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Specific Fragmentation of Thioxo Peptides Facilitates the Assignment of the Thioxylated Amino Acid

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Low-energy collision-induced dissociation (CID) product ion spectra of a series of singly protonated thioxo peptides produced by electrospray ionization (ESI) were obtained by triple-quadrupole tandem mass spectrometry. The principal feature of the spectra is a preferential cleavage of the peptide bond succeeding the thioxo peptide moiety. Thus this method provides the possibility of assigning the position of the thioxylated amino acid in oligopeptides resulting from the thioxylation procedure with Lawesson's or a related reagent. At low collision energy, dominant B ions and/or complementary Y″ ions are observed. Higher collision energy yields internal ions that consist of two adjacent amino acids encompassing the thioxo peptide bond. The formation of these internal ions and the elimination of CO from them give evidence for the position of the thioxylated amino acid. There are strong indications of a stabilization of the B and internal ions through the formation of a thiazolone. Fragment ions resulting from the cleavage of the thioxo peptide bond are either completely absent or of very low intensity. © by John Wiley & Sons, Ltd.

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INTRODUCTION

Backbone modification of peptides is an important tool to establish the mode of action of biologically active peptides. Oxygen–sulphur substitution at the peptide bond is one of the prominent backbone modifications used for this purpose. The major synthetic method to get access to thioxo peptides is the O/S exchange (thioxylation) of urethane-protected dipeptide esters with Lawesson's,[1] Davy's[2] or Yokoyama's[3] reagent and subsequent chain elongation of the resulting thioxo dipeptides. The CS—NH bond is usually planar and in *trans* (Z) conformation.[4] The energy barrier for a rotation about the CS—NH bond is 8–12 kJ mol^{-1} higher than for the CO—NH bond ($\Delta G‡$ = 80–90 kJ mol^{-1}) owing to more C=N double-bond character.[5] The C=S double bond is longer (C=S, 0.164 nm; C=O, 0.124 nm).[4,6] The larger covalent[7] and van der Waals[8] radius of sulphur restrict the allowable ϕ, ψ-angles in the vicinity of thioxo peptide groups.[9] Thioxo peptides are stronger acids[10] but weaker bases[11] than oxo peptides.

Despite these subtle structural differences between oxo and thioxo peptides, effects on the conformation of peptides[12,13] and their biological activity[14–20] have been described. Thioxo peptides often exhibit higher proteolytic stability in comparison with substrates containing oxo peptide bonds only. This was demonstrated for carboxypeptidase A,[15,17] prolyl endopeptidase,[18] aminopeptidase P and dipeptidyl peptidase IV.[19] Recently it was shown that thioxo peptides act as competitive inhibitors for the peptidyl-prolyl *cis/trans* isomerase cyclophilin A[21] known as the cytosolic receptor for the immunosuppressive undecapeptide cyclosporin A.

The presence of the thioxo peptide group can be evaluated by ^{1}H NMR and ^{13}C NMR. A downfield shift is observed for both the CS—NH protons (1.5–2 ppm) and the CS carbons (30 ppm).[19] The position of a thioxo peptide bond can be assigned by multidimensional NMR experiments along with complete assignment of the entire set of ^{1}H and ^{13}C signals.[12] However, NMR is a time- and material-intensive method. Additionally, the analysis of polypeptides may be very difficult and the investigation of polypeptide mixtures cannot be performed at all.

Mass spectrometry can overcome these limitations, providing a useful tool for the analysis of modified peptides that are hard to analyse by Edman degradation. ESI-MS/MS has been shown to be very useful in localizing side chain modifications, especially in phosphorylated peptides.[22,23] We tested the principal features of electrospray ionization in conjunction with low-energy tandem mass spectrometry for the assignment of the thioxylated amino acid in thioxo peptides. Thioxo peptides have been investigated by Deterding *et*

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al. by FAB-MS/MS so far.[24] A similar fragmentation for oxo peptides and thioxo peptides was reported. Since finishing our studies, a paper has been published describing the promotion of B_1 and Y''_n fragment ions of the $M + 2H^+$ ion of related phenylthiocarbamoyl derivatives resulting from N-terminal modification.[25]

EXPERIMENTAL

Mass spectrometry

Low-energy collision-induced dissociation (CID) mass spectra were recorded with a VG BIO-Q triple-quadrupole mass spectrometer (Fisons Instruments, Manchester, UK) equipped with an electrospray source. Peptides were dissolved at a concentration of 0.1 mg ml^{-1} in a 1 : 1 mixture of water and acetonitrile containing 1% formic acid. Samples were introduced into the electrospray ion source at a flow rate of 4 μl min^{-1} (HP 1050 pump). The needle potential was 4 kV and the skimmer potential was held at 45 V unless mentioned otherwise. Nitrogen was used as both drying and nebulizing gas. MS/MS experiments were performed between 1.2 and 1.5 mbar collision cell pressure and with argon as collision gas. The collision energy (CE) was varied between 5 and 80 eV as described in detail.

