CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Localization Of Disulfide Bridges

Purpose

Localization of disulfide bridges in peptides. LC can be used to unravel the structure of proteins by analyzing the peptide fragments produced by digestion. The final step, localization of the disulfide bridges, can be accomplished by electrochemical detection. A model system for determining the structure for synthetic rat atrial natriuretic factor (ANF, F1) is described.

Existing Methods

Traditional purification and analysis of amino acid composition, followed by some method of locating the disulfide bonds (random acid hydrolysis in the presence of thiols, or diagonal paper electrophoresis). These are neither rapid nor sensitive.

Reference

Novel Approach to Rapid and Sensitive Localization of Protein Disulfide Bridges by High-Performance Liquid Chromatography and Electrochemical Detection, C. Lazure, J. Rochemont, N.G. Seidah and M. Chretien, J. Chromatogr., 326(1985) 339-348.

Conditions

Detector: BAS Dual 4B/17
Electrode: BAS dual series Au/Hg
Potential: upstream: -1.0 V, downstream: +0.2 V vs. Ag/AgCl
Column: Reverse-phase, C 18 (300 x 3.9 mm)
Mobile Phase: A: 0.1% aqueous trifluoroacetic acid (TFA). B: 0.1% TFA: acetonitrile (10:90). Gradient elution 5-40% acetonitrile in 40 min. Flow rate was 1 mL/min.

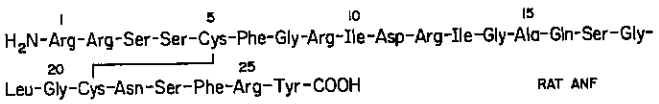

Figure 1. Rat ANF.

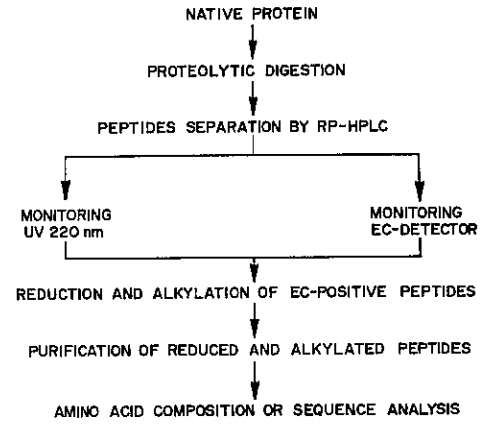

Figure 2. Scheme for protein digestion and determination of peptides with disulfide bridges.

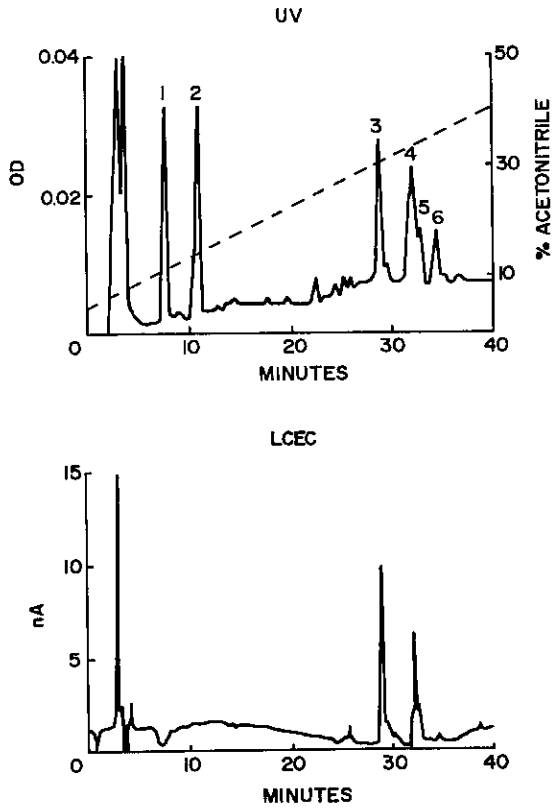

Figure 3. Chromatograms of digested ANF. The UV detector recorded 6 peaks, while the EC detector recorded only those with disulfide bridges (peaks 3, 4, and 5).

2701 Kent Ave • W. Lafayette, IN 47906 • (317) 463-4527 • TEL: 276141 • FAX (317) 497-1102

Sample Preparation
See F2.

Notes
Glutathione, vasopressin, and undigested ANF can be determined with the same chromatographic conditions.

The determination presented in this report can be duplicated utilizing the BAS 200 Problem Solver.

The information in this publication is supplied as a service to our customers. Performance of the methodology has not necessarily been verified by BAS technical staff.