Tetrahedron Letters 57 (2016) 2112–2115

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

A thiirane linker for isopeptide mimetics by peptide ligation

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ARTICLE INFO

Article history:
Received 18 February 2016
Revised 28 March 2016
Accepted 5 April 2016
Available online 6 April 2016

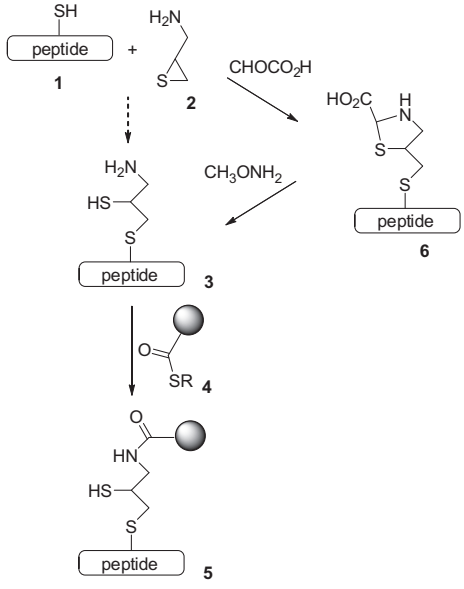
Keywords:
CPE ligation
Histone
Post-translational modification
Thiirane
Ubiquitin

ABSTRACT

2-Aminomethylthiirane was used to attach a 1,2-amino-thiol moiety at thiol groups of peptides for use in peptide ligation to produce isopeptide mimetics. To confirm the strategy, a ubiquitinated histone H3 peptide was prepared, and the ubiquitin was correctly folded judging from CD spectra with no influence by the random H3 peptide.

The site-selective modifications of proteins such as post-translational modification (PTM) and fluorescence labeling make up important methods for studies of protein functions.[1,2] Histone proteins form an octamer as a core of the nucleosome, which is a basic unit of chromatin and which consists of two copies each of four different core histones, H2A, H2B, H3, and H4 with DNA. The PTMs of histones, such as acetylation, methylation, phosphorylation, and so on, are crucial, in that they determine how the molecule will participate in the epigenetic regulation of gene expression.[3–8] We previously reported on the preparation of histone H3 containing an ε-trimethylated Lys residue.[9,10]

Ubiquitination is an important PTM, in which the C-terminus of ubiquitin (Ub), a 76 amino acid residue peptide, is attached to the side-chain amino group of Lys residues by an isopeptide bond.[11,12] Histones are also ubiquitinated to regulate their functions.[3,4,13,14] Recently the ubiquitinated histones H2A and H2B were prepared based on elegant chemistry that involved the construction of native isopeptide structures.[15–17] The overall synthetic strategies are a bit complex and require considerable skill. On the other hand, ubiquitin can be installed at a Cys residue through, for example, a disulfide or a thioether.[11,18,19] These approaches are useful because the reactions are more straightforward. Here we report on an alternative method for preparing an isopeptide-mimetic structure, in which thiiran-2-ylmethanamine (2-aminomethylthiirane **2**) is used as a linker for peptide ligation (Scheme 1). 2-Aminomethylthiirane **2** is reacted with a Cys-containing peptide **1** to give the 1,2-amino-thiol **3**,[20–22] and the peptide thioester **4**[23–25] is then reacted by native chemical ligation[26–28] to produce the isopeptide-mimetic molecule **5**. In this Letter, the ubiquitinated histone H3 peptide was prepared as a model molecule. Histone H3 was reported to be ubiquitinated at Lys18 or 23 to regulate maintenance DNA methylation by DNA methyltransferases.[13,14]



Scheme 1. Formation of an isopeptide-mimetic linkage using a 2-aminomethylthiirane linker **2**.

