Synthesis of constrained helical peptides by thioether ligation: application to analogs of gp41

Florence M. Brunel and Philip E. Dawson*

Received (in Cambridge, UK) 20th December 2004, Accepted 17th February 2005
First published as an Advance Article on the web 11th March 2005
DOI: 10.1039/b419015g

We present a straightforward and high yielding method to synthesize constrained helical peptides *via* thioether ligation; this method represents an attractive alternative to the formation of lactam bridge constraints to induce helicity in peptides.

The α-helix is the most common secondary structure in proteins.[1] However, most linear peptides do not sustain a predominantly α-helical conformation in solution. Several different methods exist to enhance the helicity of a peptide.[2] A common technique is the introduction of a constraint by covalently linking two adjacent amino acids on the surface of the helix. This constraint is usually achieved through introduction of a lactam bridge between a glutamic acid and a lysine positioned at the positions *i* and *i* + 4 or *i* and *i* + 3 using orthogonal side chain protection and on-resin cyclization.[3] In principle, chemoselective ligation approaches for tether formation would enable the crosslink to be introduced following cleavage and deprotection of the peptide. One example of this approach is the use of ring-closing olefin metathesis to synthesize side chain tethered peptides with enhanced helical character.[4,5] Although other ligation chemistries such as thioether ligation,[6] have been utilized in the high yielding synthesis of a wide number of proteins and peptidomimetics,[7] and head-to-tail cyclic peptides,[8–11] chemoselective ligation has rarely been used to synthesize constrained α-helical peptides. Here, we present a straightforward procedure for the synthesis of (*i*, *i* + 4) and (*i*, *i* + 3) side chain tethered peptides with an increased helical character.

The human immunodeficiency virus (HIV) transmembrane envelope glycoprotein, gp 41, is generally thought to exist within a trimeric complex of gp120–gp41 heterodimers on the surface of the virion.[12] Most of gp41 appears to be occluded within the native trimer, except for a highly conserved region proximal to the viral membrane. The human monoclonal antibody 4E10 appears to recognize the conserved membrane-proximal external region of gp41 and can neutralize a broad range of primary isolates of HIV-1.[13] A recent crystal structure of the complex formed between 4E10 and its peptide epitope has shown that the peptide adopts a helical conformation.[14] In order to validate the potential of this epitope for the development of an HIV vaccine, we designed mimics of the 4E10 epitope.

The helical epitope consists of residues 671–680 (according to the HXB2 sequence) of gp41, N*WF*D*IT*NWLW, where the essential amino acids for antibody binding are italicized and are presented on the same side of the helix. In order to constrain peptides into a helical conformation, we designed a side chain tether substituting amino acids on the opposite face of the helix (bold) to the binding epitope (italic) N*WF***D***IT*NWLW, N*WF***D***IT***N**WLW and **W**N*WF***D***IT*NWLW.

To test the applicability of thioether ligation to side chain tethers, peptides containing the thioether link were designed to maintain the same ring size found to be most effective for side chain lactam tethers (Fig. 1).[3] Thioether links in peptides can be formed chemoselectively between thiols and bromoacetyl groups.[8] This approach has been used for segmental ligation and head to tail cyclic peptides.[6–11] In order to yield the desired thioether tether, a Cys residue can be paired with a bromoacetylated ornithine residue (Orn) in the desired position of the peptide. Treatment of the deprotected peptide with a pH 8 buffer is expected to form the intramolecular thioether link (Fig. 2).

The peptides were synthesized using *in situ* neutralization cycles for Boc SPPS, with the coupling agent HBTU on MBHA resin.† (Fmoc SPPS could be used with appropriate side chain protection). Following chain assembly, the Orn(Fmoc) residue was deprotected with piperidine and then bromoacetylated (bromoacetic acid, 20 equiv., DIC, 10 equiv., CH_2Cl_2, preactivated 15 min at 0 °C).[8] After side chain deprotection and cleavage from the resin with HF, the peptide was precipitated and washed with ether.

