Pergamon

Tetrahedron Letters 39 (1998) 6799–6802

TETRAHEDRON LETTERS

Facile synthesis of cyclic peptides containing di-, tri-, tetra-, and pentasulfides

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Received 15 June 1998; accepted 6 July 1998

Abstract

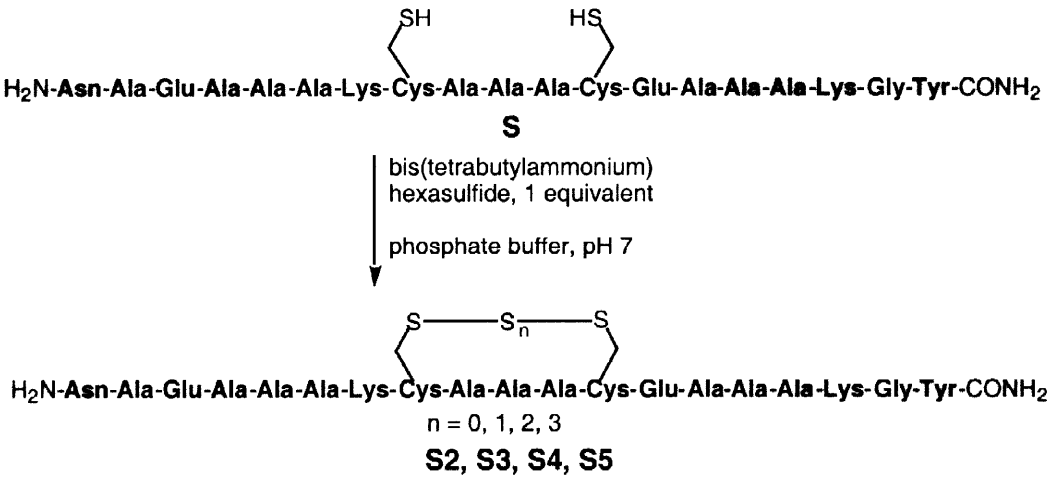
We describe an efficient method for constructing cyclic polysulfide-containing peptides. The synthesis uses commercially available bis-(tetrabutylammonium) hexasulfide (**BTH**) and can be performed in aqueous solution. Preliminary structural analyses indicate that varying the number of sulfur atoms affects the conformation of the peptide in solution.

Disulfide bonds in naturally occurring peptides and proteins have been studied for decades. Recently, examples of proteins containing *trisulfides* have been reported[1-4], prompting investigations into the synthesis and properties of trisulfide-containing peptides[5-9]. However, there appear to be no reports of naturally occurring peptides containing tetra- or pentasulfides, even though natural products containing higher-order sulfides are known[10-14], and tetra- and pentasulfide derivatized small molecules[15-23] and even a tripeptide dimer[6] have been synthesized. In all these cases the syntheses required caustic or commercially unavailable reagents or reaction conditions that would generally not be adaptable to larger peptides or proteins. Here we report that bis-(tetrabutylammonium) hexasulfide (**BTH**) can be used in aqueous solution to produce cyclic peptides containing from two to five sulfur atoms. The exact number of sulfur atoms introduced is determined in part by the ratio of peptide to **BTH**; presumably there is an equilibrium between the linear hexasulfide chain of **BTH** and the various cyclic peptides. **BTH** is a conveniently handled powder with very little odor and is available commercially from Aldrich®. It is soluble in a variety of solvents including methanol and acetonitrile.

The peptide (**S**) we chose to investigate is based closely on a peptide previously described by Baldwin and coworkers and shown to adopt an alpha helical conformation in solution[24]. We added an amino-terminal asparagine to enhance this helicity[25] and a carboxy-terminal glycine-tyrosine to facilitate quantification[26]. Previous studies have shown that constraining a peptide at positions i and i+4 with a lactam bridge[27] or metal chelate[28] can enhance alpha helicity, and we wondered whether sulfide linkages would have a similar effect. The peptide was synthesized as a carboxy-terminal amide derivative on a PerSeptive Biosystems Pioneer™

0040-4039/98/$ - see front matter
PII: S0040-4039(98)01461-0

synthesizer using conventional Fmoc-protected amino acid derivatives and HATU coupling chemistry on a Rink-amide support. The peptide was cleaved from the resin and deprotected with 5% triisopropylsilane in trifluoroacetic acid (TFA) and purified using reversed-phase High Performance Liquid Chromatography (HPLC).



Scheme 1.

