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Switch Peptide via Staudinger Reaction

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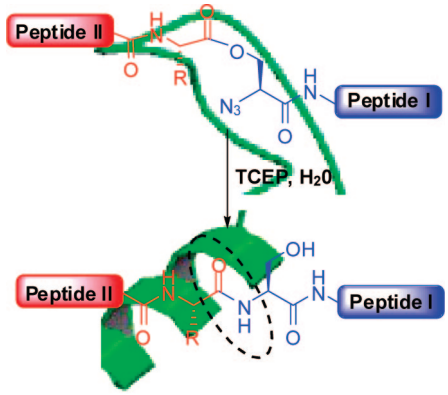
ABSTRACT



A new transformation based on the Staudinger reaction is described, and its application in the design of a novel switch element to control peptide folding is demonstrated. We found that the azide switch is activated rapidly in water to promote acyl transfer using tris(2-carboxyethyl)phosphine hydrochloride (TCEP) via the Staudinger reaction. Our findings expand the repertoire of uses of the Staudinger reaction in chemical biology and the number of available triggers for use in switch peptides.

The Staudinger reaction, discovered nearly a century ago, occurs between a phosphine and an azide to form an aza-ylide.[1] This transformation has been exploited in several reactions of high synthetic importance wherein the aza-ylide intermediate, with its highly nucleophilic nitrogen atom, serves to trap various electrophiles.[2] Staudinger ligation is one example of such reactions, in which an ester moiety is placed within a phosphine structure to capture the nucleophilic aza-ylide by intramolecular cyclization, leading to a stable amide bond.[3]

While the aza-ylide intermediate is known to be stable in organic solvents, it tends to hydrolyze rapidly in aqueous media to furnish the primary amine and the phosphine oxide products. In the traceless Staudinger ligation, however, the reduction of the azide to amine is a competing side reaction, thus reversing the capture step and leading to two peptide fragments.[4] We envisioned that placing an electrophile and an azide rather than the former and phosphine within the same molecule would allow for acyl transfer and amide formation that can be triggered upon selective azide reduction.

One possible application of this modified Staudinger reaction would be in designing new switch elements for use in "switch peptides" wherein a switch element, based on

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(1) Staudinger, H.; Meyer, *J. Helv. Chim. Acta* **1919**, *2*, 635–646.
(2) (a) Gololobov, Y. G.; Kasukhin, L. F. *Tetrahedron* **1992**, *48*, 1353. (b) Nyffeler, P. T.; Liang, C. H.; Koeller, K. M.; Wong, C. H. *J. Am. Chem. Soc.* **2002**, *124*, 10773–10778. (c) Kurosawa, W.; Kan, T.; Fukuyama, T. *J. Am. Chem. Soc.* **2003**, *125*, 8112–8113.
(3) Saxon, E.; Bertozzi, C. R. *Science* **2000**, *287*, 2007–2010.
(4) (a) Soellner, M. B.; Dickson, K. A.; Nilsson, B. L.; Raines, R. T. *J. Am. Chem. Soc.* **2003**, *125*, 11790–11791. (b) Lin, F. L.; Hoyt, H. M.; Van Halbeek, H.; Bergman, R. G.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2005**, *127*, 2686–2695. (c) Tam, A.; Soellner, M. B.; Raines, R. T. *J. Am. Chem. Soc.* **2007**, *129*, 11421–11430.

