CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Tissue Thiols and Disulfides

Purpose

Tissue Thiols and Disulfides in Non-Protein, Protein, and Protein-bound Fractions

A recent paper(1) by Dupuy and Szabo of the Harvard Medical School has provided new analytical methodology for the fractionation of biological thiols according to their disposition as free or protein-bound, thiol or disulfide. Their interest was in following the role mercaptans may play in diseases of the stomach tissue. The proposed study pointed to the need for complete fractionation of biological thiols in tissue, a step beyond the usual measurement of "free" thiol and disulfide.

Existing Methods

Spectrophotometric procedures, including fluorescence, do not allow for the simultaneous measurement of -SH vs. -SS- bonds, nor do they speciate these as to arising from glutathione, cysteine, protein, etc. All measurements are also made by difference, with the errors of each measurement compounding overall errors in accuracy and precision. The electrochemical assay of "bound" thiol, whether as part of the protein's primary structure or as disulfide cross-links, has been problematic in that the steric bulk of the biopolymer prevents electrode access to the -SH or -SS-bonds. Sensitivity is poor.

LCEC Methods

The biological sample is separated (F1) into its protein and non-protein fractions by perchloric acid precipitation. The supernatant (non-protein) is assayed directly by LCEC for cysteine, cystine, glutathione, and glutathione disulfide.

The protein pellet was further processed in two parallel paths. First, part of the pellet was subjected to acid hydrolysis at 105°C to break down the peptide backbone. A fraction of the hydrolysate was injected into the LC to determine cystine and cystine within the biopolymer. Second, for the rest of the

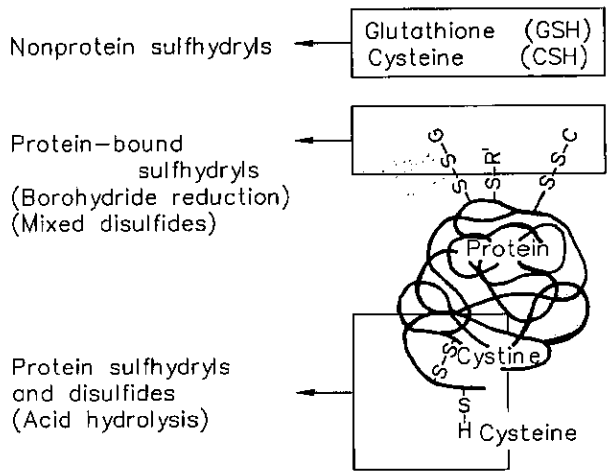

Figure 1. Origin of bound and free thiols, disulfides

pellet, the pH is raised to 9, and the protein is reacted with sodium borohydride, which reduces the -SS- bonds. Only those thiols not forming part of the protein's primary structure will be released. These, too, are injected into the same LC system.

Conditions

System: Dupuy and Szabo used a BAS LC-154 liquid chromatograph outfitted with the usual sample deoxygenation utilities. This instrument has been superceded by the BAS 400, a modular instrument introduced in 1987. The BAS 200 system is also capable of performing these assays. In the BAS 200, the deoxygenation utilities are built directly into the instrument.

Detection of thiols and disulfides is carried out by twin Hg/Au electrodes placed in series. Eluting thiols catalyze the oxidation of the downstream Hg surface poised at +0.15 V. Eluting disulfides are reduced at the upstream Hg surface at -1.0 V, and the resulting thiols are swept downstream and detected at +0.15 V as above. Spatial separation of these compounds

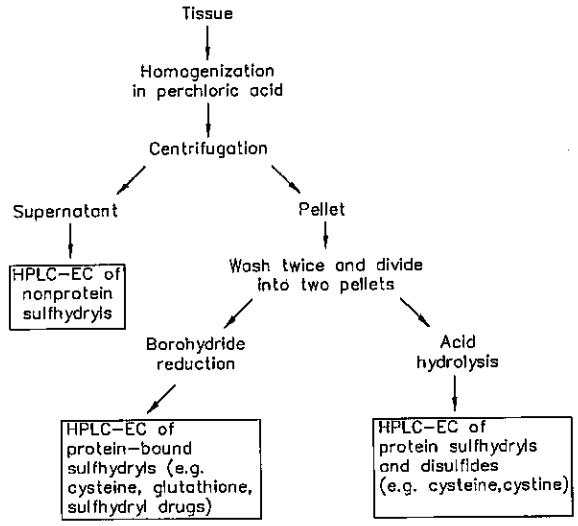

Figure 2. Schematic of sample fractionation procedure

in time via the chromatography allows individual speciation.

Mobile Phase: 96% 0.05 M monochloroacetic acid (pH 3), 4% methanol, 300 mg/L sodium octyl sulfate. This was continuously refluxed at 40°C for deoxygenation purposes.

Flow Rate: 1.5 mL/min

Column: Bioanalytical Systems Biophase® ODS 5µ (P/N MF6017, 250 x 4.6 mm)

Results

Much higher concentrations of thiol were found in the protein backbone and non-protein fraction than in the protein-bound (borohydride-susceptible) fraction. Exogenously administered thiol was detected both in the non- protein and protein-bound fractions.

Recovery of -SS- through the borohydride procedure exceeded 95%.

The isocratic procedure permitted rapid analysis of each sample in less than 10 minutes.

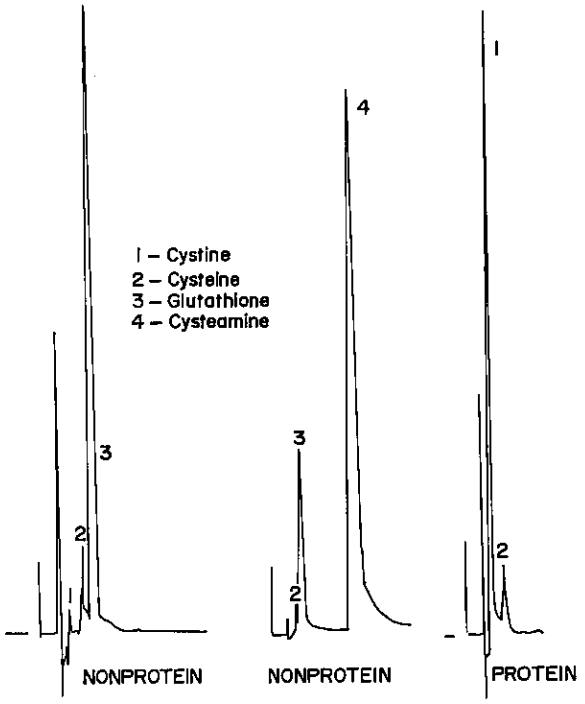

Figure 3. Chromatograms obtained from the fractionation procedure in rat gastric mucosa.

Left: non-protein fraction, which was injected into the system after the acid precipitation step.

Center: non-protein fraction, but in rat administered cysteamine.

Right: cystine and cysteine derived from protein hydrolysate of the same tissue.

Reference

1. D. Dupuy, S. Szabo, J. Liq. Chrom., 10(1987) 107-119.

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