Spectra of the deuterium-labelled peptides, in which all exchangeable hydrogens have been replaced by deuterium, were obtained by dissolving the peptide in a 1 : 1 mixture of D_2O (99.9%, Merck) and acetonitrile (non-deuterated, HPLC grade, Fluka) containing 1% formic acid (non-deuterated, 98% Merck). The nitrogen used (4.0 grade) was not additionally dried. The CID mass spectra were recorded 2 h after incubation of the thioxo peptides with the deuterated solvent mentioned above.

The fragment ion nomenclature suggested by Roepstorff and Fohlman was used.[26] Internal ions were indicated by single-letter code of the corresponding amino acids according to the nomenclature convention of Biemann.[27]

Peptide synthesis

A C-terminal modification with 4-nitroanilide was abbreviated as –4NA. Peptide sequences were written in single-letter code. The thioxo peptides derived from AGPF–4NA were obtained by oxygen–sulphur exchange with Lawesson's reagent and its derivatives as described in detail in Ref. 21.

Protection of L-tyrosine. L-Tyrosine (10 mmol) was dissolved in 2.5 ml of 4N sodium hydroxide. The solution was cooled at 0 °C and treated with 2.2 ml of allylchloroformate and 5 ml of 4N sodium hydroxide, added in five equal portions. The reaction mixture was stirred for 1 h at room temperature. The mixture was acidified to pH 2–3 using $KHSO_4$ solution. The resulting solution was then extracted with ethyl acetate (6 × 10 ml) and the combined organic phases were washed with brine (saturated NaCl solution, 6 × 15 ml) and dried over $MgSO_4$. The solvent was removed to give an amorphous solid that was recrystallized from ethylacetate/hexane (2.9 g, 83%).

Preparation of the tripeptide PFP–OtBu. Five millimoles of the amino acid Fmoc-F-OH were dissolved in 20 ml of THF and cooled to −15 °C. The solution was treated with 5 mmol of *N*-methylmorpholine and 5 mmol of iso-butylchloroformate followed by 5 mmol of the amino acid P–OtBu after an activating time of 10 min. The mixture was stirred for 3 h at −15 °C and for an additional 12 h at ambient temperature. The solvent was evaporated and the residue taken up into ethyl acetate. This mixture was washed with brine, 5% $KHSO_4$ brine, 5% $NaHCO_3$ and brine, dried over Na_2SO_4 and evaporated to give a white solid. The crude product was treated with THF/morpholine (v/v, 1 : 1) for 1 h. After removing the solvent *in vacuo*, the solution was dissolved in methanol. Removal of the precipitated Fmoc adduct by filtration gave the dipeptide FP–OtBu as a yellow oil. The crude product was reacted with Fmoc-P-OH and deprotected by THF/morpholine. The resulting oil was coupled with Alloc-Y(Alloc)-OH (Alloc, allyloxycarbonyl). The crude product was purified by preparative HPLC (42% acetonitrile, 0.05% TFA). Extraction with ethyl acetate gave a colourless oil, yield 1.7 g (47%).

Alloc-Y(Alloc)P-ψ[CS—NH]-FP–OtBu.* To a solution of 1 mmol of protected tetrapeptide-butylester in THF, 0.6 mmol of 2,4-bis(*p*-methoxyphenyl)1,3-dithiadiphosphetane-2,4-disulphide (Lawesson's reagent) was added. The resulting solution was stirred for 4 h at 50 °C. The solvent was evaporated and the thioxylated products were purified by flash chromatography using CH_2Cl_2 to remove by-products, followed by CH_2Cl_2/ethyl acetate (v/v, 8 : 2) to obtain the desired Alloc-Y(Alloc)P-ψ[CS—NH]-FP–OtBu. The resulting yellow oil was purified by preparative HPLC (45% acetonitrile, 0.05% TFA) and extracted with ethyl acetate. The product was obtained as a slightly yellow oil, yield 412 mg (55%).