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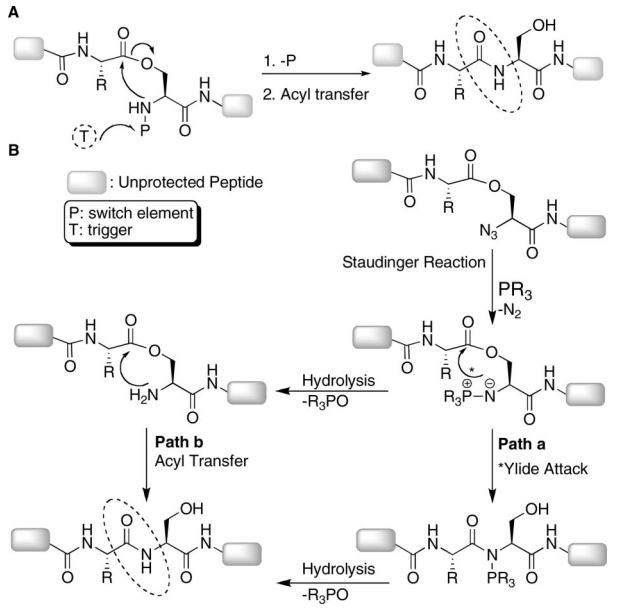
protection of the α-amino group, is placed adjacent to an ester bond (electrophile) to temporally disrupt peptide secondary structure and/or function (Scheme 1A).[5] Upon induction of acyl transfer (S_{off} to S_{on} state), via specific triggers, the peptide regains its native backbone, secondary structure, and function(s). These findings combined with recent success in the application of orthogonal triggering strategies to induce and/or reverse secondary structure transitions and peptide self-assembly have created greater interest in the development of new switch elements that could introduce greater flexibility and specificity.[5] The desired switch should be stable under physiological conditions, can efficiently be introduced into peptide systems, is amenable to rapid activation by specific and selective triggers under mild conditions, and can be operated orthogonally with other switch elements. Here we report on a new transformation based on the Staudinger reaction and its application to the development of a new switch element based on the azido ester motif (Scheme 1B), which fulfills all of the criteria mentioned above.

Scheme 1. (A) General Concept of Switch Peptides; (B) Principle of Switch Peptide via Staudinger Reaction



Activation of switch elements, based on the intramolecular O−N acyl transfer in situ, regardless of the nature of the trigger (e.g., chemical, enzymatic, photolytic) occurs through regeneration of the primary amine followed by O−N acyl transfer via a five-membered ring intermediate to yield the native amide bond.[6] By studying the Staudinger reaction, we recognized that replacing the -NH_2 of depsipeptide by an azide could allow for amide formation that can be triggered upon selective azide reduction (Scheme 1B, path b). However, a mechanism in which the aza-yilde nucleophilic amine is attacking the ester carbonyl should not be excluded (Scheme 1B, path a).

To examine the effectiveness of the reduction of the α-azido group to the amine functionality at the N-terminus, we prepared peptide **1** (N_3-SLYRAG) as a model system using standard Fmoc solid-phase peptide synthesis (SPPS) and azido-glycine as the N-terminal amino acid. Although various methods are known for the conversion of azide to amine in organic solvents, this transformation has not been fully exploited and developed under physiological-like conditions. To search for such conditions, we examined several potential azide reducing agents (e.g., borohydride reagents and propanedithiol) suitable for peptidic systems in water.[7]

However, none of these reagents gave satisfactory results, as the reactions were sluggish and gave low yields (∼15%), despite extending the reaction time for 10 h. Inspired by the known ability of substituted phosphines to reduce an azide, we tested the water-soluble phosphine TCEP.[8] We found that TCEP is an excellent azide-reducing reagent of azido-peptide in water. Reduction of **1** to give peptide **2** (H_2N-SLYRAG) was completed within 6 min using TCEP (10 equiv) in 200 mM phosphate buffer, pH 7.5 (see Supporting Information). The reduction with TCEP was dependent on the pH of the reaction mixture, increasing at a high pH, which is consistent with the p*K* value of 7.6 of TCEP.[9] Moreover, the reaction rate increased with higher amounts of TCEP, 10 equiv of which gave the best results with $t_{1/2}$ of ∼60 s.

Encouraged by these results, we designed several model peptide systems (**3**−**5**) that included the depsipeptide unit with the azido group to examine its utility as a switch element based on the intramolecular O−N acyl transfer. The synthesis of the model peptide with the azide functionality was carried out fully on solid support (see Supporting Information).

