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Analysis of Selenium Metabolites in Human Urine Using Ion Exchange Chromatography

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Urine is the major excretory route for selenium (Levander et al. 1981, Swanson et al. 1983). Trimethylselenonium ion (TMSe) has been identified as a metabolic product in urine from rats that have been dosed with selenite (Byard 1969, Palmer et al. 1969) or selenoamino acid, such as selenomethionine (Palmer et al. 1970).

Several methods have been developed for fractionating TMSe and other components in urine. Separation methods were used in the present study based on a combination of ion-exchange column and paper chromatography (Palmer et al. 1969) and precipitation with the Reinecke's reagent (Byard 1969) or cation HPLC using gradient elution (Kraus et al. 1985).

The selenium and TMSe contents in urine were determined by using radioactive ^{75}Se tracing (Byard 1969, Palmer et al. 1969, Burk 1976, Nahapetian et al. 1983, Kraus et al. 1985), neutron activation analysis (Nahapetien et al. 1984, Blotcky et al. 1985) or inductively coupled plasma mass spectrometry (Sun et al. 1987). Because of its long half-life period (118.5 days) and its hazard, the use of ^{75}Se has been limited. Fluorometry is widely used on account of its accuracy. We have improved this method for the determination of selenium at the nanogram level (Hasunuma et al. 1990), because the selenium concentrations in human tissues and urine are less than 1 $\mu g/g$ (Schroeder et al. 1970) and 0.1 $\mu g/L$ (Robberecht and Deelstra 1984), respectively.

The purpose of the present work is to develop an analytical methodology for separation and determination of TMSe and other organic selenium compounds from human urine by cation exchange chromatography and fluorometry. Using this method, we have found that selenium compounds in human urine are separated into at least five components.

MATERIALS AND METHODS

Selenium standard solution (1000 ppm, as selenious acid, for

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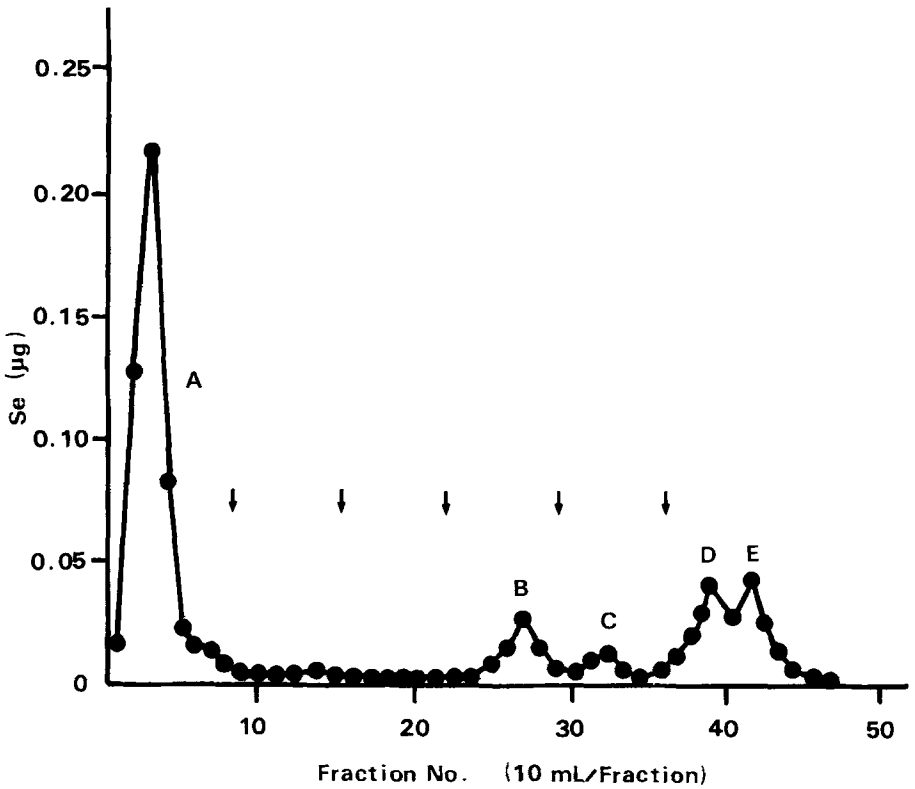

Figure 1. Separation of urinary selenium components by Dowex 50W-X4 column chromatography. Arrows indicate changes of eluents. The eluents in the order used are 0.05, 0.1, 0.5, 1 and 4 M hydrochloric acid.

atomic absorption spectrometry) was purchased from Wako (Osaka, Japan). Trimethylselenonium iodide (>99.999%) was a product of Tri Chemical Lab. (Kanagawa, Japan).

Urine samples were collected from 10 healthy adults, 5 males from 22 to 58 years of age and 5 females 21 years old.

