CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Detection of Pharmaceutical Thiols Bound to Protein

Tracing the metabolic fate of small thiols in biological systems requires a procedure which can measure the extent towards which these small molecules form mixed disulfides with proteins. The size of these biopolymers precludes the direct measurement of disulfides by any optical techniques, including fluorescence.

LCEC Method

Smolin and Schneider(1) and Dupuy and Szabo(2) have described similar extraction procedures for the measurement of the mixed disulfides which small thiols can form with proteins. Since the disulfide is typically buried within the biopolymer, direct electrochemical reduction of the -S-S-bond is unlikely. However, reducing agents such as sodium borohydride are quite effective, and the cleaved thiols may be sampled by LCEC. The measurement of these thiols before and after $NaBH_4$ treatment affords a good estimate of bound vs. free thiol in the plasma or tissue sample.

The detection of total plasma cysteamine will be described. This drug (2-mercaptoethylamine) is used therapeutically as a radioprotective agent, for controlling acetaminophen toxicity in overdose cases, and in children afflicted with nephropathic cystinosis(1). Smolin and Schneider's study allowed speciation of cysteamine as the free thiol, as well as mixed or symmetrical disulfides.

Conditions

System: As per Smolin and Schneider,(1) a BAS 400 liquid chromatograph and a BASpc Scientific Workstation was employed. Provision was made for the exclusion of oxygen from the system by replacing all PTFE tubing with stainless steel. The mobile phase was purged and then blanketed with nitrogen.

A single Hg/Au film working electrode was held at +0.15 V vs. Ag/AgCl to monitor thiols.

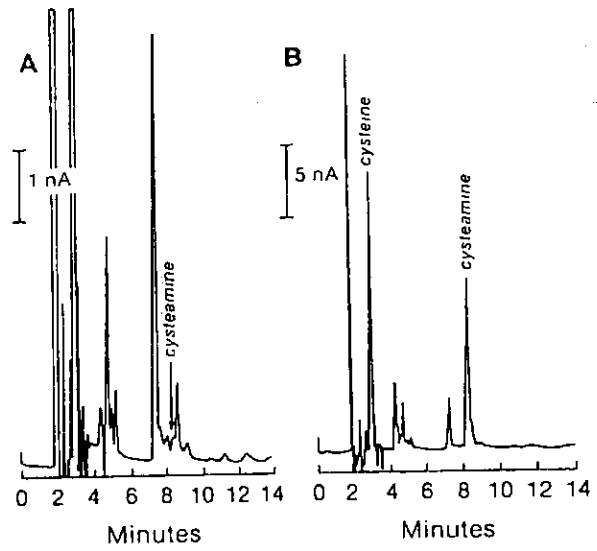

Figure 1. Left: Pre-dose plasma sample following sample preparation by $NaBH_4$ reduction, 10 nA f.s. Right: 1 hr. post-dose (0.23 mm cysteamine/kg), 50 nA f.s. (Reproduced from reference 1 with permission.)

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

Mobile Phase: 0.05 M monochloroacetic acid containing 3 mL/L ethylamine (70%, w/w aqueous) and 60 mg/L sodium octylsulfate. Adjust pH to 3.0 with monochloracetic acid, filter, and then deoxygenate in situ.

Flow Rate: 1 mL/min

Sample Preparation

Plasma samples (100 µL) were mixed with 500 µL of 7 M urea, pH 9, to which 1 drop of 1-octanol was added to prevent foaming. 50 µL of 100 mg/mL $NaBH_4$ (in 0.1 M NaOH) was added. The reduction reaction proceeded at 50°C for 30 min, after which 500 µL of cold 10% trichloracetic acid was slowly

added. After centrifugation, supernatants were filtered and then sparged with N_2 prior to injection.

Standards

Cysteamine HCl and cystamine dihydrochloride were prepared to 1 mM concentrations in 1 g/L Na_2EDTA solution, and stored at 4°C.

Effectiveness Of $NaBH_4$ Reduction

Apparently the presence of protein during the borohydride reduction is protective of cysteamine. Recoveries from plasma or from standards spiked with bovine serum albumen were 85-100% for both cysteamine and cystamine, whereas this dropped to 55% in the absence of protein.

Pharmacokinetic Study

Blood plasma was collected before and 30-360 minutes after administration of 0.23 mmol/kg cysteamine HCl. In F1, chromatograms of plasma before (left) and 60 min after (right) the dose were treated as above. Note the difference in full scale sensitivity.

The dose response curve is presented in F2, further substantiating the analytical effectiveness of the method.

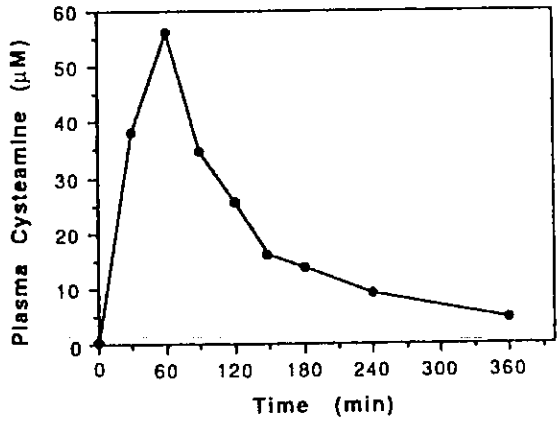

Figure 2. Plasma cysteamine vs. time for 0.23 mm/kg cysteamine dose. (Reproduced from reference 1 with permission.)

References

1. L.A. Smolin, J.A. Schneider, Anal. Biochem., 168(1988) 374-379.

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