Determination Of Thiols And Disulfides In Urine

Purpose
Determination of thiols and disulfides in urine.

High concentrations of cystine and cysteine in the urine are characteristic of the childhood disorder, cystinuria (1). An accurate determination of urinary cysteine and cystine is therefore crucial in diagnosing these cases. Individuals with high concentrations of cystine in their urine may develop kidney stones as well as other disorders. Homocystinuria, another genetic disorder, is characterized by the excretion of a mixed disulfide of homocysteine and cysteine (1,2).

Existing Methods
The conventional method for the determination of cysteine and cystine in urine is a colorimetric assay (3). This involves measuring the cysteine first, then reducing the disulfide and remeasuring the thiol concentration. Many non-thiol substances in the urine react to produce a high background.

LCEC Method
LCEC provides a much easier and more reliable method for the determination of not only cysteine and cystine but also other thiols and disulfides which may be present in the urine. The thiols and disulfides can be measured simultaneously without any chemical reduction step or sample clean-up.

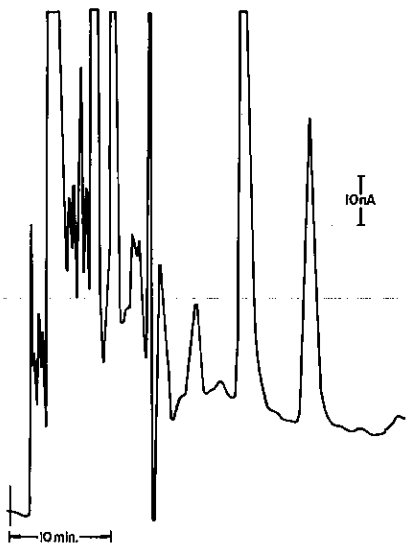

Figure 1. Chromatogram of a direct injection of urine using a glassy carbon electrode. Potential +1.0 V vs. Ag/AgCl.

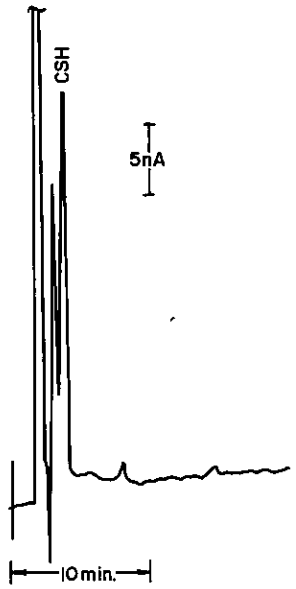

Figure 2. Chromatogram of a direct injection of urine (same as for F1) using a Hg/Au electrode. Potential +0.15 V vs. Ag/AgCl.

Conditions
System: Both the BAS 200 and BAS 400 liquid chromatographs are appropriate for this work. In the BAS 200, the correct systems are designated by MF200- X2X11X, which includes the necessary dual detector electronics and solvent deoxygenation hardware. In the BAS 400 series, two LC-4B Amperometric Controllers are used to control the potentials at the two mercury/gold (Hg/Au) thin film electrodes. Another cell containing one glassy carbon (GC) electrode and one Hg/Au electrode was used to illustrate the selectivity of the Hg/Au electrode. Preparation of the Hg/Au amalgam has been previously described (BAS, CAPSULE 192). Oxygen must be excluded from

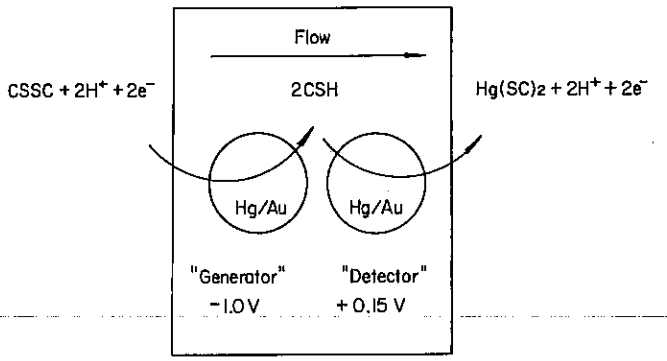

Figure 3. Schematic diagram of a series dual Hg/Au electrode and reactions which occur at each electrode. Potentials relative to a Ag/AgCl reference electrode.

the system by utilizing all stainless steel tubing in the system and sparging the mobile phase and sample with N_2 or helium. In the BAS 200 this is a standard hardware feature; in the BAS 400 series, a reflux manifold constructed from ground-glass labware is necessary. Consult the detector manual for construction plans.

Column: Biophase ODS, 5 µm, C_{18} (250 x 4.6 mm) (BAS, P/N MF- 6017). A guard (precolumn) column of similar packing material was included between the injector and analytical column; part numbers MF-6020, MF-6033.

Mobile Phase: 96% monochloroacetic acid (pH 3.0) and 4% methanol, containing 1 mM sodium octyl sulfate as ion- pairing agent. Flow rate was 1 mL/min.

Electrode: Dual Hg/Au (BAS, P/N MF-1002)

Potential: Upstream -1.0 V and downstream +0.15 V vs. Ag/AgCl.