The peptides AAPF-ψ[CS—NH]-AY and AFG-ψ[CS—NH]-AFG-ψ[CS—NH]-AAPF-ψ[CS—NH]-GG were synthesized by treatment of AAPF-ψ[CS—NH]-4NA with subtilisin C (Sigma) and the dipeptide AY or GG.[28] Subsequently, the resulting peptide AAPF-ψ[CS—NH]-GG was treated with Papain (Sigma) and the peptide AFG-ψ[CS—NH]-4NA, leading to the modified peptide AFG-ψ[CS—NH]-AFG-ψ[CS—NH]-AAPF-ψ[CS—NH]-GG thioxylated at multiple sites.[28]

Ab initio calculations

The *ab initio* self-consistent field (SCF) calculations for the respective B ions were carried out with the TURBOMOLE program (Biosym/MSI, San Diego, CA, USA,

* Alterations of a peptide bond are represented by the ψ nomenclature system. A ψ is followed by the structure of the new bond in brackets. The nomenclature of the compounds is in accordance with the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature, *Pure Appl. Chem.* **5B**, 595 (1984).

version 95.0/3.0.0) at the 6-31G basis level.[29] Four model ions of a corresponding oxo and thioxo peptide were chosen to calculate the energetic difference between the cyclic and the corresponding acyclic structure (see Fig. 3). The acyclic structures were constrained to an all-*trans* conformation. The energy for the isolated ions was calculated in the gas phase at 0 K.

RESULTS AND DISCUSSION

Figure 1 shows the daughter ion spectra of the $[M + H]^+$ ions of the model tetrapeptide AGPF–4NA (4-nitroanilide) and its four monothioxylated derivatives (A-ψ[CS—NH]-GPF–4NA, AG-ψ[CS—NH]-PF–4NA, AGP-ψ[CS—NH]-F–4NA and AGPF-ψ[CS—NH]-4NA) at low collision energy (CE = 5 eV), where only initial fragmentation occurred. Fragmentation of the $[M + H]^+$ ion of the oxo peptide preferentially yielded a proline-directed Y_2'' ion (Fig. 1A). In comparison with the thioxo peptides, the low yield of fragment ions of the oxo peptide at the same collision energy is obvious.

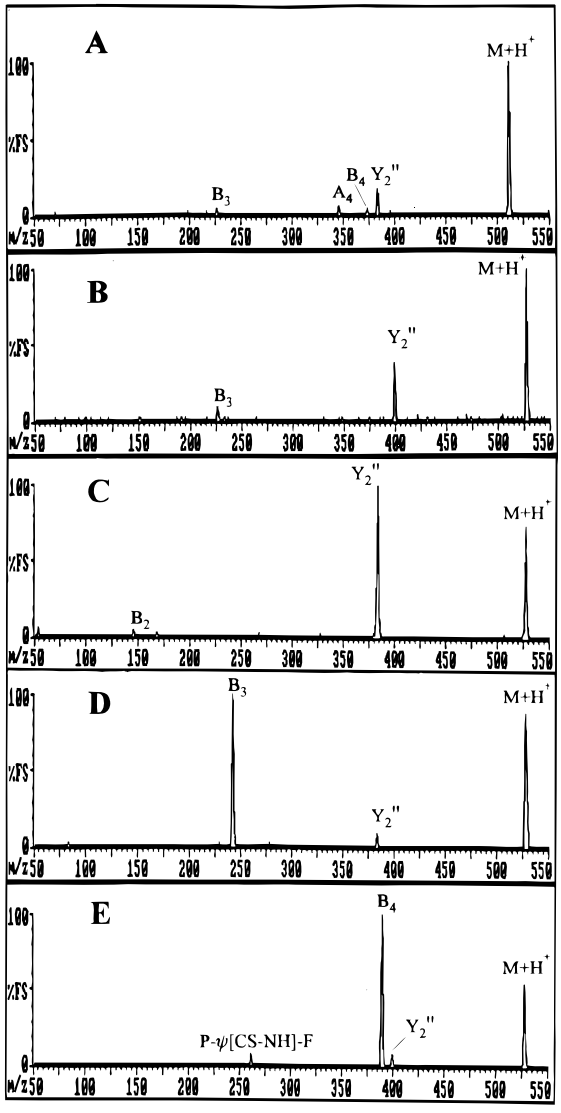


Figure 1. CID mass spectra of $[M + H]^+$ ion of AGPF–4NA and four monothioxylated peptides: (A) AGPF—4NA; (B) AGPF-ψ[CS—NH]-4NA; (C) A-ψ[CS—NH]-GPF–4NA; (D) AG-ψ[CS—N]-PF–4NA; (E) AGP-ψ[CS—NH]-F–4NA (CE = 5 eV, p = 1.2 mbar).

The singly protonated thioxo peptides formed dominant fragment ions (Figs 1C–1E) resulting from the preferential cleavage of the adjacent peptide bond succeeding the thioxo peptide bond. This strongly indicates a sulphur-controlled pathway of fragmentation. The protonated molecular ions of the peptides AG-ψ[CS—N]-PF–4NA and AGP-ψ[CS—NH]-F–4NA (Figs 1D and 1E) gave intense signals of B ions, while the Y_2'' ion dominated in the CID mass spectrum of the protonated peptide A-ψ[CS—NH]-GPF–4NA (Fig. 1C). A plausible explanation for the preferred formation of B ions (Figs 1D and 1E) is related to the stabilization of these ions by cyclization to a protonated thiazolone (Fig. 2). Recently a similar pathway has been described for $M + 2H^+$ ions of N-terminal phenylthiocarbamoyl (PTC) derivatives of peptides that produce a related fragmentation pattern.[25] Hunt, Harrison and co-workers suggested an analogous stabilization of B ions for oxo peptides.[30–32] The higher nucleophilic character of sulphur compared with oxygen is the most likely reason for the preferential fragmentation of thioxo peptides.