The reaction of peptide **S** with **BTH** is depicted in Scheme 1. Preliminary experiments suggested that the reaction occurs very rapidly: when a 0.9 mM solution of peptide **S** is mixed with 1 equivalent of **BTH** in 10 mM sodium phosphate (pH 7) and immediately analyzed by electrospray mass spectroscopy, peaks corresponding to **S2**, **S3**, **S4**, and **S5** are prominent, as shown in Figure 1A. There is some dependence on stoichiometry; when the ratio of **BTH** to **S** is less than 0.2, no **S4** and **S5** are observed, although **S2** and **S3** are formed.

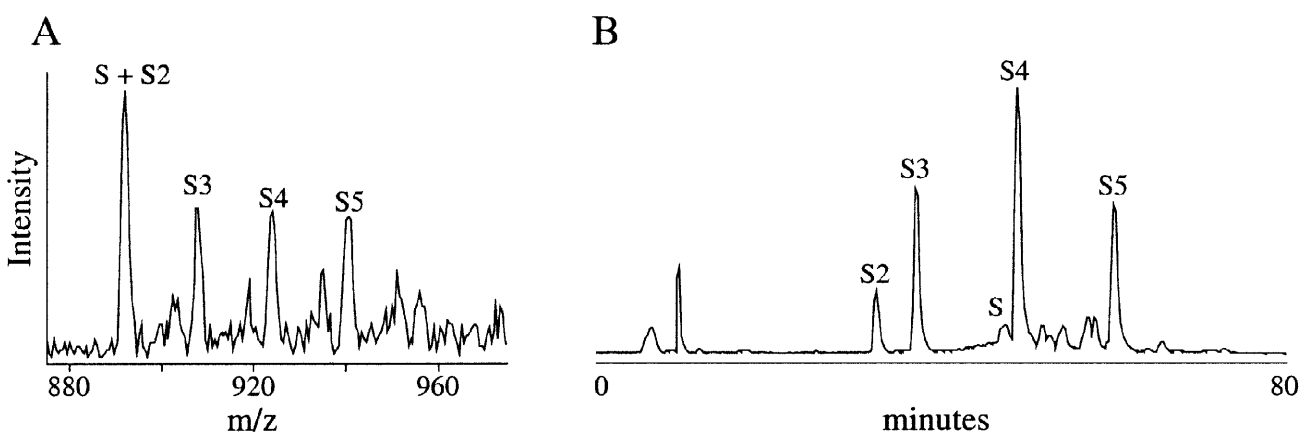


Figure 1. **A**: A portion of the electrospray mass spectrum of the reaction between peptide **S** and **BTH**. This region shows doubly charged peptide ions; the small peaks to the right of the larger peaks are due to sodium adducts. **B**: HPLC chromatogram showing a typical purification. The gradient is 5% acetonitrile in water to 50% acetonitrile in water with 0.1 % TFA over 80 minutes on a C18 column. Subsequent analytical HPLC analyses of the isolated compounds confirmed that they are homogeneous. Matrix-assisted laser desorption ionization mass spectroscopy further confirmed the assignments: **S2** expected mono-isotopic weight (M+H) 1780.81, found 1780.74; **S3** expected 1812.78, found 1812.78; **S4** expected 1844.76, found 1844.87; **S5** expected 1876.73, found 1876.93.

In order to obtain analytical amounts of these sulfide derivatives, peptide **S** was dissolved in 4.5 mL of an 11.1 mM sodium phosphate (pH 7) aqueous buffer to a final concentration of 0.21 mM. To this was added 0.5 mL of a 2 mM solution of **BTH** in methanol. After 45 minutes the reaction was quenched with 5 mL of 0.1% TFA in water, filtered, and purified using reversed-phase HPLC. As shown in Figure 1B, peptide **S** is almost completely consumed, and four major products are visible. The masses of these products, as well as their increasing hydrophobicity, identify them as cyclic sulfides **S2**, **S3**, **S4**, and **S5**.

To determine whether the introduction of the sulfide tethers has any structural effects on the peptides, circular dichroism (CD) measurements were performed, as shown in Figure 2. All of the peptides show some alpha helicity, as indicated by the maximum at 192 nm and the two minima at 208 and 222 nm. However, there are significant differences among the spectra. These are most likely due to structural effects of the sulfide linkage on the peptides, although a direct contribution from the sulfide tethers themselves has not been ruled out. Intriguingly, the mean residue ellipticity at 222 nm, commonly taken as an indicator of alpha helicity, is more negative for peptides **S4** and **S5** than for the control peptide, suggesting that these longer sulfide tethers may enhance alpha helicity. Consistent with this interpretation, peptide **S2** is less helical than the control peptide by this criterion, as predicted by modeling studies which show that a disulfide linkage is too short to be accommodated in an alpha helix. Nuclear magnetic resonance studies are necessary to more accurately determine the nature of the structural changes.

