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A novel ring opening reaction of peptide N-terminal thiazolidine with 2,2′-dipyridyl disulfide (DPDS) efficient for protein chemical synthesis

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ABSTRACT

In the protein chemical synthesis via native chemical ligation (NCL) method with three peptide segments, the N-terminal cysteine residue of middle segment is generally protected by thiazolidine ring. In this paper, we show the novel method for thiazolidine ring opening using 2,2′-dipyridyl disulfide (DPDS). The N-terminal thiazolidine was converted into *S*-pyridylsulfenylated cysteine residue with DPDS under acidic conditions, and this N-terminally Cys peptide protected with disulfide was applicable to NCL reaction without purification and deprotection steps. DPDS treatment did not remove other Cys protecting groups generally used for regioselective disulfide bond formation reactions. These results indicate that this thiazolidine ring opening reaction is quite useful for the protein chemical synthesis with three-segment NCL strategy.

1. Introduction

Solid-phase peptide synthesis (SPPS) method is a powerful tool for preparing peptides,[1] whereas it is usually limited to a length of 40–50 amino acid residues. To overcome this limitation, the native chemical ligation (NCL) method developed by Dawson and co-workers is generally used, in which a peptide α-thioester is site-specifically condensed with an N-terminally Cys peptide.[2,3] Since this reaction requires no protecting group at peptide side chains, the peptide segments can be prepared with the ordinary SPPS method.

The synthesis of long peptide chains (>100 amino acid residues) with NCL often requires three or more peptide segments, in which middle segment(s) must have both the N-terminal Cys residue and the C-terminal thioester functionality. To prevent the cyclization of the middle segment, the N-terminal Cys residue must be protected. For such purpose, several protection schemes have been proposed; the amino group protection by methylsulfonylethoxycarbonyl (Msc),[4] 4-(dimethylamino)phenacyloxycarbonyl (Mapoc),[5] 9-fluorenylmethoxycarbonyl (Fmoc),[6] *p*-boronobenzyloxycarbonyl (Dobz),[7] azido[8] or acetoacetyl (AcA) group,[9] and the thiol group protection by acetamidomethyl (Acm),[10] thiazolidine ring (Thz)[11] or trifluoroacetamidomethyl (Tfacm) group.[12] Among them, Thz has been used most frequently worldwide, and this protecting scheme has been utilized for the synthesis of various proteins, such as erythropoietin[13] and modified histones.[14]

It has been reported that the N-terminal Thz ring can be opened by methoxyamine treatment under weakly acidic conditions to convert Cys residue.[11] This reaction is usually complete within several hours time. On the other hand, methoxyamine often cause side reactions when using a protecting group with carbonyl moiety, such as pheacyl group.[15] Actually. It has been reported that the phenacyl group at Cys side chain was converted in part to 2-(methoxyimino)-2-phenylethyl group by methoxyamine treatment.[15] Recently, it has been reported that Thz could be deprotected by the treatment of excess amount of allylpalladium(II) chloride dimmer.[16] This deprotection reaction proceeds under NCL reaction conditions within only 15 min, and is applicable to the one-pot three-segment condensation method. The palladium(II) complex is, however, very expensive, and is an irritant heavy metal compound. Therefore, alternative deprotection method of Thz is desired.

In our previous study, we synthesized the N-terminally sulfanylated disulfide-rich peptide for producing a KLH-peptide conjugate.[17] On the synthesis processes, we found that the N-terminal Thz ring was opened with 2,2′-dipyridyl disulfide (DPDS) treatment under acidic conditions, forming *S*-pyridylsulfenylated Cys residue (Scheme 1). DPDS is cheaper than the palladium reagent, and therefore this ring opening reaction might be useful for chemical synthesis of proteins via NCL reactions. In this paper, we show the

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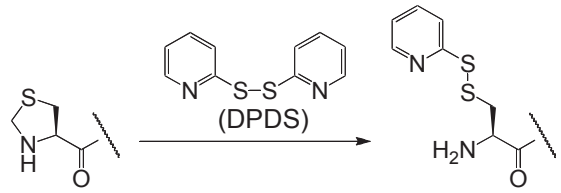


Scheme 1. Thiazolidine ring opening with 2,2′-dipyridyl disulfide.

utility of this Thz ring opening reaction to three-segment NCL reaction.

2. Results and discussion

2.1. Preparation of peptide segments

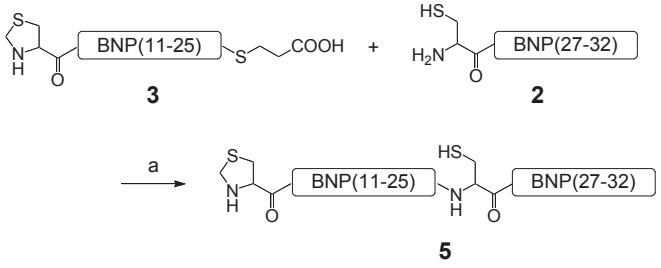
In order to demonstrate the utility of the Thz ring opening reaction with DPDS, we synthesized human brain natriuretic peptide (BNP) (**1**) as a model. BNP consists of 32 amino acid residues including two Cys forming an intramolecular disulfide bond.[18] To apply three-segment NCL reaction, the sequence was divided into three segments, (1–9), (10–25) and (26–32), and these were separately synthesized by Fmoc-based SPPS.