Ab initio calculations comparing the energy level of cyclic and acyclic B ions with thioxo peptide and oxo peptide bonds were performed to verify the higher stability of the B ions of thioxo peptides (Fig. 3). Under the conditions used (see Experimental), the energy gain is 170 kJ mol^{-1} for the protonated oxazolone and 188 kJ mol^{-1} for the protonated thiazolone with respect to the corresponding acyclic structure. The results show that

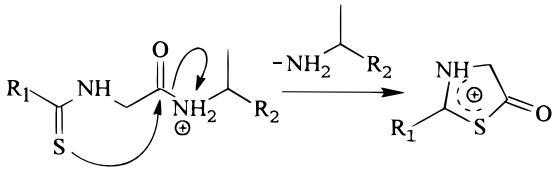


Figure 2. Possible formation of protonated thiazolone.

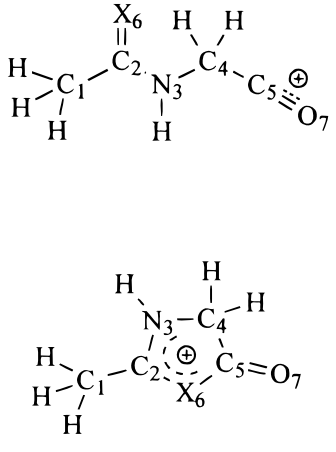


Figure 3. Acyclic and cyclic model of B ions (X ≡ 0,S).

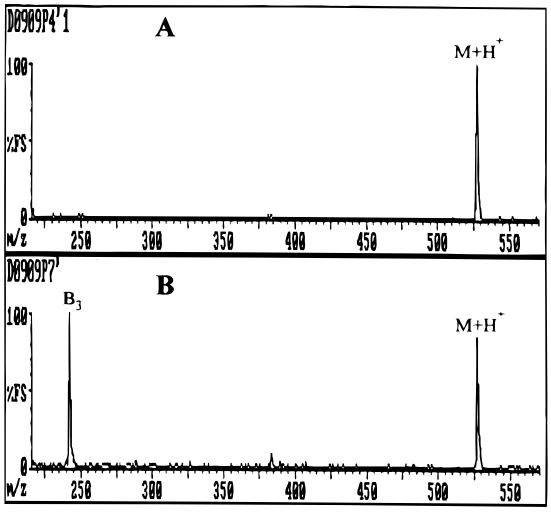


Figure 4. Mass spectra of AG-ψ[CS—N]-PF–4NA recorded with (A) 40 V and (B) 75 V skimmer potential.

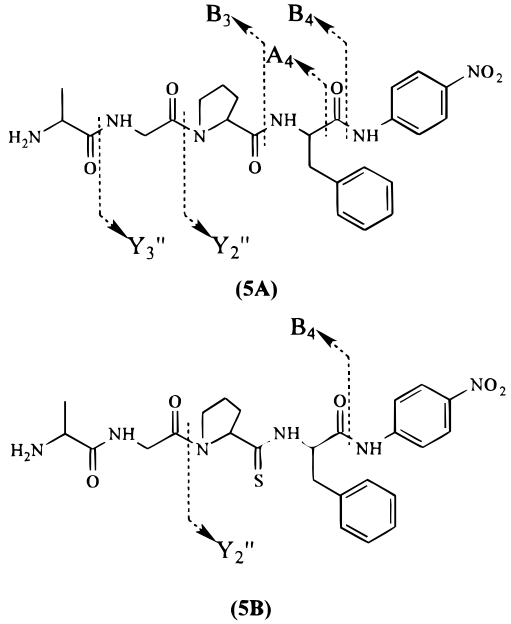
the protonated thiazolone is more stable than the oxygen analogue. There is a significant charge transfer from the C_5 atom to the sulphur of the cyclic structure in the case of the thiazolone (data not shown). The preferential localization of the positive charge at the sulphur atom seems to be the major reason for the higher stability of the thiazolone in comparison with the oxazolone.

