Pergamon

Tetrahedron Letters 41 (2000) 827–829

TETRAHEDRON LETTERS

α-Chloroacetyl capping of peptides: an N-terminal capping strategy suitable for Edman sequencing

Michael A. Shogren-Knaak, Kevin A. McDonnell and Barbara Imperiali *

Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

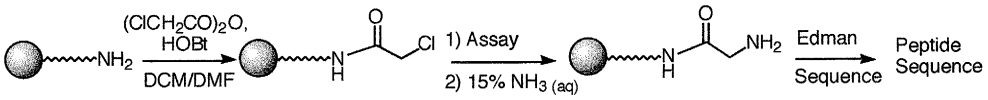
Received 21 October 1999; revised 18 November 1999; accepted 19 November 1999

Abstract

Use of an α-chloroacetyl group allows for the conditional N-terminal capping of peptides. This capping group is easily introduced and stable over a broad pH range, but can be converted to glycine by aqueous ammonia treatment in order to perform N-terminal Edman peptide sequencing. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: amino acids; amino acid derivatives; combinatorial chemistry; peptides; polypeptides; solid-phase synthesis.

The screening of large, synthetic peptide libraries requires means to determine peptide sequences. Edman degradation sequencing offers an effective method for identifying the sequence of picomoles of unknown peptides with a free amino terminus and has been used with resin-bound peptide libraries.[1] However, the presence of a free N-terminus, especially in its charged form, can be undesirable due to its potential to influence the structural and functional properties of the peptides. In this context, standard removable amine protecting groups are often unsuitable because of their steric bulk or incompatibility with common screening conditions. These conflicting needs necessitated the development of a conditional N-terminal peptide capping strategy (Scheme 1). In this strategy, the N-terminus of a full-length peptide on solid support is capped with the small α-chloroacetyl group prior to side chain deprotection. This capped peptide can then be used in screening assays as the N-terminally capped form. When sequence determination is desired, the peptide is subjected to aqueous ammonia treatment to convert the cap to glycine.[2] This new glycine-capped peptide may then be sequenced by Edman peptide degradation methods.



Scheme 1. Glycine conversion capping strategy

* Corresponding author. Fax: 617-452-2419; e-mail: imper@mit.edu (B. Imperiali)

PII: S0040-4039(99)02202-9

The desired chemical stability of the α-chloroacetyl cap was demonstrated with the peptide YRVG (Tyr-Arg-Val-Gly). This peptide was generated by standard Fmoc solid-phase peptide synthetic protocols on PAL-PEG-PS resin support (0.01 mmol).[2] Following standard Fmoc–amine deprotection, the α-chloroacetyl group was coupled using 1.0 ml of a 9:1 DMF:DCM solution of monochloro-acetic anhydride (51.6 mg, 0.30 M) and HOBt (40.6 mg, 0.27 M) for 2 h. Coupling went to completion based on the Kaiser test analysis.[3] The capped peptide was side chain deprotected and cleaved from the resin using standard protocols with reagent K (trifluoroacetic acid:phenol:H_2O:thioanisole:ethanedithiol, 82.5:5:5:5:2.5), to afford peptide **1**, $ClCH_2CONH$-[YRVG]-$CONH_2$. Reversed-phase analytical HPLC and electrospray mass spectral analysis showed the desired α-chloroacetyl capped peptide as the major product (Fig. 1), both confirming the coupling of the capping group, as well as demonstrating its stability toward acidic cleavage conditions. Subjecting capped peptide **1** to aqueous basic conditions (pH 10.5, 0.1 M CAPSO buffer) for 24 h resulted in no new products as detected by HPLC. This result, in conjunction with the acid cleavage result, suggests that the α-chloroacetyl capping group is stable and suitable for assays performed over a wide pH range.

