Waters
Lab Highlights
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RAPID IDENTIFICATION OF CYSTEINE-CONTAINING PEPTIDES IN PROTEIN DIGESTS USING μBONDAPAK™ C_{18} RADIAL-PAK™ CARTRIDGES

The amino acids cysteine (CYS) and tryptophan (TRP) are generally regarded as being the most difficult to deal with in terms of protein compositional analysis and sequencing. The determination of TRP requires the use of non-volatile, costly reagents for hydrolysis. This problem is mitigated by the fact that tryptophan-containing peptides can be readily identified by their strong absorbance at 280 nm; thus quantitative determination of TRP need only be performed on those peptides exhibiting significant 280 nm absorbance. Cysteine presents additional problems due to the reactivity of the -SH group. These are generally dealt with by oxidation to cysteic acid, or modification of CYS to a variety of stable derivatives.

A particularly useful procedure is the reaction of CYS residues with 4-vinyl-pyridine following reduction of any disulfide bonds to produce the S-β-(4-pyridylethyl) cysteine (PEC) derivative. This adduct is stable to acid hydrolysis, highly specific for cysteine, and compatible with subsequent protein modification procedures. Additionally, it provides for the introduction of a "tag" into cysteine-containing peptides, allowing for sensitive, instantaneous detection at 254 nm during peptide mapping.

Dr. Curtis Fullmer (Cornell University) recently reported (1) the application of this technique to calcium-binding protein (CaBP), demonstrating the utility of the method, as well as the benefits of μBONDAPAK™ C_{18} Radial-PAK™ cartridges for peptide mapping. Figure 1 shows the tryptic map of PEC-CaBP, monitored at 220, 280, and 254 nm. The two TRP-containing peptides are clearly evident ("T"). Peaks 1-4, which show strong absorbance at 254 nm, represent the cysteine-containing peptides (confirmed by amino acid analysis). These peaks are absent from the tryptic map of underivatized CaBP.

This technique protects the cysteine-containing peptides, while allowing for their rapid identification. The author also concluded that "...use of the radially compressed C_{18} μBONDAPAK™ column provided separation similar to conventional stainless steel columns of the same packing, but at greatly reduced operating pressure... The result is...improved pump performance."

Tom Tarvin

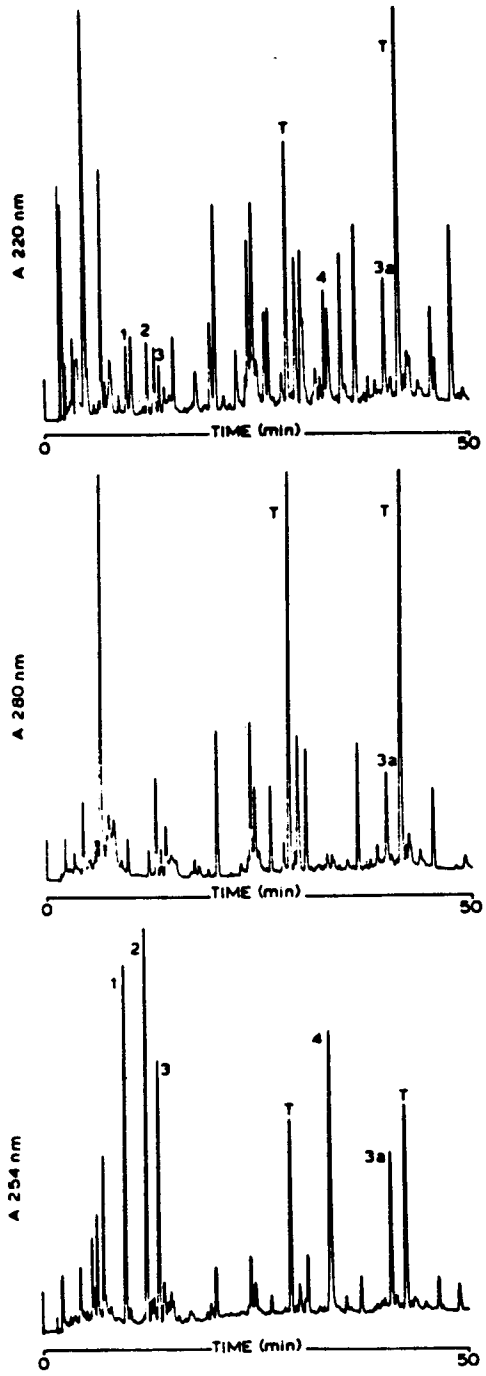
FIGURE 1

TRYPTIC MAP OF PEC-CaBP

Conditions

Sample: Tryptic digest of PEC-Calcium Binding Protein
Column: μBONDAPAK™ C_{18} Radial-PAK™ 8 mm X 10 cm
Detector: M440 DC, 280 nm and 254 nm; M450, 220 nm
Mobile Phase: A: 0.1% TFA
B: 0.1% TFA in CH_3CN
Flow Rate: 2 ml/min
Gradient: 0-60% B, linear, 60 minutes

T = TRP-containing peptides
1-4 = PEC-containing peptides



1. Curtis S. Fullmer, Anal. Biochem. 142, 336-339 (1984).