Isotachophoretic Analysis of Mercaptoundecahydrododecaborate Anion in Human Serum and Urine

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An isotachophoretic assay method was developed for sodium mercaptoundecahydrododecaborate (1), $\text{Na}_2^{10}\text{B}_{11}\text{H}_{11}\text{SH}$, which is used for boron neutron-capture therapy. The method is simple, specific and suitable for the determination of 1 as an anion in human serum and urine.

Compound 1 was separated from serum and urine constituents with 0.01 M hydrochloric acid and β-alanine (leading electrolyte, pH 3.70) and 0.01 M n-caproic acid (terminating electrolyte). The recoveries of 1 from serum and urine were about 85 and 101%, respectively. The detection limit was $7 \times 10^{-7}$ mol/ml in serum and urine. The procedure could also detect the oxidation product, sodium di(thioundecahydrododecaborate), at $5 \times 10^{-7}$ mol/ml or more in urine.

The present method was used to determine the levels of 1 in urine samples of patients with malignant brain tumour being treated by boron neutron-capture therapy.

Keywords — isotachophoresis; sodium mercaptoundecahydrododecaborate; boron neutron-capture therapy; patients’ urine; sodium di(thioundecahydrododecaborate)

Boron neutron-capture therapy of malignant brain tumors has recently been developing rapidly and has attracted the attention of many investigators. At present, sodium mercaptoundecahydrododecaborate (1), $\text{Na}_2^{10}\text{B}_{11}\text{H}_{11}\text{SH}$, is the best agent for boron neutron-capture therapy, which requires the selective accumulation of $^{10}\text{B}$ at high concentration in the malignant tissue. In a previous paper, we reported a colorimetric method with curcumine for determining boron levels in biological samples.

In this paper, we describe a simple and specific isotachophoretic method for the determination of 1 in human serum and urine.

Experimental

Apparatus and Conditions — Isotachophoretic analysis was performed with a Shimadzu IP-1B isotachophoretic analyzer equipped with a PG-1 potential detector. The separation was run in a Teflon capillary tube (length 20 or 40 cm, i.d. 0.5 mm) maintained at 20° C. The driving current was stabilized at 100 μA. Chart speed was 20 mm/min. The leading electrolyte was 0.01 M HCl adjusted to pH 3.70 by adding β-alanine; 1.5% Triton X-100 was also added to prevent diffusion of the electrophoretic zone. The terminating electrolyte was 0.01 M n-caproic acid.

Materials — Compound 1 is highly so hygroscopic and also liable to be transformed to its disulfide in air. Therefore, nonhygroscopic cesium mercaptoundecahydrododecaborate monohydrate (2), $\text{Cs}_2\text{B}_{12}\text{H}_{12}\text{SH} \cdot \text{H}_2\text{O}$, and cesium di(thioundecahydrododecaborate) (3), $\text{Cs}_3\text{B}_{12}\text{H}_{12}\text{S}_2$, were used instead of 1 and its disulfide as standard samples, respectively. Other chemicals were of reagent grade.

As human serum, Hyland Control Serum (Travenol Laboratories) and Moni-trol (American Hospital Supply Co.) were used. The urine samples from patients with malignant brain tumor were provided by Teikyo University Hospital.

Standard Solutions — A series of standard solutions for obtaining calibration curves were prepared by dissolving about 15 mg of 2 or 3 in 10 ml of distilled water, followed by dilution to obtain the desired concentrations. These standard solutions of 2 and 3 were freshly prepared each time.

Procedure — A 0.1-ml serum sample in a 2-ml centrifuge tube was mixed well with 0.2 ml of ethanol. After centrifugation of the mixture for 5 min, 5 μl of the supernatant was introduced into the apparatus.
A urine sample was diluted ten-fold with distilled water and 10 μl of the sample solution was directly introduced into the apparatus.

**Results and Discussion**

Compound 2 is easily converted in alkaline solution to its disulfide 3. Therefore, the analytical conditions for the determination of 2 were examined with respect to potential unit \((PU)\) value\(^3\) using 0.01 M hydrochloric acid and \(\beta\)-alanine (pH 3.0—4.2), 0.01 M hydrochloric acid and histidine (pH 5.6) and 0.01 M hydrochloric acid and ammediol (pH 6.8) as leading electrolytes and 0.01 M \(n\)-caproic acid and 0.01 M glutamic acid as terminating electrolytes. A combination of 0.01 M hydrochloric acid and \(\beta\)-alanine (pH 3.70) and 0.01 M \(n\)-caproic acid was the most appropriate for detecting 2 and 3 without interference from serum and urine constituents based on comparison of the \(PU\) value. Under the analytical conditions, compound 2 was distinctly separated from 3 and the \(PU\) value was determined to be 0.09 for 2 and 0.05 for 3. Addition of Triton X-100 improved the sharpness of the zone boundary between 2 and the serum or urine constituents.

