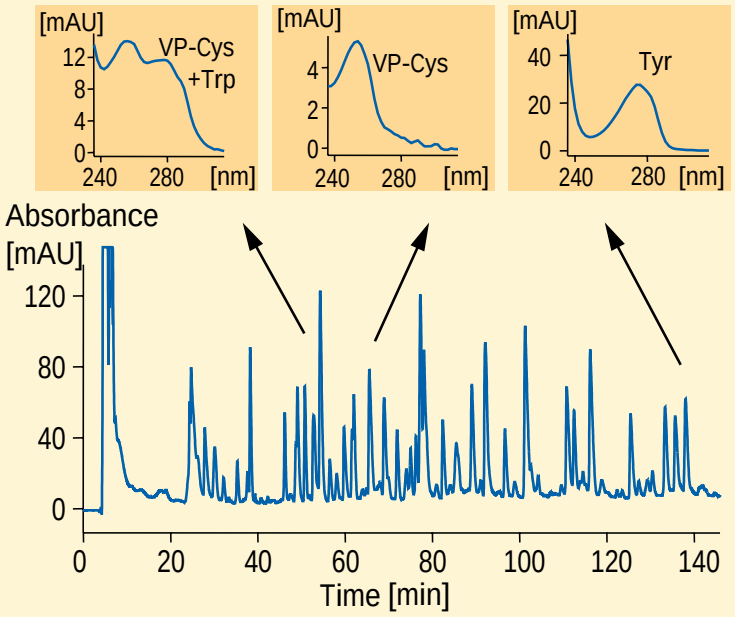
Identification of Pyridylethylated Cysteine Residues in Peptide Maps Using Diode-array Detection

Biopharmaceutical

Cysteine and cystine residues are important amino acid residues because they are involved in the correct formation of the tertiary and quaternary structure of peptides and proteins. The full biological acitivity relies on correctly synthesized intra- and intermolecular disulfide bridges between two particular cysteine residues. As a consequence of disulfide bridge formation and hence a globular structure many proteins are hardly succeptible to proteolytic attack. Therefore, the protein chemical characterization of these proteins is often not possible without prior modification of the cysteine/cystine residues.



Conditions

Column
0.3 ˘ 250 mm Vydac C-18
Mobile phase
A = water, 0.05 % TFA
B = acetonitrile, 0.045 % TFA
Flow rate
4 µl/min after split from 100 µl/min
Gradient
at 0 min 2 % B
at 140 min 45 % B
UV detector
diode-array detector
206 nm, 254 nm, 280 nm
spectra 200–320 nm
500 nl micro flow cell
Temperature
ambient

Figure 1
Tryptic digest of a bacterial neurotoxin protein after reduction and alklation with vinylpyridine

HPLC Performance

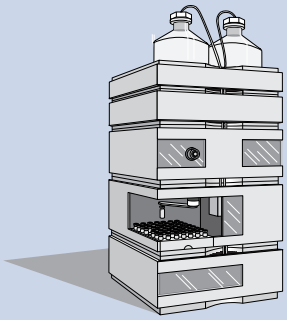
Typically, reduction with a variety of reagents, such as mercapto-ethanol or dithiodithreithol is carried out followed by alkylation using iodoacetic acid, iodoacetamide or vinylpyridine. Afterwards peptides are separated by HPLC and identified by Edman sequencing or MS-MS sequencing.

Prior to sequence analysis peptide maps are routinely separated by microbore or capillary HPLC. Identification of modified cysteine residues is best performed using vinylpyridine as alkylation reagent, since it adds additional spectral characteristics to this residue. As shown in figure 1, using diode-array detection vinylpyridinilated cysteine residues can be unambiguously identified in peptides separated by reversed-phase capillary HPLC by their characteristic absorption maximum at 254 nm They can thus be easily distinguished from tyrosine and tryptophan residues which have an absorption maximum around 280 nm, even if they are present in the same peptide. With this approach collected peptide fractions can be easily analyzed further by protein sequencing or alternatively online by MS-MS sequence analysis.

Equipment

Agilent 1100 Series

- Binary pump
- Vacuum degasser
- Standard autosampler
- Diode-array detector
  micro flow cell
  10-mm path length, 500 nl
- Agilent ChemStation
  + 3D software
  LC Packings flow splitter,
  IC-100-VAR precolumn
  microflow processor*



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Released 03/99
Publication Number 5968-4739E

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