Conversion of *N*-Terminal Cysteine to Thiazolidine Carboxylic Acid during Hydrogen Fluoride Deprotection of Peptides containing N^{π}-Bom protected Histidine

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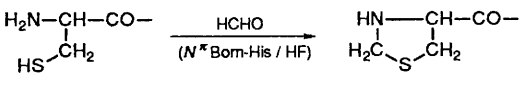
Formaldehyde released during hydrogen fluoride deprotection of peptides containing a histidine residue protected by the N^{π}-benzyloxymethyl (Bom) group induces cyclisation of *N*-terminal cysteine to thiazolidine carboxylic acid (Thz).

The recently introduced N^{π}-Bom (benzyloxymethyl) group has become widely used for the protection of histidine in the Boc/benzyl (Boc = t-butoxycarbonyl) strategy for solid-phase peptide synthesis.[1] It offers the advantage of being removed together with other benzyl masking groups during hydrogen fluoride (HF) treatment. Other protecting groups for histidine require an additional deprotection step [*e.g.* thiolysis for N^{im}-DNP (DNP = 2,4-dinitrophenyl)], or are not stable towards some of the reagents used during the synthesis as the tosyl group, being readily cleaved by hydroxybenzotriazole.[2] We report here that when N^{π}-Bom protected histidine is used during the synthesis of a peptide having a cysteine residue as NH_2-terminus the major product, isolated in high yield after HF treatment, is not the expected peptide but a peptide in which cysteine is converted to thiazolidine carboxylic acid (Thz) by reaction with formaldehyde that is released during the cleavage of the Bom group (Scheme 1). Such a reaction is of particular importance as cysteine is often added to the *N*-terminus of peptides in order to couple them to carrier proteins and to raise antipeptides antibodies.

During the synthesis of Cys–Lys–Gly–Val–His–Ala, the following HF-sensitive side-chain protecting groups were used: Cys (MeBzl); Lys (ClZ); His (N^{π}-Bom) (MeBzl = 4-methylbenzyl, ClZ = 2-chlorobenzyloxycarbonyl); the protected peptidyl resin was treated with anhydrous HF at 0 °C for 1 h in the presence of *para*-cresol and *para*-thiocresol. After removal of HF, the crude peptide was analysed by reverse phase HPLC and shown to elute as a major peak (Fig. 1*a*). The results of an amino acid analysis, performed after acid hydrolysis (HCl 5.6 M, 110 °C, 24 h) and mild oxidation were in good agreement with the theoretical values except for cysteine (Cys = 0.44/1), however, this low value is not unusual as this amino acid is known to be partly destroyed during acid hydrolysis.

Molecular weight determined by plasma desorption mass spectrometry (BioIon) indicated a value *m/z* 12 in excess of the expected value (determined: 626.0 and calculated: 613.7). Taking advantage of the presence of a lysine residue, the peptide was treated with trypsin and the molecular weights of the resulting tryptic fragments were determined. The fragment which contains His (Gly–Val–His–Ala) had the expected molecular weight (determined: 383.0 and calculated: 382.4) indicating that the *m/z* 12 excess is located in the NH_2-terminus dipeptide. Owing to the specificity of trypsin for basic side chains, it is unlikely that this enzyme could have cleaved a peptide containing a modified lysine residue, thus, modification of cysteine was suspected. The peptide was then submitted to Edman degradation in a gas-phase microsequencer (ABI 470A). This method, which requires a free amino group at the *N*-terminus could be carried out classically and confirmed the sequence Xxx–Lys–Gly–Val–His–Ala. Under the conditions of Edman degradation, unmodified cysteine is known to be destroyed thus, no phenylthiohydantoin (PTH) amino acid was expected at the end of the first cycle of degradation. However, in this case, a peak was observed (which elutes very close to diphenylthiourea (DPU), a classical side product of Edman degradation). When an authentic sample of thiazolidine-4-carboxylic acid (Thz) was submitted to Edman degradation, its PTH-derivative co-eluted with the one observed previously.

