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Short communication

Qualitative detection of selenium in fortified soil and water samples by a paper chromatographic–carboxyl esterase enzyme inhibition technique

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Abstract

Thevetia peruviana seed carboxyl esterase was employed as a biosensor for the detection of selenium compounds by an enzyme inhibition technique on paper chromatograms. The selenium compounds (sodium selenite and selenium dioxide) appeared as white spots on a magenta background due to the inhibition of *Thevetia peruviana* seed carboxyl esterase (substrate 1-naphthyl acetate, coupling reagent Fast blue B salt). The minimum detectable amounts were about 5 μg of sodium selenite and 5 μg of selenium dioxide. Many other animal and plant carboxyl esterases gave no inhibition spot under the same conditions. Soil and water samples were fortified with sodium selenite and selenium dioxide. A procedure for preparing test solutions and conditions for paper chromatography was established.

Keywords: Soil; Water analysis; Biosensors; *Thevetia peruviana*; Selenium; Enzymes; Metals

1. Introduction

Selenium is an essential trace element which plays a vital role in many metabolic functions. Its concentration in foods, however, may not exceed certain limits. Dietary allowed (or recommended) intake of selenium for humans is 41–92 fg/day [1]. Elemental selenium is present in some soils due to bacteria and fungi which are capable of reducing selenites and selenates to the elemental form [2]. Water from seleniferous areas may contain high concentrations of selenium but this water is generally unpalatable to livestock [3,4]. The presence of selenate in soil was reported as being responsible for highly poisonous vegetation indigenous to the seleniferous areas [5]. The active factors of seleniferous soil formation are precipitation and temperature, interrelated with these are evaporation, humidity, wind, microorganisms in soil, plants and animals [6]. Selenium in water is due to leaching of some selenium from seleniferous plants [7,8]. Selenium toxicity also affects the sediment based water quality [9]. The vertical distribution of selenium in lake waters was related to the growth of algae [10]. Reports also showed the presence of selenium in potable, irrigation and waste waters [11].

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Selenium in biological samples can be determined by using a gas chromatographic assay with electron-capture detection; the minimum detectable limits by this method are <2 ng/200 μl [12]. Selenium in organic compounds like selenomethionine and selenocystine can be determined on a silica gel sintered thin-layer chromatographic plate by using 2,3-diaminonaphthalene reagent and detected by a TLC scanner; the detection limit is 0.4 ng of selenium [13]. Selenium can be determined by ion chromatography with a detection limit of 48 μg/l [14]. Selenium in biological matrices was determined by high-performance TLC with in situ fluorometric detection [15]. Biological tissues were analysed for selenium by TLC and densitometry [16].

Selenium concentrations were detected in liver of hares and milk of cows by gas chromatography–electron-capture detection [17]. Selenium present in steroids is separated and estimated by spectrophotometry at 330 nm, the range of selenium determined was 10–150 μg [18]. Arsenic and selenium in soil vegetation, water, sediment and industrial wastes are determined by flow-injection analysis in conjunction with automated hydride generation and atomic absorption spectrometry [19]. The selenium content of thyroid and liver was estimated by hydride generation atomic absorption spectrophotometry [20].

In view of the importance of selenium, an attempt was made to develop a simple and sensitive biosensor method for the detection of selenium compounds in fortified soil and water samples. The seleniferous locations are very meagre in India, so fortified samples of soil and water were used. The chromatographic–enzymatic inhibition methods using paper and thin-layer chromatography were developed earlier for the detection of heavy metal compounds like mercuric chloride, copper sulphate, cadmium sulphate and silver nitrate with mammalian liver succinate dehydrogenase as a biodetector [21]. While a few heavy metals could be detected by a liver dehydrogenase, the trace metal selenium could be detected by *Thevetia peruviana* seed carboxyl esterase in fortified soil and water samples. *Thevetia peruviana* is a poisonous plant commonly known as yellow or exile oleander and its seeds were used as a carboxyl esterase source for the determination of selenium in the present investigation.

2. Experimental

2.1. Reagents and materials

The seeds of *Thevetia peruviana* were isolated by breaking the hard kernels and were crushed in a homogenizer into a semisolid paste and kept for freeze-drying in freeze drier (Heto, FD-3, Japan) at −40°C. The freeze-dried powder was removed and preserved in an airtight container and kept frozen at −20°C. The powder is stable for 6 months. A 10% (w/v) enzyme extract was prepared from the freeze-dried powder in distilled water and used as the enzyme source.

The chromogenic salt fast blue B and the substrate 1-naphthyl acetate were provided by Sigma (St. Louis, MO, USA). Ethanol, chloroform and acetone were provided by Fisher Inorganics & Aromatics (Madras, India). Glacial acetic acid, sodium selenite, selenium dioxide, sodium chloride and methyl cyanide were provided by Loba Chemie (Bombay, India). Sodium dihydrogenphosphate was provided by E. Merck (Bombay, India). Whatman No. 3 filter paper strips for paper chromatography were used; this yielded a better resolution than cellulose TLC plates.