The preferential formation of the corresponding B ion was also observed in the product ion spectrum of the singly protonated peptide AG-ψ[CS—N]-PF–4NA (Fig. 1D), although unmodified oxo peptides containing proline usually yield abundant Y″-type fragment ions by CID, arising from the cleavage of the peptide bond preceding proline.[33–36]

In the CID mass spectrum of the $[M + H]^+$ ion of A-ψ[CS—NH]-GPF–4NA (Fig. 1C) the intense signal of the proline-directed fragment ion Y_2'' indicated its increased abundance when compared with the Y_2'' ion of the oxo peptide AGPF–4NA (Fig. 1A). The B ion discussed above was detected with very low intensity. The reason for the preferential formation of Y_2'' resides in the high acidity of the NH proton of the thioxo peptide group.[37] Additionally, the proline nitrogen has a higher proton affinity in comparison with the nitrogen of other peptide groups[35] and is able to support the migration of a proton to the adjacent peptide group.

For AGPF-ψ[CS—NH]-4NA (Fig. 1B) the preferential fragmentation of an adjacent C-terminal peptide bond is impossible. The fragmentation pattern obtained with 5 eV CE is similar to that of the oxo peptide.

In principle, it is possible that thiazolones are preformed during acidic treatment in solution.[38] A similar cleavage takes place during the first step of the Edman degradation, the standard procedure for sequencing peptides and proteins.[39] It is important to demonstrate that the easily formed thiazolones are not present in solution. In order to exclude a possible preformation of thiazolones in the solvent used containing 1% formic acid, the skimmer potential was varied between 40 and 75 V. The signal of the product ion B_3 arising from the preferential cleavage of the adjacent peptide bond C-terminal to the thioxo peptide bond of



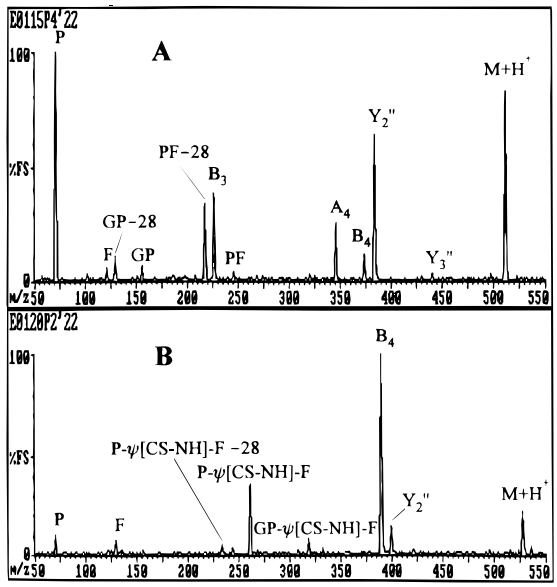


Figure 5. Comparison between CID mass spectra of $[M + H]^+$ ion of (A) AGPF–4NA and (B) AGP-ψ[CS—NH]-F–4NA (CE = 20 eV, p = 1.2 mbar).

AG-ψ[CS—NH]-PF–4NA disappears in the spectrum measured with 40 V skimmer potential (Fig. 4A). Therefore thiazolone species are not present in solution.

Positional assignment of the thioxylated amino acids

A preferential fragmentation of the adjacent peptide bond succeeding the thioxo peptide bond was observed for thioxo peptides derived from AGPF–4NA, except for AGPF-ψ[CS—NH]-4NA where this favoured fragmentation is impossible. This paves the way to localize the thioxylated amino acid in the peptide chain. The formation of a specific internal ion of the structure X-ψ[CS—NH]-Y[CO+] (X and Y represent an amino acid) and the elimination of CO from it at higher activation in the collision cell provide further evidence

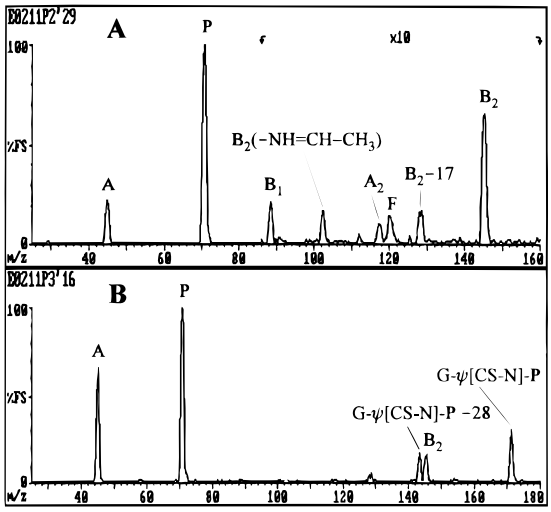


Figure 6. CID mass spectra (detail) of $[M+H]^+$ ion of (A) A-ψ[CS—NH]-GPE–4NA and (B) AG-ψ[CS—NH]-PF–4NA (CE = 50 eV, p = 1.5 mbar).

for the position of the thioxylated amino acid. The alternative internal ion of the structure X[CO—NH]Y-ψ[CS+] with the same mass is expected to expel CS, which was not detected in our experiments. Additionally, the cleavage of the thioxo peptide bond is infrequent for the peptides under investigation. The internal ion X-ψ[CS—NH]-Y[CO+] mentioned above is consistent with a thiazolone and demonstrates the high stability of this structure.