The standard curves for 2 and 3 between the zone length and the injection amount were linear in the ranges of 2.5—48 × 10\(^{-9}\) and 2.5—20 × 10\(^{-9}\) mol, respectively. As no oxidized anions from 2 were formed in standard solutions, we concluded that oxidation of 2 did not occur during analysis.

Ethanol was selected as a deproteinizing agent for serum samples because it was easy to handle and did not interfere with the detection of 2 under the analytical conditions described above (Fig. 1). The recovery of 2 which had been added to human serum was about 85\% in the range of 1.0 to 10.1 × 10\(^{-6}\) mol/ml upon addition of two volumes of ethanol. About 15\% of the added 2 may have bound to the serum protein. It was possible to detect 7 × 10\(^{-7}\) mol/ml of 2 in the serum.

On the other hand, compound 3 was scarcely recovered from the serum deproteinized with ethanol. This result probably reflects the occurrence of coprecipitation with the serum protein.

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**Fig. 1. Isotachophoretic Separation of 2 in Human Serum**

A serum containing 4.03 × 10\(^{-6}\) mol/ml of 2 was treated as described in the text.

- a, Cl\(^{-}\); b, \(\text{B}_{12}\text{H}_{15}\text{SH}^{2+}\) (2, \(PU\) value 0.09); c, \(n\)-caproic acid.

**Fig. 2. Isotachophoretic Separation of 2 and 3 in Human Urine**

Urine containing 1.83 × 10\(^{-5}\) mol/ml of 2 and 6.16 × 10\(^{-6}\) mol/ml of 3 was treated as described in the text.

- a, Cl\(^{-}\); b, \(\text{B}_{12}\text{H}_{15}\text{SH}^{2+}\) (3, \(PU\) value 0.05); c, \(\text{B}_{12}\text{H}_{15}\text{SH}^{2+}\) (2, \(PU\) value 0.09); d, \(n\)-caproic acid.
Figure 2 shows a typical isotachopherogram for the separation of 2 and 3 in human urine. Compounds 2 and 3 were separated completely from each other and were well resolved from urine constituents under the analytical conditions.

Regression analysis for the determination of 2 in human urine was examined in the concentration range of $1.5 \times 10^{-6}$ to $3.1 \times 10^{-5}$ mol/ml using mixed urine samples in which 3 was present in 0.3- to 10-fold molar excess over 2. The relationship between the added (x) and found (y) values of 2 was linear; $y = 1.013x - 0.17$, standard deviation ($s$) = 0.28, coefficient of variation (c.v.) = 1.79%, $n = 16$. The regression equation indicated that the present method is accurate to within about 2% coefficient of variation.

In addition, regression analysis for the determination of 3 detected simultaneously was examined in the concentration range of $1.6 \times 10^{-5}$ to $7.7 \times 10^{-7}$ mol/ml. The relationship between the added (x) and found (y) values of 3 was also linear; $y = 1.009x - 0.04$, $s = 0.25$, c.v. = 3.16%, $n = 16$. The regression equation indicates that the present method could determine 3 with about 3.2% coefficient of variation.

These results confirmed that the present method could be used for simultaneous determination of 2 and 3 in urine. The detection limit was $7 \times 10^{-7}$ mol/ml for 2 and $5 \times 10^{-7}$ mol/ml for 3.

The present method was applied to the determination of 1 in urine samples from patients. Compound 1 was administered to patients with malignant brain tumor at 50 mg of boron per kg of body weight by intra-arterial infusion. Urine samples from five patients were analyzed by the present method and the colorimetric method using curcumine.\textsuperscript{2} Compound 1 was clearly detected in all urine samples collected within 17 h after administration while the disulfide was scarcely detectable. The urinary concentrations of 1 ranged from $1.41 \times 10^{-6}$ to $1.43 \times 10^{-5}$ mol/ml (183–1855 μg B/ml). The values found by the present method were compared with those from the colorimetric method, and as shown in Fig. 3, a close correlation was found (correlation coefficient, $r = 0.9942$).

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References