The thioether link was formed by adding 6 M guanidine HCl 100 mM NaH_2PO_4, pH = 8.4 to the mixture of precipitated peptide and resin (<1 mg mL^{-1}) which was stirred at RT for 2 h (Fig. 2). The cyclic thioether peptides were formed in high yields and purity without apparent polymerization. The pH of the solution was important since use of a non-buffered 6 M guanidine HCl solution resulted in conversion of the bromoacetyl group to a chloroacetyl group without ligation. However, the purified chloroacetylated peptide underwent efficient cyclization upon stirring in the buffered solution. Controls were synthesized where an acetyl group was introduced on the side-chain of Orn using acetic anhydride. The corresponding lactams were also formed for comparison.‡ Purity of the cyclic thioether was high and similar to

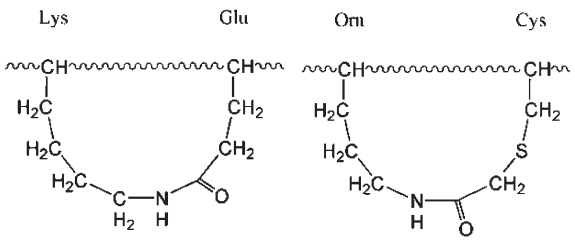

Fig. 1 Schematic representation of the rings in lactam and thioether compounds.

*dawson@scripps.edu

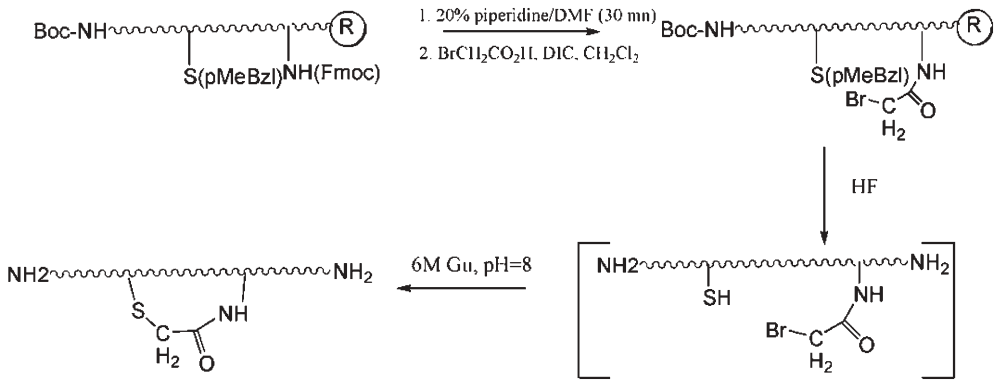

Fig. 2 General synthetic scheme for the formation of thioethers.

the linear control. The thioether cyclization yielded a cleaner crude synthetic product than the corresponding lactam formation. (Fig. 3, a compared to b). Cyclic thioether peptides representing i, $i + 3$ and i, $i + 4$ links in both orientations are presented in Table 1.

To assess the helicity of the peptides, circular dichroism (CD) experiments were performed at 25 °C in 20 mM phosphate buffer pH = 7.4.§ For solubility reasons, 20% TFE (trifluoroethanol) phosphate buffer was used with the (i, $i + 4$) series. (i, $i + 4$) cyclic thioether spectrum presented two minima at 207 and 222 nm with higher molar ellipticity (data not shown). Both spectra are consistent with a helical conformation, and the increase in molar ellipticity for the tethered peptide demonstrates that the introduction of the thioether bridge results in an increase in helicity compared to the linear analog.

The (i, $i + 3$) substituted peptides provide a direct comparison of thioether and lactam tethers with the linear peptide. The CD spectrum of the cyclic thioether in aqueous buffer presented two minima shifted to the right compared to the acyclic analog, and had a higher molar ellipticity. (Fig. 4, first two compounds) This shift is consistent with an increase of helical character. The corresponding lactam tethered peptide was analyzed for comparison. As shown in Fig. 4, curves 2 and 4, the spectrum of the cyclic thioether c(CWFO)ITNWLWKKKK-NH_2 is more consistent with an α-helix than the spectrum of the corresponding lactam c(EWFK)ITNWLWKKKK-NH_2: the two minima are closer to the values for an idealized helix (207 and 222 nm) and the molar ellipticity for the minimum around 222 nm is considerably increased. In this case, not only did the introduction of the thioether bridge result in an increase of helicity compared to the

Table 1 Yields of cyclization of different analogs[a]

Structure of the compound	Cyclization yield[a]	Overall yield
c(CAWFO)IT-NH_2	86	56
WFc(OITAC)	ND	70
c(CNWFO)ITNWLWRR-NH_2	ND	71
c(CWFO)ITNWLWKK-NH_2	88	30
c(CWFO)ITNWLWKKKK-NH_2	ND	85
Wc(CWFO)ITNWLWKKKK-NH_2	78	50
NWFc(CITO)WLWKKKK-NH_2	93	38

[a] Yields were calculated by comparing the integration of the HPLC spectra of the cyclic derivatives with their linear analogs.