2.2. Fortified soil samples

One hundred grams of red soil collected from agricultural land was fortified with 20 mg of soluble selenium compound (sodium selenite or selenium dioxide) and was kept in laboratory for 15 days (200 mg/kg). The soil was thoroughly mixed, and 10 g of the soil sample was shaken with 100 ml of distilled water and kept at room temperature (29–33°C) for an hour to settle. The contents were centrifuged and the supernatant of the soil mixture was filtered through Whatman No. 1 filter paper.

2.3. Fortified water samples

First, 20 mg/l of sodium selenite or selenium dioxide was dissolved in potable water (river water); then, 100 ml of this water sample was employed in the present investigation.

2.4. Clean-up procedure

The filtrates of the fortified soil extract and water samples were kept in a hot air oven at 80°C and evaporated to get a final volume of 5 ml. To this 1 ml of 0.4 *M* sodium dihydrogenphosphate buffer of pH 8.4 was added to precipitate heavy metals if these are present. It does not affect the enzyme activities [21]. The solutions were further subjected to clean-up procedure by employing *n*-hexane for elimination of organophosphorus and organochlorine insecticides [23], if present, by a liquid–liquid partition technique [22]. The final solutions were again evaporated to a volume of 1–2 ml to enhance the concentration of selenium and were used for a paper chromatographic–enzymatic method of detection (Figs. 1–3).

2.5. Solvent systems for selenium compounds (sodium selenite and selenium dioxide)

For chromatographic movement of soluble selenium compounds on Whatman No.3 filter paper a number of solvent systems in different combinations were tried. Thirty-six solvent systems for sodium selenite and 41 solvent systems for selenium dioxide in different combinations and ratios of water and organic solvents were examined. The recommended solvent systems were methyl cyanide–1% NaCl (1:1, v/v) for sodium selenite and methyl cyanide–1.5% NaCl (1:1, v/v) for selenium dioxide. They allowed appropriate solubility, movement of compounds and compactness of spots. Their R_f values were 0.6 ($\pm$0.07) for sodium selenite and 0.65 ($\pm$0.03) for selenium dioxide (Fig. 1).

2.6. Chromatographic–enzymatic detection

Selenium-containing extracts obtained after the clean-up procedure were spotted with a fine graduated micro capillary on Whatman No.3 filter paper strips (7.5×2.5 cm). The solution containing 20 μg of selenium was spotted about 1.5 cm above the bottom line. The extract was spotted through a capillary and was dried either by blowing air or by using a hot air blower (hair drier). The Whatman No. 3 paper strips were placed in a small coupling jar

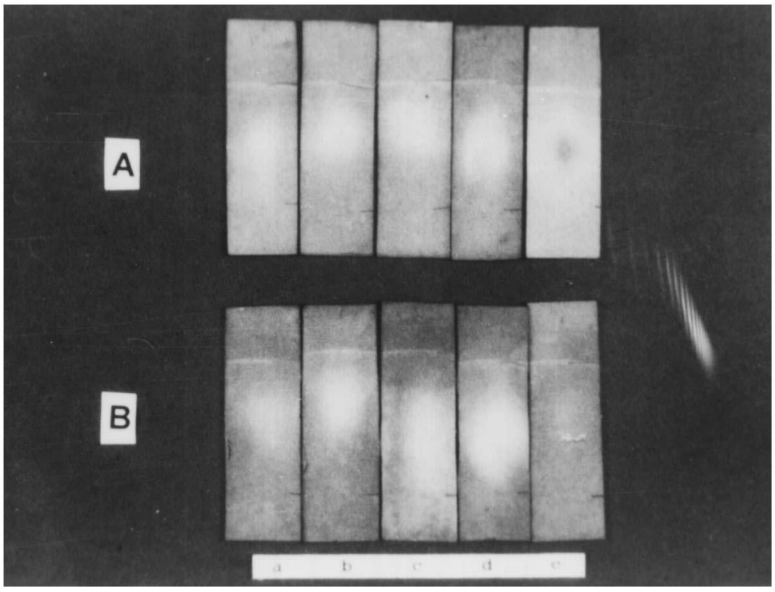

Fig. 1. Detection of selenium by chromatographic *Thevetia peruviana* seed carboxyl esterase inhibition method. (A) Sodium selenite fortified water sample; (B) selenium dioxide fortified water sample; (a–e) different concentrations of selenium compounds from 5 to 25 μg/μl.

