A Comparison of N-terminal Sequencing Using Different Sulphonication Reagents with MALDI-PSD-TOFMS

David J Evason, Mark D Mills, Victor C Parr, Alexis J Polley : Scientific Analysis Instruments, Manchester, UK.

Overview

Charge directed fragmentation via N-terminal sulphonication

- Fragmentation occurs more readily
- Predictable ladder sequence (y ion only, for tryptics)
- Via a "harmonic field reflectron" MALDI-TOFMS

APPROACHES

1) 4-sulfophenyl isothiocyanate (SPITC)
2) 2-sulfophenyl benzoic acid cyclic anhydride (SBAn)
3) 2-sulfopropionic acid NHS-ester (CAF)

CONCLUSIONS

- SBAn is difficult to use, derivatives aren't seen in reflectron
- SPITC had the best performance, is more robust than CAF and is cheaper

Introduction

Charge directed fragmentation (CDF) increases the fragmentation rate of peptides and directs their fragmentation into a predictable pattern. For this purpose, sulphonication has proved useful. The following presentation compares three methods of sulphonication for the purpose of peptide sequencing by a harmonic field reflectron MALDI-PSD-TOFMS.

Methods

All materials were from Sigma, Poole, UK, except for CAF reagent (gift from M Liminga, Amersham Biosciences).

Protein digested with 5% w/v DPCC treated trypsin, in 0.05M ammonium bicarbonate (AMBC).

Sulphonication a) *SPITC* : sample diluted 1:1 with 50mg/ml 4-sulfophenyl isothiocyanate in 0.04M AMBC. b) *SBAn:* sample diluted 1:1 with 10mg/ml 2-sulfobenzoic acid cyclic anhydride in dry acetone. c) *CAF:* sample diluted 1:1 in 10mg/ml CAF reagent in 0.25M Na_2CO_3 pH 9.4. Samples incubated for 2 hours at room temperature. Sample clean up prior to analysis on C-18 z-tips (Millipore) .

All MALDI-TOFMS was performed on a ***LaserTof RS***, SAI, UK, using a high performance, harmonic field reflectron MALDI-TOFMS (Thompson et al).

Results

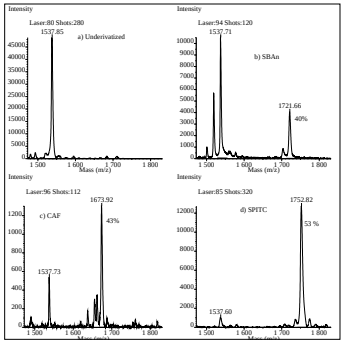

Figure 1: Yield of derivatization in linear

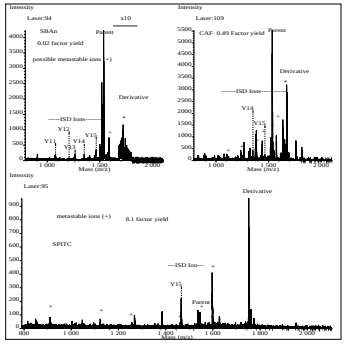

Figure 2: Yield of derivatization in reflectron

	1	2	3	4	5
None	77.9	14, 11, 9, 7 and 1	na	na	Sig
SBAn	78.3	15, 14, 13 and 11	40	0.02	Sig
SPITC	100.0	All	53	8.1	Y 15
CAF	100.0	15 only	43	0.49	none

Table 1: Comparison of Sulphonication Treatments on PSD Efficacy

1 - Percentage of ions that are C-terminal in PSD of 8 pmol peptide
2 - Y ions present at the lowest dilution tested. (8 fmol for all derivatizations, 16 fmol for underivatized)
3 - Ratio of derivatized to underivatized linear mode
4 - Ratio of derivatized to underivatized reflectron mode
5 - ISD - In Source Decay. Sig = significant

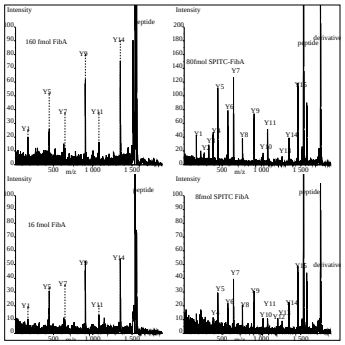

Figure 3: PSD of (top left, clockwise) 160fmol, FibA, 80fmol SPITC-FibA, 16fmol FibA, 8fmol SPITC-FibA

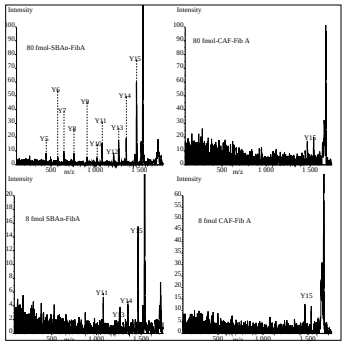

Figure 4: PSD of (top left, clockwise) 80fmol SBAn-FibA, 80fmol SPA-FibA, 8 fmol SBAn-FibA, 8fmol SPA-FibA

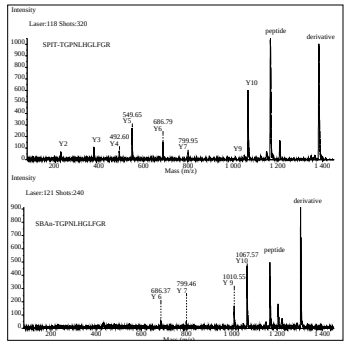

Figure 5: CDF of a tryptic peptide from digested cytochrome c. SPITC v SBAn

Discussion

Figures 1 and 2 compare the sulphonication chemistries in linear and reflectron modes. The linear mode cannot distinguish between a derivative ion and its in-flight fragments (metastable ions) . It therefore gives a better picture of yield since the derivative peak will be composed of the derivative ions at the time of their ionisation in the source. The reflectron spectra is more complicated. It will have peaks of the derivative, and should it fragment during its flight before the reflectron, it will have peaks of these fragments (metastable ions). The reflectron spectra also shows that sulphonication has caused the molecules to fragment in the source (ISD ions). Timing is everything. To be of maximum use in PSD, the derivative must fragment, during its flight to the reflectron, completely, into an equal distribution of all possible y-ions. From the reflectron spectra it appears that CAF and SBAn have done this. However, both have poorer distributions and yields of y-ions at low peptide concentrations (Figure 4). For SBAn derivatives, this is because fragmentation occurs too early, in the source (ISD ions Fig 2).

Conclusion

A good sulphonication (CDF) agent should: give y-only spectra, give readily-identifiable derivatives (for automation), have good y-ion sequences at low concentrations of peptide. In this study SPITC was found to be the best sulphonication agent for PSD. It gave a good yield of readily identifiable derivatives (Fig 1 & 2), had little in source decay (Fig 2), gave a continuous and full y-ion sequence at 80 fmol (Fig 3) and it gave a continuous y-ion sequence from y-15 to y-4 at 8 fmol (Fig 3).

As a demonstration SPITC and SBAn derivatization of a cytochrome c tryptic peptide are shown (Fig 5). The native peptide had less useful PSD (data not shown).

References

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