The urine sample (10 mL), adjusted to pH 2.2 to 2.4 with 6 M hydrochloric acid, was applied to a Dowex 50W-X4 (200 to 400 mesh, H^+ form) column (12 x 155 mm). The column was washed stepwise with 70 mL each of water and 0.05, 0.1, 0.5 and 1 M hydrochloric acid and finally with 100 mL of 4 M hydrochloric acid. Ten-mL portions of the eluate were collected. Ten-mL of the first fractions (peak A) adjusted to pH 7 with 6 M sodium hydroxide were applied to an AG1-X8 (200 to 400 mesh, Cl^- form) column (12 x 155 mm). The column was washed stepwise with 70 mL each of water and 0.1, 1 and 4 M hydrochloric acid. Ten-mL portions of the eluate were collected.

Each fraction was neutralized, desalted, evaporated and applied to

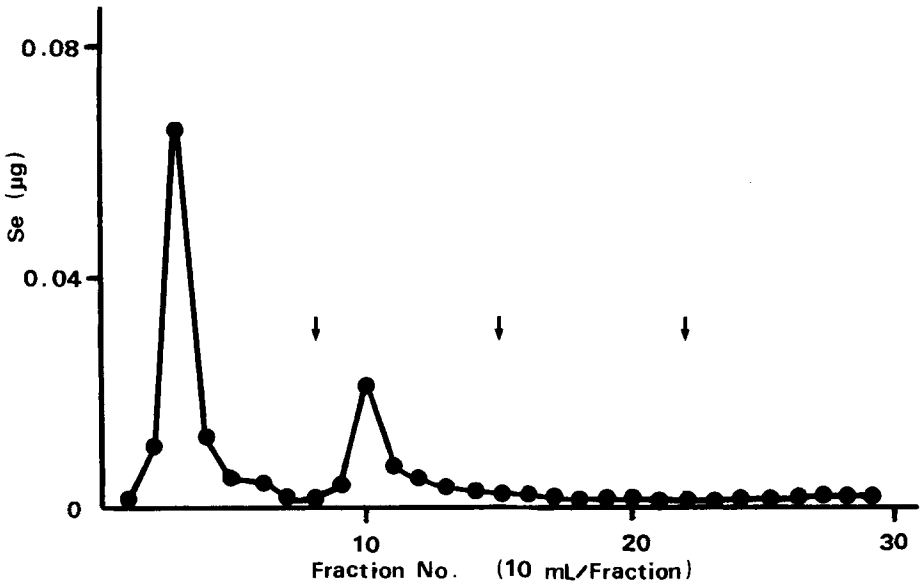

Figure 2. Separation of peak A in Figure 1 by AG1-X8 column chromatography. Arrows indicate changes of eluents. The eluents are 0.1, 1 and 4 M hydrochloric acid in the elution order.

a silica gel sintered plate (Hasunuma et al. 1991). Thin-layer chromatography was performed in (1) n-butanol/ acetic acid/ water (4:1:1, v/v) or (2) phenol/ water (5:1, v/v, ammonia vapor saturated).

Selenium was determined fluorometrically using 2,3-diaminonaphthalene (DAN) according to the method described previously (Hasunuma et al. 1982, 1990).

RESULTS AND DISCUSSION

Cation exchange column chromatography was performed for separation of urinary selenium components. As shown in Figure 1, there are five major peaks. The peaks were named A, B, C, D and E in their order of elution. Peak A was further separated by anion exchange column chromatography into selenite (fraction no. 10, a few percent of the total urinary selenium) and an unknown selenium component (Figure 2). Peaks B and C were eluted with 0.5 M and 1 M hydrochloric acid, respectively. Peaks D and E were eluted with 4 M hydrochloric acid. Peak C was identified as TMSe by Dowex 50W-X4 column chromatography and TLC with an authentic sample.

The selenium distribution among the fractions is given in Table 1. All the urine samples showed similarly five elution peaks irrespectively of sex and age, though the area of each peak varied a little with subjects. The total selenium content in urine strongly correlated with each area of peaks A, B, D and E, except for peak C (TMSe). We found that the relative concentration of TMSe varied from 3 to 20% of the total selenium content in the

urine samples. So far the reported values of the ratio of TMSe to the total urinary selenium vary widely, ranging from 6 to 30% in Japanese (Oyamada and Ishizaki 1982) and 0.3 to 80% in North Americans (Nahapetien et al. 1984, Sun et al. 1987, Blotcky et al. 1988). This discrepancy might be due to the difference of the environmental selenium levels and the dietary habit of the regional inhabitants.

Table 1. Distribution of selenium components in single-void urine separated by Dowex 50W-X4 column chromatography.