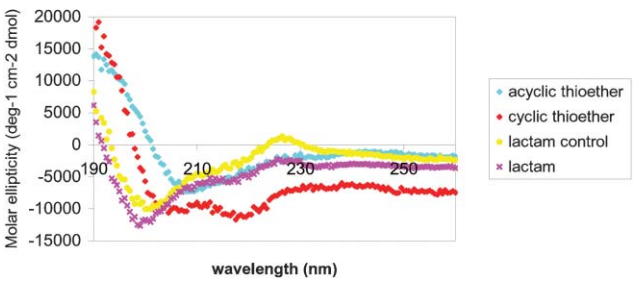

Fig. 4 CD spectra of a cyclic thioether, the corresponding lactam and the acyclic analogs.

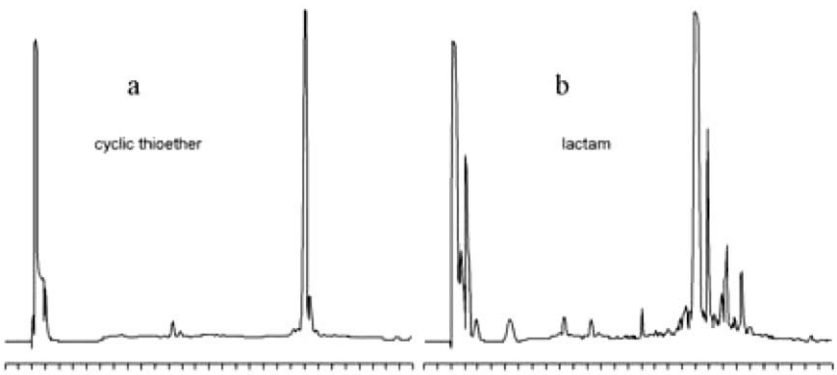

Fig. 3 HPLC spectra of a cyclic thioether (c(CWFO)ITNWLWKKKK-NH2) and the corresponding lactam (c(EWFK)ITNWLWKKKK-NH2). A Rainin HPLC system equipped with a Vydac C18 column (10 μm, 1.0 × 25 cm, flow rate 1 mL min^{-1}) was used.

control, it also induced a higher helicity than the introduction of a lactam bridge.

We have presented a straightforward synthesis of sidechain tethered peptides by thioether ligation as a means to increase the helicity of a peptide. This method was used to form (i, $i+3$) and (i, $i+4$) rings, and the orientation of the thioether tether can be reversed in a straightforward manner. The reaction is rapid, efficient and in our hands, the products are more homogeneous than the corresponding lactam peptides. Thioether tethers provide an attractive addition to the available methods used for increasing the helicity of a peptide. Moreover, the ease of formation should allow for screening of tethers having different lengths by replacing the amino acids Orn and Cys with longer or shorter homologs.

We thank Michael Zwick and Dennis Burton for advice and discussions, Rosa Cardoso and Ian Wilson for sharing unpublished observations, Linda Tennant and Peter Wright for assistance with CD; this study was supported in part by AmFAR (American Foundation for Aids Research) (fellowship 106587 36 RFVA), and The Skaggs Insitute for Chemical Biology and NIH MH062261.

Florence M. Brunel and Philip E. Dawson*
The Scripps Research Institute, Department of Chemistry and Cell Biology, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA. E-mail: dawson@scripps.edu; Fax: 858-784-7319; Tel: 858-784-7052

Notes and references

† Boc–Orn(Fmoc)–OH was used. The peptide couplings were carried out with a 7-fold excess of activated amino acid for a period of 20 min. Only 2.5-fold excess were used when coupling Lys(Fmoc) and Orn(Fmoc). Completion of those couplings was assessed by qualitative Kaiser test and double couplings were performed when necessary.