The following procedure for the assignment of the thioxylated amino acid is recommended: (i) fragmentation of the protonated thioxo peptide at low collision energy to induce the preferential cleavage; (ii) increase of the collision energy to form the internal ions of the structure X-ψ[CS—NH]-Y[CO+] resulting from the initially formed B ions; (iii) further activation of the fragment ions in the collision cell leading to elimination of CO from these internal ions.

The general strategy in assigning the thioxylated amino acid has been tested for several thioxo peptides. The preferential formation of the B_4 ion of the protonated peptide AGP-ψ[CS—NH]-F–4NA can be derived from the CID mass spectrum in Fig. 1E. Further fragmentation resulted in the formation of the internal ion P-ψ[CS—NH]-F and the elimination of CO from it (Fig. 5B). This clearly points to a thioxylated proline residue. In contrast with the oxo peptide (Fig. 5A), the formation of the ion A_4 as well as the elimination of CO from the internal ion P-ψ[CS—NH]-F was diminished (Fig. 5B). The $[M+H]^+$ ion of A-ψ[CS—NH]-GPF–4NA preferentially yielded the Y_2'' ion (Fig. 1C). The generation of the corresponding B_2 ion was less favoured but was sufficient to yield the fragment ion A_2 at higher collision energy (Fig. 6A). Furthermore, the loss of the neutral molecules NH_3 and $NH{=}CH{-}CH_3$ from the ion B_2 was observed. The formation of these fragment ions is consistent with our H/D exchange experiments and is illustrated for the deuterated B_2 fragment ion in Fig. 7. The B_1 ion (Fig. 6A) may be stabilized by delocalizing the positive charge of the thioacylium ion over carbon and sulphur. B_1 ions formed by the amino acids of oxo peptides are mostly not observable in the CID mass spectra, because additional stabilization by functional groups is lacking.[30,40,41]

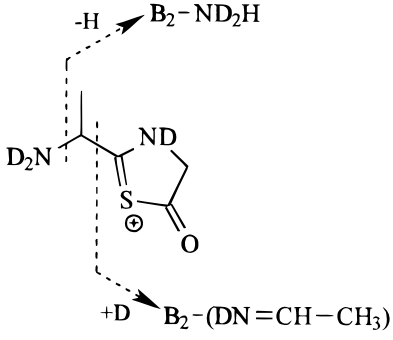
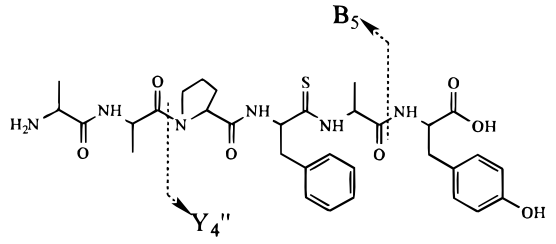


Figure 7. Fragmentation scheme for formation of unusual fragment ions of B_2 fragment ion of deuterated peptide A-ψ[CS—NH]-GPF–4NA.



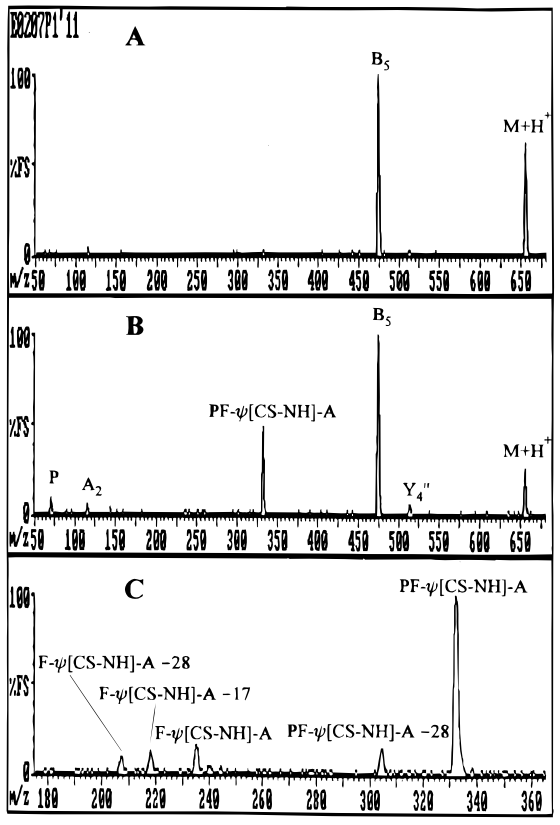


Figure 8. CID mass spectra of $[M+H]^+$ ion of AAPF-ψ[CS—NH]-AY: (A) CE = 10 eV; (B) CE = 20 eV; (C) detail of CID mass spectrum of $[M+H]^+$ ion of AAPF-ψ[CS—NH]-AY showing most interesting internal fragment ions, CE = 80 eV (p = 1.2 mbar).