Subject	Selenium amount from each peak* (%)**					Total Se*
Sex/Age	A	B	C	D	E	
Male						
22	88 (36.5)	24 (10.1)	21 (8.7)	28 (11.7)	29 (11.9)	241
36	145 (50.5)	26 (8.9)	34 (12.0)	37 (12.9)	13 (4.5)	287
37	204 (29.4)	92 (13.3)	48 (6.9)	136 (19.6)	144 (20.8)	694
49	120 (35.9)	29 (8.7)	59 (17.6)	43 (12.9)	29 (8.6)	333
58	112 (27.2)	55 (13.4)	73 (17.6)	35 (8.6)	47 (11.4)	412
Mean	134 (35.9)	45 (10.9)	47 (12.6)	56 (13.1)	52 (11.4)	393
S.D.	40 (8.1)	26 (2.1)	18 (4.4)	40 (3.6)	47 (5.4)	161
Female						
21	246 (20.4)	111 (9.2)	58 (4.8)	164 (13.6)	175 (14.5)	1205
21	138 (26.7)	45 (8.7)	88 (17.1)	79 (15.3)	56 (10.8)	517
21	285 (47.5)	42 (7.0)	37 (6.1)	82 (13.7)	61 (10.2)	600
21	131 (33.1)	37 (9.4)	23 (5.7)	48 (12.2)	72 (18.3)	396
21	236 (62.8)	29 (7.7)	11 (2.9)	39 (10.4)	27 (7.2)	376
Mean	207 (38.1)	53 (8.4)	43 (7.3)	82 (13.0)	78 (12.2)	619
S.D.	62 (15.3)	30 (0.9)	27 (5.0)	44 (1.7)	51 (3.8)	304
Total						
Mean	170 (37.0)	49 (9.6)	45 (9.9)	69 (13.1)	65 (11.8)	506
S.D.	62 (15.3)	30 (0.9)	27 (5.0)	44 (1.7)	51 (3.8)	304
$r^{\$}$	0.628	0.919#	0.320	0.943#	0.917#	

*: nanograms.
**: The ratio to the total urinary selenium (%).
$: Correlation coefficient of each peak to total selenium.
#: $p<0.01$.

In conclusion, by Dowex 50W-X4 column chromatography, five major selenium-containing fractions were found in human urine. Peak A, the first fraction, was separated further into two subfractions, an unknown peak and a minor peak of selenite. The chemical identities of the separated compounds are now under investigation.

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REFERENCES

Blotcky AJ, Hansen GT, Operanio-Buencamino LR, Rack EP (1985) Determination of trimethylselenonium ion in urine by ion-exchange chromatography and molecular neutron activating analysis. Anal Chem 57:1937-1941

Blotcky AJ, Ebrahim A, Rack EP (1988) Determination selenium metabolites in biological fluids using instrumental and molecular neutron activation analysis. Anal Chem 60:2734-2737

Burk RF (1976) Selenium in man. In: Prasad AS, Oberleas D (eds) Trace Elements in Human Health and Disease, vol 2. Essential and Toxic Elements, Academic Press, New York, p 105

Byard JL, (1969) Trimethyl selenide. A Urinary metabolite of selenite. Arch Biochem Biophys 130:556-560

Hasunuma R, Ogawa T, Kawanishi Y (1982) Fluorometric determination of selenium in nanogram amounts in biological materials using 2,3-diaminonaphthalene. Anal Biochem 126:242-245

Hasunuma R, Tsuda M, Ogawa T, Kawanishi Y (1990) Urinary selenium levels in Japanese males and females. Bull Environ Contam Toxicol 44:501-507

Hasunuma R, Ogawa T, Ishii J, Kawanishi Y (1991) Determination of selenium in organic compounds on a silica gel sintered thin-layer chromatographic plate with 2,3-diaminonaphthalene after direct digestion. J Chromatogr 537:397-405

Kraus RJ, Foster SJ, Ganther HE (1985) Analysis of trimethylselenonium ion in urine by high performance liquid chromatography. Anal Biochem 147:432-436

Levander OA, Sutherland B, Morris VC, King JC (1981) Selenium balance in young men during selenium depletion and repletion. Am J Clin Nutr 34:2662-2669

Nahapetian AT, Janghorbani M, Young VR (1983) Urinary trimethylselenonium excretion by the rat: effect of level and source of selenium-75. J Nutr 113:401-411

Nahapetian AT, Young VR, Janghorbani M (1984) Measurement of trimethylselenonium ion in human urine. Anal Biochem 140:56-62

Oyamada N, Ishizaki M (1982) Determination of trimethylselenonium ion and total selenium in human urine by graphite furnace atomic absorption spectrometry. Bunseki Kagaku 31:17-22

Palmer IS, Fischer DD, Halverson AW, Olson OE (1969) Identification of a major selenium excretory product in rat urine. Biochim Biophys Acta 177:336-342

Palmer IS, Gunsalus RP, Halverson AW, Olson OE (1970) Trimethylselenonium ion as a general excretory product from selenium metabolism in the rat. Biochim Biophys Acta 208:260-266

Robberecht HJ, Deelstra HA (1984) Selenium in human urine: concentration levels and medical implications. Clin Chem Acta 136:107-120

Schroeder HA, Frost DV, Balassa JJ (1970) Essential trace metals in man:selenium. J Chron Dis 23:227-243

Sun XF, Ting BTG, Janghorbani M (1987) Excretion of trimethylselenonium ion in human urine. Anal Biochem 167:304-311

Swanson CA, Reamer DC, Veillon C, King JC, Levander OA (1983) Quantitative and qualitative aspects of selenium utilization in pregnant and nonpregnant women: an application of stable isotope methodology. Am J Clin Nutr 38:169-180

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