C-terminal segment (26–32) (**2**) was synthesized using H-His(Trt)-Trt(2-Cl)-resin as a starting material. Peptide chain was elongated manually by the ordinary Fmoc-SPPS method using *N,N'*-dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt) as condensation reagents. After cleaving peptide from the solid support by trifluoroacetic acid (TFA) treatment, peptide **2** was obtained in 42% yield.

The middle segment (10–25) (**3**) must have both the N-terminal Thz moiety and the C-terminal thioester functionality. To prepare peptide thioesters, we used *N*-alkylcysteine-assisted thioesterification method.[18] Fmoc-(Et)Cys(Trt)-OH, which was prepared as described previously,[19] was introduced to H-Arg(Pbf)-Arg(Pbf)-NH-resin, and peptide chain was elongated by the ordinary Fmoc chemistry. At Cys[10] position, Boc-thiazolidine-4-carboxylic acid was introduced. After the deprotection with TFA, the thioesterification reaction was carried out with 3-mercaptopropionic acid (MPA), giving peptide thioester **3** in 16% yield. The N-terminal segment (1–9) (**4**) was also prepared by essentially the same method as for peptide **4**, and was obtained as MPA thioester in 23% yield.

2.2. First NCL reaction

The first NCL was performed with peptide segments **2** and **3**. In the NCL reaction, an aryl thiol compound is generally required as an additive, and thiophenol was originally used.[2] Several thiol additives have also been proposed, such as 4-mercaptophenylacetic acid (MPAA)[20] and trifluoroethanethiol.[21] Recently, it was demonstrated that no thiol additive was required when Cys-rich peptide segments were used in NCL reaction.[22] In our case, since the peptide segments were not Cys-rich peptides, thiol additive-free NCL reaction was not thought to be applicable. Therefore, we used MPAA as a thiol additive in the first NCL. Equimolar amounts of the segments were dissolved in a phosphate buffer containing 1% MPAA, and the solution was kept at room temperature for 2 h, giving BNP(10–32) segment **5** which had N-terminal Thz moiety in 78% yield (Scheme 2).

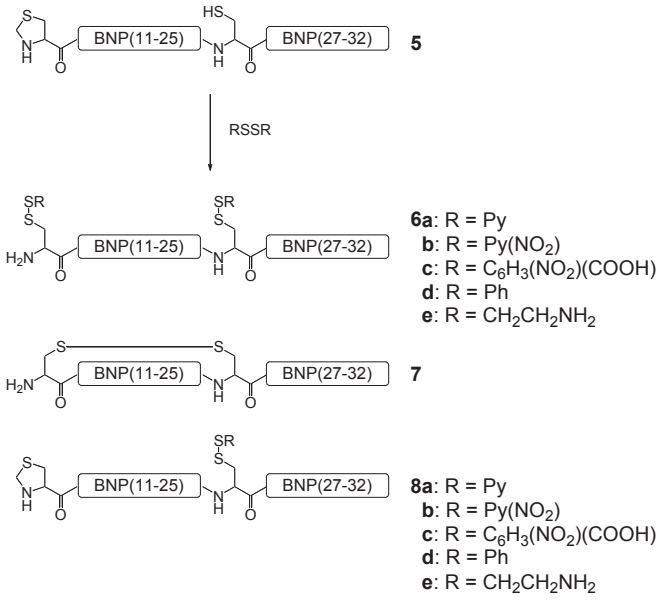


Scheme 2. NCL reaction with peptides **2** and **3**. Reaction conditions: (a) 1.6 M guanidine-HCl/100 mM sodium phosphate buffer (pH 7.4) containing 1% MPAA, rt, 2 h; 2.1% TCEP, rt, 20 min.

2.3. Thiazolidine ring opening

Using **5**, we tried to open the N-terminal Thz ring with DPDS (Scheme 3). To optimize the reaction conditions, various acidity of solvent was examined at first. Peptide **5** was dissolved at a concentration of 1 mM in 20 mM DPDS/50% acetonitrile aqueous solution without acidic compound, or containing 0.1% acetic acid or 0.1%, 1% or 10% TFA, and the solutions were gently mixed at room temperature. After the reaction for 24 h, the reactions were monitored by reversed-phase (RP)-HPLC (Fig. 1). As a result, the conversion of Thz to *S*-pyridylsulfenylated Cys was observed under acidic conditions, and bis(*S*-pyridylsulfenylated)-peptide **6a** was yielded. The Thz ring-opened peptide with an intrachain disulfide bond **7** was also observed. Especially, the Thz-peptide almost completely disappeared within 24 h under 0.1% TFA solution conditions. On the other hand, increment of acidity made reaction rate slower. This may be caused by the decrease of nucleophilicity of Thz sulfur atom under strong acidic conditions. Under a neutral pH, Thz-ring was not opened with DPDS within 24 h, and only mono-(*S*-pyridylsulfenylated)-peptide **8a** was observed on the RP-HPLC chromatogram. When the reaction temperature was changed to 50 °C, the conversion in 0.1% TFA solution was complete within 2 h without significant decomposition of peptide structure.

It has been reported that 2,2′-dithiobis(5-nitropyridine) (DTNP) acts as a deprotectant for various cysteine protecting groups.[23] In order to investigate whether disulfide additives other than DPDS



Scheme 3. Deprotection of peptide **5** with various disulfide compounds. DPDS (**a**), DTNP (**b**), DTNB (**c**), DPhDS (**d**) and cystamine (**e**) were examined as RSSR.