An unequivocal synthesis of the Thz containing peptide was performed. In this case, the histidine side chain was protected by a DNP group and Boc–Thz was incorporated in place of



Scheme 1

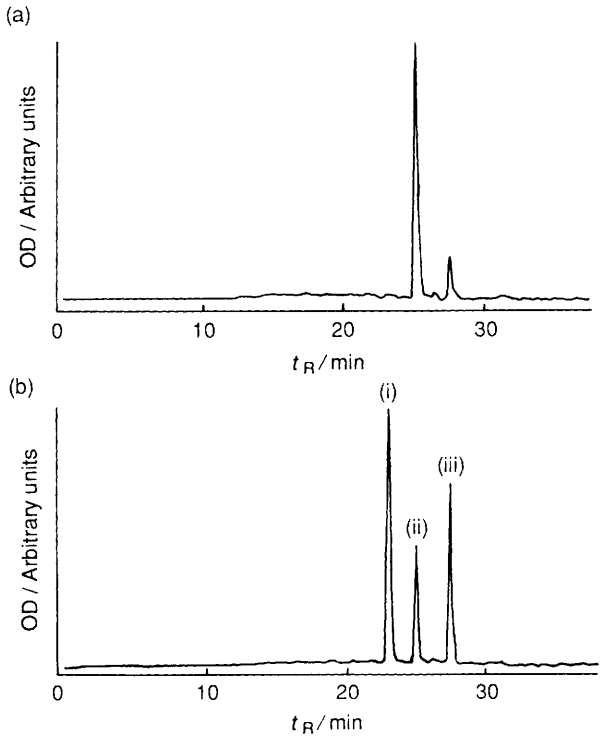


Fig. 1 Analytical HPLC analysis; (*a*) Crude product obtained after HF cleavage of Cys(MeBzl)–Lys(ClZ)–Gly–Val–His(Bom)–Ala–Resin. (*b*) Separation of a mixture containing: (i) Cys-containing peptide prepared by HF cleavage after thiolysis of Cys(MeBzl)–Lys(ClZ)–Gly–Val–His(DNP)–Ala–Resin; (ii) Thz-containing peptide prepared by HF cleavage after thiolysis of Thz–Lys(ClZ)–Gly–Val–His(DNP)–Ala–Resin; (iii) Disulphide of (i) prepared by mild air oxidation of (i) at pH 8. *HPLC conditions*: eluant (*A*) 0.05% TFA, (*B*) MeCN (0.05% TFA) flowrate 0.7 ml min^{-1}, column Nucléosil C18/5 μm/100 Å (4.6 × 250 mm), linear gradient 0–100% (*B*) (30 min).

cysteine. After thiolysis and HF cleavage, the Thz-containing peptide proved to be indistinguishable from the product of the initial synthesis (Fig. 2*b*). In contrast, when the synthesis was performed using DNP-protected histidine and MeBzl-protected cysteine, the Cys-containing peptide which was obtained, eluted in a different position in HPLC (Fig. 2*b*) and did not have a *m/z* 12 excess.

Conversion of cysteine to thiazolidine derivatives by reaction with aldehydes or ketones in acidic conditions has been described previously[3] and has even been proposed as a convenient new protective system for cysteine.[4,5] In the present case, formaldehyde, which is formed during cleavage of the Bom group, reacts both with the NH_2-terminus and the thiol groups of cysteine to yield the thiazolidine cycle.

Another peptide containing an NH_2-terminus cysteine (Cys–Leu–Pro–Arg–Cys–Pro–Pro–His–Ser–His–Gly–Ala–Leu–Lys–Arg–Met) had been previously synthesised using the same combination of protecting groups: Cys(MeBzl) and His(Bom). Determination of its molecular weight showed an excess of *m/z* 12 (determined: 1821.7 and calculated: 1809.1), indicating the generality of the reaction. The peptide was treated with trypsin and the molecular weight of the resulting fragments determined without separation. As expected, the molecular weight of the NH_2-terminus fragment was in excess of *m/z* 12 (determined: 498.9 and calculated: 487.6) while the CO_2H-terminus fragment which contains a cysteine initially located at an internal position in the complete peptide was not modified, confirming that a free NH_2-terminus on cysteine is required for the cyclisation during HF treatment.

This new type of reaction is of particular importance because it could remain unnoticed: in both cases the Thz-containing peptide was the major product and, owing to the fact that Thz regenerates cysteine during acidic hydrolysis, amino acid analysis gave satisfactory ratios.

In conclusion, N^{π}-Bom protected histidine should not be used during the synthesis of peptides having an *N*-terminus cysteine. In this case, the classical DNP protecting group, which requires an additional deprotection step (thiolysis) should be preferred.

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