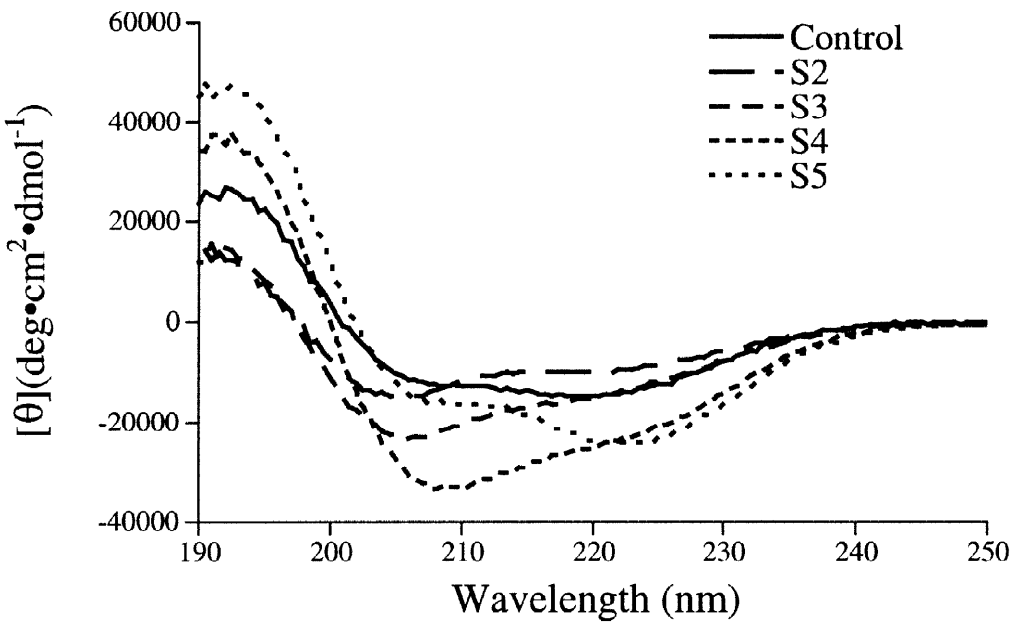


Figure 2. Circular dichroism (CD) measurements of peptides **S2**, **S3**, **S4**, and **S5**, as well as a control peptide which contains serines instead of cysteines. All measurements were performed at a constant temperature of 22 °C in 10 mM sodium phosphate buffer (pH 7) with 25% trifluoroethanol added on an Aviv Model 62DS CD spectrometer. Each trace is the average of 20 scans taken in an 0.05 cm path-length cell. Peptide concentrations were determined by quantitative amino acid analyses, and range between 0.1 and 0.18 mM.

The peptides **S2**, **S3**, and **S4** are remarkably stable in aqueous buffer at neutral pH, but **S5** slowly decomposes to **S4** over a period of days at neutral pH and room temperature. In the presence of 5 mM dithiothreitol (DTT) at pH 7, all four sulfide-containing peptides are reduced quantitatively to S within ten minutes, as determined by HPLC.

In conclusion, we have developed a simple, rapid, and effective method to produce disulfides, trisulfides, tetrasulfides, and pentasulfides from cysteine-containing peptides in buffered aqueous solutions, and have shown that the length of the sulfide tether appears to affect the conformations of the peptides. The mildness of the present method is such that it can likely be applied directly to cysteine-containing proteins or phage-displayed peptides without denaturing them. The ability to precisely tune the conformation of peptides and proteins in a reversible manner has significant potential for drug discovery and furthering the biological understanding of macromolecules. Additionally, the recent discovery that polysulfides can promote DNA strand cleavage[29] raises the intriguing possibility that polysulfide-containing peptides or proteins targeted to specific DNA binding sites could be used as site-specific nucleases.

Acknowledgments

We thank Michael G. Mulkerrin for assistance with the CD measurements, Jim Bourell for the MALDI measurements, and Allan Padua and Bill Henzel for the quantitative amino acid analyses. DAE is supported by a grant from the U. S. National Institute of Health (#1 F32 DK09755-01).

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