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http://dx.doi.org/10.1016/j.tetlet.2016.04.006
0040-4039/

When a model peptide, Ala-Lys-Gly-Thr-Arg-Ala-Val-Thr-Cys-Tyr-Thr-Ser-NH_2 (**1a**), was reacted with an excess amount of 2-aminomethylthiirane **2** in sodium phosphate buffer (pH 8.1), oligomers of **2**, as a result of the subsequent reaction of the 1,2-amino-thiol peptide **3a** with **2**, were observed, while peptide **1a** remained (Fig. S3 in Supplemental data). Thiiranes are known to readily undergo oligomerization.[22] Therefore, we attempted to terminate the 1,2-amino-thiol moiety as the thiazolidine **6** by reaction with aldehydes (Scheme 1). Although formaldehyde was not effective, the thiazolidine **6a**, which contains two chiral carbons, was produced in the presence of glyoxylic acid, and the subsequent addition of *O*-methylhydroxylamine to the reaction mixture directly produced the desired 1,2-amino-thiol **3a** in a yield of 68% from peptide **1a** with negligible amounts of the dimeric product **3a**′ (Fig. 1A and B). Regiochemistry of the ring opening site of the thiirane was determined by the NMR of a product, obtained by the reaction of *N*-*tert*-buthoxycarbonyl-2-aminomethylthiirane and ethyl 3-mercaptopropionate, and amino acid analysis of **3a** showed that the Cys residue was modified (peptides are listed in the Supplemental data).

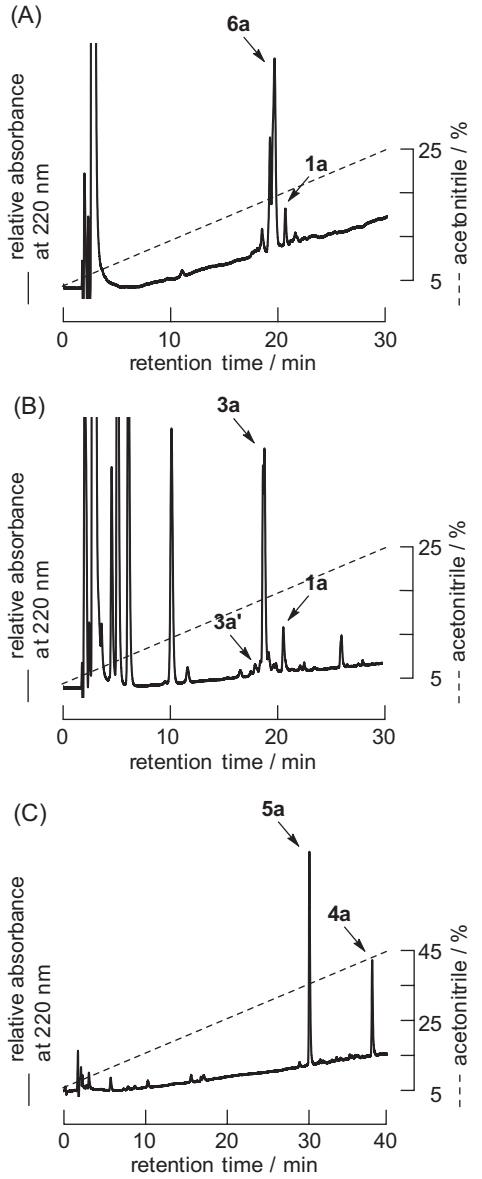

Figure 1. RP-HPLC of reaction mixtures of peptide **1a**. (A) Reaction mixture of peptide **1a** and thiirane **2** in sodium phosphate buffer (pH 8.0) containing glyoxylic acid at 37 °C after 24 h; (B) reaction mixture of peptide **6a** at 37 °C after an additional 24 h, after adding *O*-methylhydroxylamine; (C) reaction mixture of peptide **3a** and the CPE peptide **4a** in sodium phosphate buffer (pH 7.9) containing TFET at 37 °C after 8 h. Column: YMC-Pack ProC18 (4.6 × 150 mm), eluent: aq. acetonitrile containing 0.1% TFA, flow rate: 1.0 mL/min.