Having these precursors at hand, we then focused on the O−N acyl transfer step using the TCEP reduction conditions. Thus, peptide **3** was dissolved in 200 mM phosphate buffer, pH 7.5 followed by the addition of TCEP (10 equiv). The progress of the reaction was followed using HPLC and mass spectrometry by monitoring the appearance of the product, which showed a decrease of 26 mass units upon reduction of the azide product (Figure 2). Under these conditions, the reaction was successfully completed within 7 min with $t_{1/2}$ of ∼80 s, thus the azide switch fulfils an important criterion of the desired switch element. Authentic samples of the amine intermediate **6** and the product **7** were prepared for

(5) (a) Sohma, Y.; Yoshiya, T.; Taniguchi, A.; Kimura, T.; Hayashi, Y.; Kiso, Y. *Biopolymers* **2007**, *88*, 253–262. (b) Tuchscherer, G.; Chandravarkar, A.; Camus, M.-S.; Berard, J.; Murat, K.; Schmid, A.; Mimna, R.; Lashuel, H. A.; Mutter, M. *Biopolymers* **2007**, *88*, 239–252. (c) Santos, S. D.; Chandravarkar, A.; Mandal, B.; Mimna, R.; Murat, K.; Saucède, L.; Tella, P.; Tuchscherer, G.; Mutter, M. *J. Am. Chem. Soc.* **2005**, *127*, 11888–11889. (d) Taniguchi, A.; Sohma, Y.; Kimura, M.; Okada, T.; Ikeda, K.; Hayashi, Y.; Kimura, T.; Hirota, S.; Matsuzaki, K.; Kiso, Y. *J. Am. Chem. Soc.* **2006**, *128*, 696–699.

(6) This step is quite reminiscent of the S- to N-acyl transfer intrinsic to native chemical ligation: Dawson, P. E.; Muir, T. W.; Clarklewis, I.; Kent, S. B. H. *Science* **1994**, *266*, 776–779.

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(9) Cline, D. J.; Redding, S. E.; Brohawn, S. G.; Psathas, J. N.; Schneider, J. P.; Thorpe, C. *Biochemistry* **2004**, *43*, 15195–15203.

comparison. The intermediate **6** and product **7** have identical mass but exhibited different retention time with the amine intermediate eluting earlier on a C18 reverse phase column (Figure 2). The peak appearing slightly before the product corresponds to the amine intermediate (**6**) and elutes with the same retention time as the authentic sample. The reaction of the peptide bearing the more sterically hindered amino acid (e.g., Ala (**4**) and Val (**5**), Figure 1) on the ester moiety

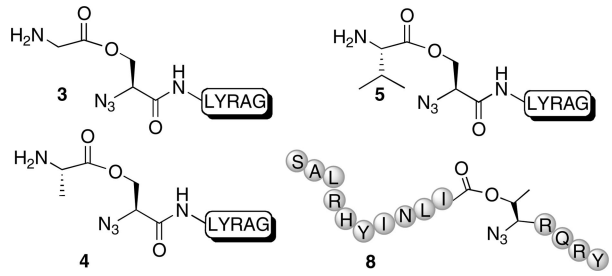

Figure 1. Model peptides used in this study.

gave slightly slower rates (within 1- to 2-fold) and similar pH-dependent properties, indicating that steric hindrance does not affect rate of the reaction dramatically.