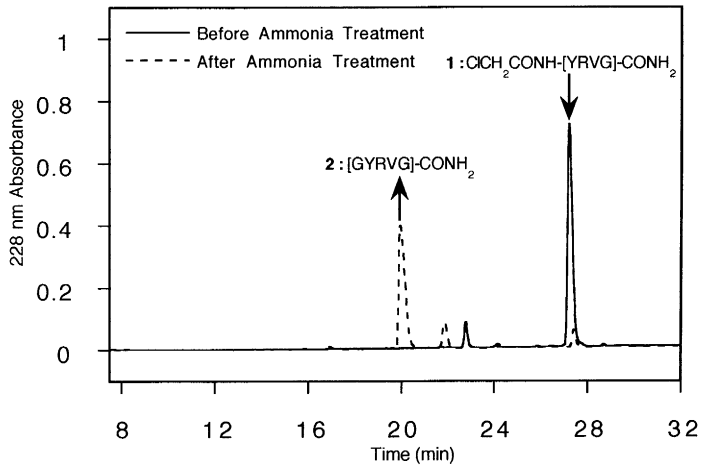


Fig. 1. Reversed-phase analytical HPLC trace of peptide **1** before and after 12 h treatment with 15% aqueous ammonia

Experiments with peptide **1** also demonstrated that the α-chloroacetyl group could be cleanly converted to the glycine amino acid. Treatment of the peptide with 15% aqueous ammonia for 12 h resulted in nearly complete conversion of the peptide to the glycine-capped peptide **2**, [GYRVG]-$CONH_2$ (Fig. 1). While other methods are available for the removal of the chloroacetamide groups,[4] the aqueous ammonia modification of this group was more gentle and simpler than the alternatives.

The generality of the method was further tested on a library of 23-residue peptides bound to PEGA-resin support. This library of over 100 000 different sequences was generated by split-and-pool[5] solid-phase peptide synthesis and was capped with the α-chloroacetyl group as described above. Following side chain deprotection and extensive washing, three random resin beads from this library were subjected to the 15% aqueous ammonia treatment for 12 h. These beads were then analyzed by Edman degradation sequencing and shown to possess an N-terminal glycine. Furthermore, full length sequencing proceeded easily and cleanly to afford peptide sequences, **3**–**5**, which were expected in the peptide library (Table 1).[6]

In conclusion, use of an α-chloroacetyl N-terminal capping group provides a robust strategy to protect and then regenerate the N-terminus of peptides for the purpose of Edman peptide sequencing.

Table 1
Sequencing results of randomly selected peptide library members

Peptide	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
3	**G**	F	R	T	P	S	Y	D	R	S	R	S	D	E	K	A	K	W	N	R	Q	H	A	G
4	**G**	I	R	T	P	S	Y	D	Y	S	R	S	D	E	Dap[7]	A	K	F	R	R	Q	H	A	G
5	**G**	Y	R	I	P	S	Y	D	R	S	R	S	D	E	K	A	K	W	I	R	Q	H	A	G

Acknowledgements

This work was supported by NIH grant number GM 53098. Additional support for M.S.K. was provided by a Howard Hughes Medical Institute predoctoral fellowship. Additional support for K.A.M. was provided by an NSERC (Canada) PGS-B fellowship.

References

1. Meldal, M.; Svendsen, I.; Breddam, K.; Auzanneau, F. *Proc. Natl. Acad. Sci. USA.* **1994**, *91*, 3314–3318.
2. Fields, G. B.; Noble, R. L. *Int. J. Peptide Protein Res.* **1990**, 35, 161–214.; Shogren-Knaak, M. A.; Imperiali, B. *Bioorg. Med. Chem.* **1999**, 7, 1993–2002.
3. Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595–598.
4. Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis, Third Ed.*; John Wiley & Sons: New York, 1999; pp. 555–556.
5. Lam, K. S.; Lebl, M.; Krchnak, V. *Chem. Rev.* **1997**, *97*, 411–448.
6. Sequencing of the peptide beads was performed either at the Caltech Protein Microanalytical Laboratory or at the University of British Columbia Protein Service Laboratory. Individual beads were submitted whole in 1:1 methanol:water, transferred to a filter support, and sequenced on a 476A protein sequenator (Perkin Elmer/Applied Biosystems Inc.) using standard Edman degradation protocols. Hydrolysis of side chain amides has been documented following aqueous ammonia treatment. However, in our sequences this was not a concern, because no position contained both an asparagine and aspartic acid or a glutamine and glutamic acid.
7. The presence of unnatural amino acid β-diaminoproprionic acid (Dap) was inferred, because it could not be detected directly under standard sequencing conditions. Furthermore, the presence of Dap resulted in truncated side product starting from the Dap residue. This product was shown to result from the ammonia treatment, but did not occur when the Dap residue was replaced by γ-diaminobutyric acid, ornithine, or lysine amino acids. The truncated product was not the major species and did not effect the ability to assign the peptide sequence.