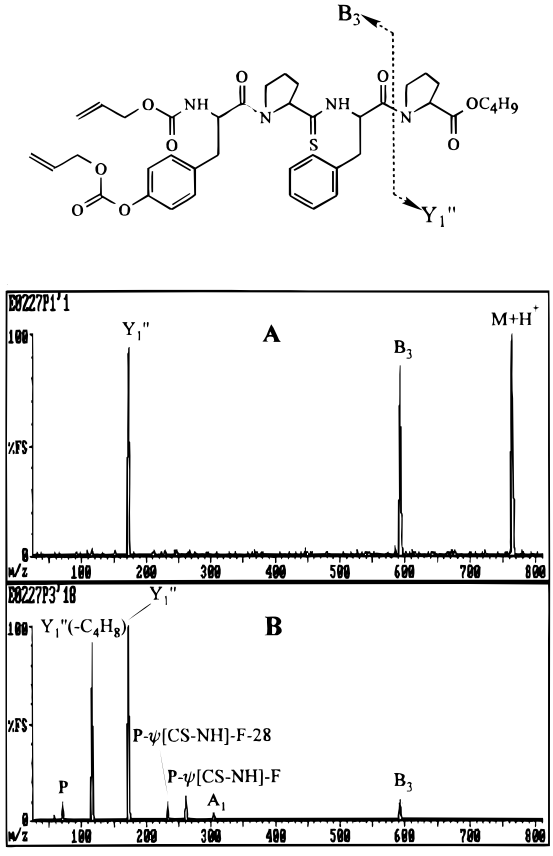


Figure 9. CID mass spectra of $[M + H]^+$ ion of Alloc-Y(Alloc) P-ψ[CS—NH]-FP–OtBu: (A) CE = 0 eV; (B) CE = 40 eV (p = 1.2 mbar).

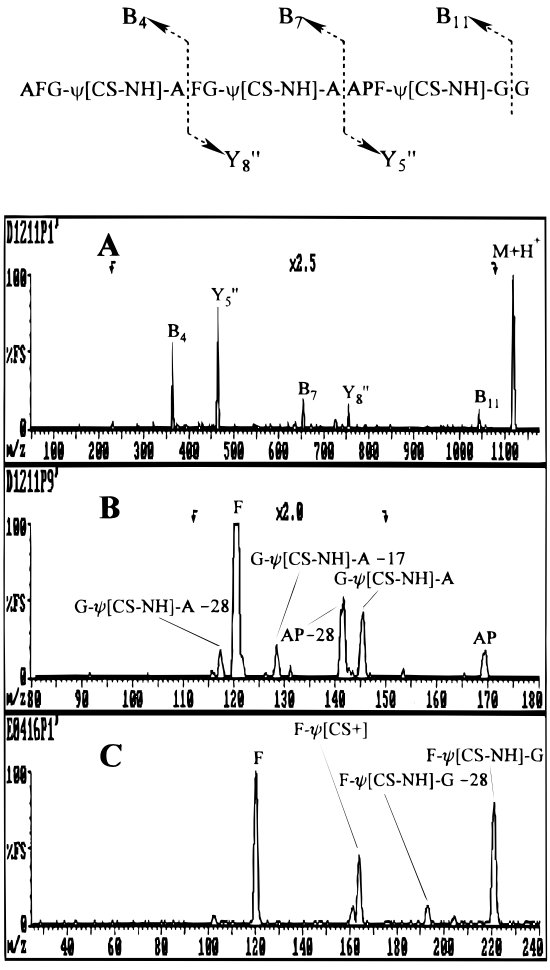


Figure 10. CID mass spectra of $[M + H]^+$ ion of thioxo peptide AFG-ψ[CS—NH]-AFG-ψ[CS—NH]-AAPF-ψ[CS—NH]-GG: (A) CE = 30 eV; (B) (detail) CE = 60 eV; (C) CID mass spectrum of internal ion F-ψ[CS—NH]-G initially formed by source fragmentation, skimmer potential 110 V, CE = 5 eV (p = 1.5 mbar).

The protonated molecular ion of AG-ψ[CS—N]-PF–4NA preferentially generated the B_3 ion at low collision energy, indicating a thioxylated glycine (Fig. 1D). Further fragmentation of the ion B_3 at higher collision energy resulted in the formation of the internal ion G-ψ[CS—N]-P and the elimination of CO from it, providing information about the position of the thioxylated amino acid (Fig. 6B).

