asher, *J. Am. Chem. Soc.*, 1999, **121**, 3762.
13 C. Schiene-Fischer and G. Fischer, *J. Am. Chem. Soc.*, 2001, **123**, 6227.
14 S. Meyer, A. Jabs, M. Schutkowski and G. Fischer, *Electrophoresis*, 1994, **15**, 1151.
15 D. Lauvergnat and P. C. Hiberty, *J. Am. Chem. Soc.*, 1997, **119**, 9478.