In principle, part of the O−N acyl transfer product could be obtained through an attack of the nucleophilic amine of the aza-ylide formed between the azide and TCEP, resembling Staudinger ligation (Scheme 1B, path a). Recent studies by Arndt and co-workers have shown that when similar peptide systems were exposed to PPh_3, in THF or THF/H_2O (4:1) aza-Wittig ring-closure products were obtained in high yields.[11] In these studies, no premature hydrolysis of the putative iminophosphorane intermediate was observed, i.e., amine, even in the presence of 25% v/v water. Our results show that when TCEP is applied, under physiological-like conditions, the cyclized product could not be detected by HPLC or mass spectrometry. This probably occurs as a result of the instability of the aza-ylide formed with TCEP in water leading to the primary amine, which subsequently attacks the ester carbonyl followed by the O−N acyl transfer (Scheme 1B, path b). Moreover, Raines and co-workers reported that the use of the water-soluble bis(2-carboxyethyl)thiomethyl phosphine, resembling the structure of TCEP, to mediate traceless Staudinger ligation in water predominately afforded the amine byproduct.[4c] These studies coupled with our results of the rapid reduction of peptide **1** and the appearance of the amine intermediate in the conversion of peptide **3** to **7** support that the O−N acyl transfer product is obtained primarily through an attack of the reduced primary amine (Scheme 1B, path b). The rapid reduction of the azide to the amine intermediate (<1 min) followed by a slower O−N acyl transfer step (~6 min) suggests that the latter step is rate-limiting.

(10) Low, W.; Kang, J.; DiGruccio, M.; Kirby, D.; Perrin, M.; Fischer, W. H. *J. Am. Soc. Mass. Spectrom.* **2004**, *15*, 1156–1160.

(11) Riedrich, M.; Harkal, S.; Arndt, H. D. *Angew. Chem., Int. Ed.* **2007**, *46*, 2701–2703.

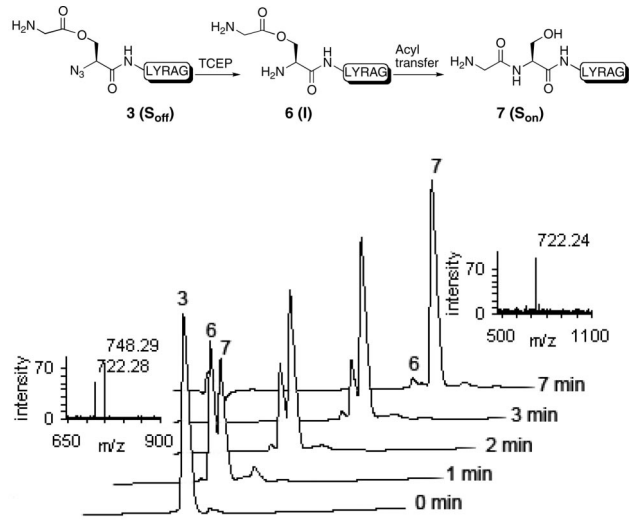

Figure 2. Analytical HPLC of the time-course for the Staudinger reaction of model peptide **3** with a mass of 748.29 Da (calculated 748.36 Da). The MALDI-TOF analysis of peptide **3** also shows a mass of 722.28 Da corresponding to azide fragmentation, with a loss of N_2 and an addition of two hydrogen atoms, during mass analysis.[10] The product of the O−N acyl transfer reaction **7** and the amine intermediate **6** shows a mass of 722.24 Da (calculated 722.39 Da).

Encouraged by the results obtained with the model peptides **3**−**5**, we sought to explore the application of our depsipeptide unit with the azide switch element to control secondary structure transitions. For this purpose we chose the C-terminal peptide (**9**, SALRHYINLITRQRY) of the neuropeptide analogue NPY as a model system.[12] This peptide represents the minimal chain length for retaining significant binding capacity to NPY receptor Y2 with the α-helical state as the bioactive conformation. A switch peptide **8** (Figure 1) was designed to include the isopeptide unit with the azido functionality at the Ile31-Thr32 position. The synthesis of peptide **8** with the azide moiety was fully achieved on solid support, which after cleavage and purification steps gave the desired peptide in 22% isolated yield (Supporting Information). It is noteworthy that despite the fact that the esterification in this case was performed between two sterically hindered amino acids (Thr and Ile) the synthesis was efficient and low racemization levels (<5%) were observed (Supporting Information).