In the case of AGPF-ψ[CS—NH]-4NA the preferential fragmentation mentioned above was impossible, because there is no adjacent C-terminal peptide group. Nevertheless, the position of the sulphur is obvious from Fig. 1B. The existence of the B_3 ion devoid of sulphur proves a thioxo anilide moiety.

The peptide AAPF-ψ[CS—NH]-AY was studied to demonstrate that the applied strategy can also be extended to thioxo peptides with free terminal positions. The CID mass spectrum obtained with 10 eV CE is shown in Fig. 8A. Preferential formation of the ion B_5 containing the CS—NH group indicates a thioxylated phenylalanine. The internal ion PF-ψ[CS—NH]-A (Fig. 8B), as well as the formation of the internal ion F-ψ[CS—NH]-A and the elimination of CO and NH_3 from it, was generated at 80 eV CE (Fig. 8C).

The position of the thioxylated amino acid was independently assigned by NMR spectroscopy for the peptides discussed above.[21] However, the following peptides treated with Lawesson's reagent gave thioxylated derivatives with unknown position of the thioxylated amino acid.

Alloc-Y(Alloc)P-ψ[CS—NH]-FP–OtBu represents a typical intermediate on the synthetic route to extended chains of thioxo peptides. Both the N-terminus and the hydroxyl group of Tyr are blocked by the allyloxycarbonyl protecting group (Alloc); the C terminus is modified as *tert*-butylester. These protecting groups are essential because of the side reaction of Lawesson's reagent with nucleophilic groups. The CID mass spectra of the $[M + H]^+$ ion of the thioxo peptide Alloc-Y(Alloc)P-ψ[CS—NH]-FP–OtBu are shown in Fig. 9. At 0 eV CE a preferential cleavage of the peptide bond between phenylalanine and proline was observed. There was competition between the formation of the ions B_3 and Y_1'' (Fig. 9A). The product ion spectrum taken at 40 eV CE showed the generation of the internal ion P-ψ[CS—NH]-F as well as the elimination of CO from it (Fig. 9B).

The tandem mass spectrometry-assisted assignment of the thioxylated amino acids can also be performed with peptides thioxylated at multiple sites, e.g. for the thioxo peptide AFG-ψ[CS—NH]-AFG-ψ[CS—NH]-AAPF-ψ[CS—NH]-GG. The modified positions can be similarly inferred from the CID spectrum of the $[M + H]^+$ ion measured at low CE (Fig. 10A).

The preferential formation of the ions B_4/Y''_8, B_7/Y''_5 and B_{11} strongly indicated the thioxylated amino acids glycine (position 3), glycine (position 6) and phenylalanine (position 10). Further evidence was given by the formation of the internal ion G-ψ[CS—NH]-A and the elimination of CO from this internal ion at high collision energy (Fig. 10B). The elimination of CO from the internal ion F-ψ[CS—NH]-G was not observed in the CID mass spectra of the protonated thioxo peptide (data not shown). In this special case it was possible to show the elimination of CO by means of a daughter ion scan of the internal ion F-ψ[CS—NH]-G initially formed by source fragmentation (Fig. 10C). This clearly demonstrated the conversion of the phenylalanine to a thioxylated amino acid.

An interesting aspect of the preferential formation of the B_3 ion of AG-ψ[CS—NH]-PF–4NA is that this fragmentation is only possible from the *trans* thioxo prolyl peptide bond conformer. In the *cis* conformer the CS group cannot approach the succeeding peptide bond as was suggested by using molecular models. Therefore the formation of the corresponding B ions should only be observed for the *trans* conformer. The same problem can be discussed for the oxo prolyl peptide bond. In this case, however, the formation of the discussed B ion is less favoured.[32,36]

CONCLUSION

In thioxo peptides the low-energy collision-induced dissociation of protonated molecular ions causes a preferential cleavage of the peptide bond succeeding the thioxo peptide bond. This is suggested owing to the formation of a stable thiazolone by nucleophile attack of the sulphur on the succeeding peptide bond. A competition between two pathways leading to the B ion and the complementary Y″ ion is observed. Subsequent fragmentation of the B ion results in the formation of internal ions containing the thioxo peptide bond. The cleavage of the thioxo peptide bond itself is rarely observed.

Two experimental steps are recommended for the assignment of the position of the thioxylated amino acid in oligopeptides. The preferential cleavage of the peptide bond succeeding the thioxo peptide moiety at low collision energy provides initial information about the modified position. In a second step the collision energy has to be increased to fragment the initially produced B ion. As a result, the formation of the internal ion of the structure X-ψ[CS—NH]-Y[CO+] and the elimination of CO from the internal ion occur. This provides unequivocal evidence for the nature of the thioxylated amino acid. The positional assignment of thioxo peptide bonds is of general interest for the characterization of reaction products yielded from the Lawesson procedure of oligopeptide thioxylation. Therefore we are going to test this method for other thioxo peptides as well.

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