‡ Formation of the lactams: after chain assembly, Lys(Fmoc) and Glu(OFm) were deprotected with 10% piperidine in DMF for 30 min. Ring formation was initiated by adding BOP, HOBt and DIEA (2 : 2 : 4) and stirring overnight.

§ An Aviv spectropolarimeter Model 203-02 was used, with cells of 0.1 cm length. Instruments parameters used were a wavelength step of 0.5 nm, a bandwidth of 1.0 nm, and an average of 3 scans reported. The exact peptide concentrations were determined by Ultra-Violet measurements at 280 nm on a Gilson UV detector, model 116.

1 T. E. Creighton, *Proteins: Structures and Molecular Properties*, Freeman, New York, 2nd edn., 1984, pp. 182–188.
2 E. Dovalsantos and A. C. Satterthwait, *Houben-Weyl Methods of Organic Chemistry*, Thieme Stuttgart, New York, vol. E22c, pp. 759–792.
3 (*a*) A. M. Felix, E. P. Heimer, C. T. Wang, T. J. Lambros, A. Fournier, T. F. Mowles, S. Maines, R. M. Campbell, B. B. Wegrzynski, V. Toome, D. Fry and V. S. Madison, *Int. J. Pept. Protein Res.*, 1982, **32**, 441–454; (*b*) G. Osapay and J. W. Taylor, *J. Am. Chem. Soc.*, 1990, **112**, 6046–6051; (*c*) For a review: J. W. Taylor, *Biopolymers*, 2002, **66**, 49–75.
4 (*a*) A. H. E. Blackwell and R. H. Grubbs, *Angew. Chem., Int. Ed.*, 1998, **37**, 3281–3284; (*b*) H. E. Blackwell, J. D. Sadowsky, R. J. Howard, J. N. Sampson, J. A. Chao, W. E. Steinmetz, D. J. O'Leary and R. H. Grubbs, *J. Org. Chem.*, 2001, **66**, 5291–5302.
5 C. E. Schafmeister, J. Po and G. L. Verdine, *J. Am. Chem. Soc.*, 2000, **122**, 5891–5892.
6 D. R. Englebretsen, B. G. Garnham, D. A. Bergman and P. F. Alewood, *Tetrahedron Lett.*, 1995, **36**, 8871–8874.
7 P. E. Dawson and S. B. H. Kent, *Annu. Rev. Biochem.*, 2000, **69**, 923–960.
8 F. A. Robey and R. L. Fields, *Anal. Biochem.*, 1989, **177**, 373–377.
9 S. J. Wood and R. Wetzel, *Int. J. Pept. Protein Res.*, 1992, **39**, 533–539.
10 J. Tulla-Puche and G. Barany, *J. Org. Chem.*, 2004, **69**, 4101–4107.
11 P. Botti, T. D. Pallin and J. P. Tam, *J. Am. Chem. Soc.*, 1996, **118**, 10018–10024.
12 D. C. Chan, D. Fass, J. M. Berger and P. S. Kim, *Cell*, 1997, **89**, 263–273.
13 (*a*) M. B. Zwick, A. F. Labrijn, M. Wang, C. Spenlehauer, E. O. Saphire, J. M. Binley, J. P. Moore, G. Stiegler, H. Katinger, D. R. Burton and W. H. I. Parren, *J. Virol.*, 2001, **75**, 10892–10905; (*b*) G. Stiegler, R. Kunert, M. Purtscher, S. Wolbank, R. Voglauer, F. Steindl and H. Katinger, *AIDS Res. Hum. Retroviruses*, 2001, **17**, 1757–1765; (*c*) J. M. Binley, T. Wrin, B. Korber, M. B. Zwick, M. Wang, C. Chappey, G. Stiegler, R. Kunert, S. Zolla-Pazner, H. Katinger, C. J. Petropoulos and D. R. Burton, *J. Virol.*, 2004, **78**, 13232–13252; (*d*) S. Mehandru, T. Wrin, J. Galovich, G. Stiegler, B. Vcelar, A. Hurley, C. Hogan, S. Vasan, H. Katinger, C. J. Petropoulos and M. Markowitz, *J. Virol.*, 2004, **78**, 14039–14042.
14 R. M. F. Cardoso, M. B. Zwick, R. L. Stanfield, R. Kunert, J. M. Binley, H. Katinger, D. R. Burton and I. A. Wilson, *Immunity*, in press.