Initially, we studied the kinetics of the reduction and the acyl transfer by LCMS. Similar to the model systems, the reduction step was rapid wherein a complete disappearance of the starting material **8**, resulting in the amine intermediate, was observed in 3 min (Supporting Information). Despite the fact that the acyl transfer step occurs at a sterically hindered center, i.e., Ile-Thr, the rate of the reaction was still rapid, and complete acyl transfer, to give the unmodified NPY analogue **9**, was accomplished within 30 min. As measured by circular dichroism (CD), the resulting switch peptide adopts a random-coil conformation; however, upon activa-

(12) Mutter, M.; Chandravarkar, A.; Boyat, C.; Lopez, J.; Dos Santos, S.; Mandal, B.; Mimna, R.; Murat, K.; Patiny, L.; Saucede, L.; Tuchscherer, G. *Angew. Chem., Int. Ed.* **2004**, *43*, 4172–4178.

tion with TCEP a random-coil to α-helix transition was observed (Figure 3A). The structural change of NPY monitored by CD at 220 nm yields $t_{1/2}$ of ~6 min at 37 °C in presence of 1 mM TCEP (Figure 3B), which is in a good agreement with the time of conversion as measured using HPLC.

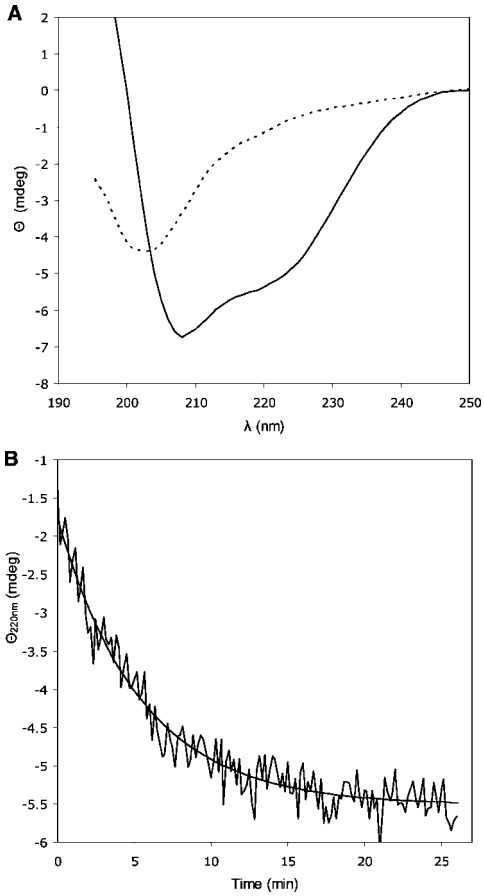

Figure 3. Triggering and characterization of the switch peptide of the C-terminal NPY. (A) CD spectra recorded before (dashed line) and after (straight line) 30 min of incubation with 10 equiv of TCEP. (B) The kinetics of the structural change monitored at 220 nm yields $t_{1/2}$ of ~6 min.

We have shown a new transformation based on the Staudinger reaction and demonstrated its application in the design of a novel switch element to control the folding of the NPY peptide from random coil to α-helix conformation. The azido functionality in the depsipeptide unit is activated rapidly in water using TCEP via the Staudinger reaction.[13] Our findings expand the repertoire of uses of the Staudinger reaction in chemical biology and the number of available triggers for use in switch peptides. Current efforts in our laboratories are focused on applying the azide switch, with other known switches, in the design and characterization of switch proteins[14] and self-assembling systems.

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Supporting Information Available: Synthetic procedures for peptides (**3**−**5**, **8**), procedures for triggering, pH rate profiles. This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) Along these lines, it was reported that TCEP can induce a mild and chemoselective peptide-bond cleavage of peptides and proteins at azido homoalanine by initiating the reaction via reduction of the azide to triazene: Back, J. W.; David, O.; Kramer, G.; Masson, G.; Kasper, P. T.; de Koning, L. J.; de Jong, L.; van Maarseveen, J. H.; de Koster, C. G. *Angew. Chem., Int. Ed.* **2005**, *44*, 7946.

(14) Vila-Perelló, M.; Hori, Y.; Ribó, M.; Muir, T. W. *Angew. Chem., Int. Ed.* **2008**, *47*, 7764–7767.