The ligation of peptide **3a** at the side chain 1,2-amino-thiol moiety with the model peptide, Fmoc-Arg-Gly-Asn-Tyr-Asp-Ala-Cys-Pro-OCH_2CO-Tle-NH_2 (**4a**) (Tle: *tert*-leucine), in which the sequence, -Cys-Pro-OCH_2CO- (CPE), at the C-terminus constitutes a thioester-forming structure,[29–33] in phosphate buffer (pH 7.9) in the presence of 2,2,2-trifluoroethanethiol (TFET)[34] produced the isopeptide-mimetic peptide **5a** in a yield of 79% (Fig. 1C). Attempts to remove the thiol group under desulfurization conditions[35] resulted in the cleavage at the isopeptide-mimetic moiety to produce the following peptides: Ala-Lys-Gly-Thr-Arg-Ala-Val-Thr-**Ala**-Tyr-Thr-Ser-NH_2 (**1a**′) and the Fmoc-Arg-Gly-Asn-Tyr-Asp-Ala-NH_2 derivative **4a**′, a mass analysis of which indicated a C-terminal allyl amide (Supplemental data), which might be due to radical β-fragmentation.[36] Although it was not possible to remove the thiol group of the isopeptide-mimetic structure, the thiol functionality can be used for further labeling such as fluorescent probes.

The histone H3 peptide, with Ub attached at the 23 position[13] through the isopeptide-mimetic structure, [Cys($CH_2CH(SH)CH_2$NHCO-Ub)^{23}]H3(1-35)-NH_2 (**5b**), was synthesized next. The amino acid sequences are shown in Figure 2. Histone H3 contains 135 amino acid residues, and its N-terminal region is known to be flexible and to accept various types of PTM.[5] The 1,2-amino-thiol peptide, [Cys($CH_2CH(SH)CH_2NH_2$)^{23}]H3(1-35)-NH_2 (**3b**), was synthesized by the reaction of [Cys^{23}]H3(1-35)-NH_2 (**1b**) and thiirane **2** via the thiazolidine **6b** by a one-pot procedure in a yield of 47% after RP-HPLC purification (Fig. 3A and B). Only a small amount of the oligomer **3b**′ of **2**, attached to the H3 peptide, was observed. The Ub-CPE, Ub-Cys-Pro-OCH_2CO-Tle-NH_2 (**4b**), which was prepared by Fmoc solid phase peptide synthesis (SPPS),[37] was then ligated with the 1,2-amino-thiol peptide **3b** in the presence of 4-mercaptophenylacetic acid (MPAA) as a catalyst[38] to give the H3-Ub isopeptide-mimetic peptide **5b** in a yield of 56% after RP-HPLC purification (Fig. 3C).

(A)

1
ARTKQTARKS TGGKAPRKQL ATKAARKSAP
ATGGV 35

(B)

1
MQIFVKTLTG KTITLEVEPS DTIENVKAKI
QDKEGIPPDQ QRLIFAGKQL EDGRTLSDYN
IQKESTLHLV LRLRGG 76

Figure 2. Amino acid sequence of (A) H3 (1–35), in which Lys23, shown in red, was replaced by a Cys residue; (B) Ub, in which the amino acid residues, shown in red or blue, were introduced as a pseudoproline dipeptide or a Dmb dipeptide, respectively, during SPPS.[37]

A far-UV circular dichroism (CD) spectrum of H3-Ub peptide **5b** was measured and the spectrum was compared with those of recombinant Ub and synthetic Ub, which was prepared by the hydrolysis of Ub-CPE **4b** (Fig. 4). The pattern of spectra of the recombinant and synthetic Ubs was identical, and was also very similar to previously reported ones.[39] The spectrum of the H3-Ub peptide **5b** was similar to spectra of Ubs without the H3 peptide in the 207–250 nm region, and was closely similar to the spectrum of a mixture of synthetic Ub and H3 peptide **1b** (Fig. S6), which indicated that Ub was correctly folded without any influence of the H3 peptide. The increase in the negative CD signal at a wavelength shorter than 207 nm further can be attributed to the presence of a random structure derived from H3 peptide.