Sample Preparation

Urine was collected from volunteers on two separate days. No sample preparation was required in the case of fresh urine. If the urine is to be stored, it should be acidified with HCl or some other acid in order to prevent further oxidation of endogenous thiols. The injection volume was 20 µL.

Results And Discussion

In LCEC, thiols can be detected on either a glassy carbon or Hg/Au electrode. Bare gold electrodes have also been utilized. In order to detect thiols using a glassy carbon or gold electrode, potentials of +0.8 to +1.0 V must be used. F1 shows a chromatogram of a urine sample using a glassy carbon electrode. At +1.0 V it is difficult to distinguish cysteine from the other early eluting peaks. The chromatogram also contains a number of late eluters.

On a Hg/Au amalgam electrode, mercury is oxidized in the presence of thiols by the following reaction:

$$2RSH + Hg \rightarrow Hg(SR)_2 + 2e^- + 2H^+$$

This reaction occurs at a much lower potential than the direct oxidation of thiols on glassy carbon. By setting the detector at +0.15 V this reaction can be used to selectively detect thiols, halides and chelating agents. Very few, if any, compounds will interfere. The power of this method of detection is illustrated by F2, where the separation of the urine

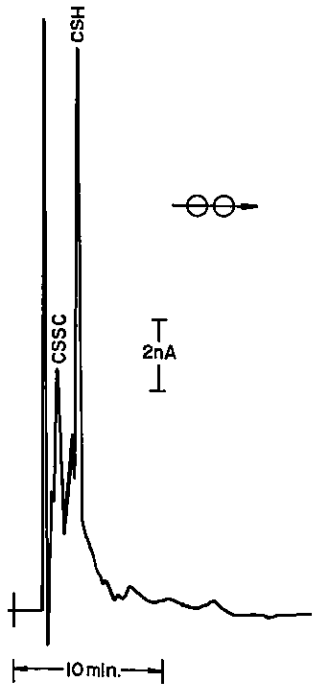

Figure 4. Chromatogram of a direct injection of urine using a series dual Hg/Au electrode. Potential of electrodes as described in F3.

sample as used in F1 is monitored on a Hg/Au electrode. The urine sample was injected directly into the system with no preconcentraton and no clean-up.

The addition of a second electrode, as shown in F3, allows one to detect not only thiols but also disulfides. Disulfides are reduced to their corresponding thiols at the upstream electrode. The resulting thiols can then be selectively detected downstream (oxidized). F4 and 5 show the use of this detection scheme for a urine sample. With the first electrode on, both cystine and cysteine are detectable. If only the downstream electrode is utilized, only cysteine and other endogenous thiols will be detected.

Comments

The use of dual Hg/Au electrodes for the determination of thiols in urine and other complex samples offers several advantages over other methods. Both the thiols and disulfides can be determined simultaneously. No chemical reduction, preconcentration, or clean-up steps are required. Urine can be injected directly into the LC (provided a guard column or filter is used). The results shown here for urine can be extrapolated to other biological samples such as serum and tissue.

Related References

1. L.H. Smith and H.E. Williams, in M.B. Strauss and L.G. Welt (ed.), "Diseases of the Kidney," Boston, Little Brown, (1963) 984.

2. G.W. Frimpter, J. Biol. Chem., 1961, PC51, 236.

3. J.F. Kackmar, in Fundamentals of Clinical Chemistry, N. Tietz (ed.), W.B. Saunders Co., Philadelphia, London, Toronto, 1979, pp. 248-250.

4. R. Eggli and R. Asper, Anal. Chim. Acta., 101(1978) 253.

5. L.A. Allison and R.E. Shoup, Anal. Chem., 55(1983) 12.

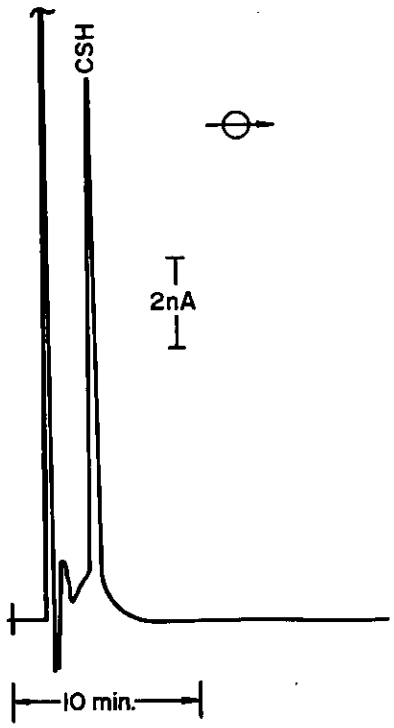

Figure 5. Same as in F4, but upstream (generator) electrode is off.

Related BAS Publications

Thiols

CAPSULE 192. Preparation and Use of Mercury Film Electrodes.

CAPSULE 194. Measuring Penicillamine in Plasma and Urine.

Disulfides and Thiols

CAPSULE 195. Chromatography of Biological Thiols and Disulfides

CAPSULE 235. Oxidized and Reduced Glutathione.

CAPSULE 234. Captopril and Captopril Disulfide in Plasma and Urine.