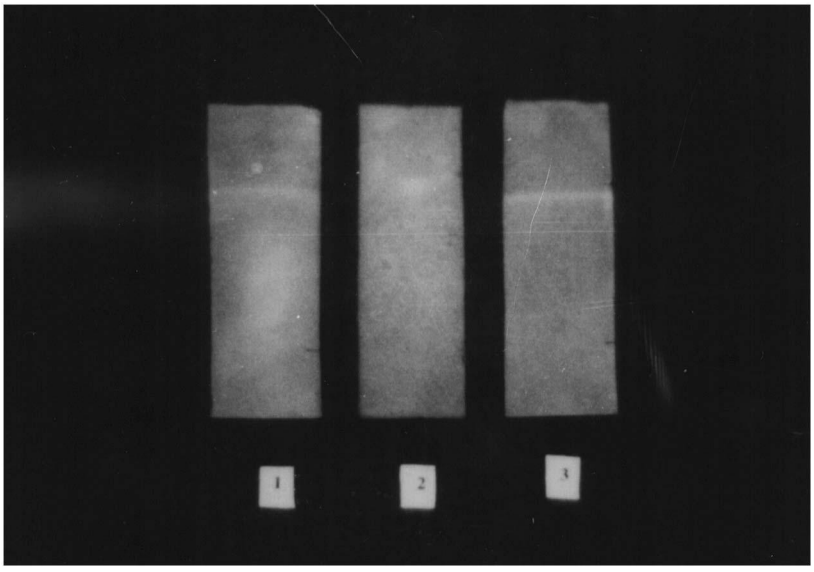

Fig. 2. Detection of sodium selenite by chromatographic *Thevetia peruviana* seed carboxyl esterase inhibition from fortified soil sample. (1) Detection of sodium selenite from fortified soil sample, 200 mg/kg; (2) detection of organophosphorus insecticide (methyl parathion) in the fortified soil, 10 mg/kg; (3) water extract of soil devoid of sodium selenite and organophosphorus pesticide (methyl parathion).

containing methyl cyanide–1% NaCl (1:1, v/v) for sodium selenite and methyl cyanide–1.5% NaCl (1:1, v/v) for selenium dioxide. After the solvent run, the paper strips were removed and dried over a clear glass plate at room temperature; alternatively a hot air blower could be used. The paper strips were taken and sprayed with *Thevetia peruviana* seed carboxyl esterase just to wet the paper strips. Excess spraying of enzyme caused leaching and possible distortion of chromatogram. The paper strips were then placed over a slide-warmer at 35°C. The paper strips were removed after 2 min and sprayed with chromogenic substrate reagent mixture containing 0.4% fast blue B salt in distilled water and 0.2 *M* solution of 1-napthyl acetate in acetone [26]. They were then observed for the development of white inhibition spots due to inhibition of carboxyl esterase enzyme of *Thevetia peruviana* seed against a magenta-coloured background. The magenta colour represents enzyme activity. The chromatograms get partially masked and turn into a whitish brown colour at higher concentrations of selenium compounds (Fig. 1).

3. Results and discussion

Table 1 shows several enzyme sources of animals and plants which were tried for the detection of selenium compounds. *Thevetia peruviana* seed carboxyl esterase was found sensitive and was inhibited by soluble selenium compounds (sodium selenite and selenium dioxide). A procedure was also developed to extract selenium from the fortified soil and water samples which eliminates the presence of interfering substances. The minimum detection limit for both sodium selenite and selenium dioxide by a solvent run on Whatman No. 3 paper is 5 μg/1 μl and can be detected after concentrating 50 mg/l of fortified soil sample or 50 mg/kg soil to 5 ml either from water or soil sample extract (see Sections 2.2–2.4). Similarly the minimum detection limit is 2 μg/μl for fortified water or for fortified soil sample extract by spot test on Whatman No. 3 paper without solvent run.

The principle of the detection of selenium by enzyme inhibition on paper chromatography is as follows. The substrate 1-naphthyl acetate is metabo-

Table 1
Biological sources, enzyme systems and inhibition response for soluble selenium

Serial no.	Biological source	Enzyme system	Response on chromatographic paper
1	Beef	Liver carboxylesterase	Negative
2	Pig	Liver carboxylesterase	Negative
3	Horse	Liver carboxylesterase	Negative
4	Rat	Liver carboxylesterase	Negative
5	Sheep	Liver carboxylesterase	Negative
6	Calotes	Liver carboxylesterase	Negative
7	Fish	Liver carboxylesterase	Negative
8	Field rat	Liver carboxylesterase	Negative
9	Frog	Liver carboxylesterase	Negative
10	*Pila globasa*	Liver carboxylesterase	Negative
11	*Thevetia peruviana*	Seed carboxylesterase	Positive [**recommended**]
12	*Zyzyphus zuzuba*	Seed carboxylesterase	Negative
13	*Mumardica caranshia*	Raw fruit carboxyl esterase	Negative
14	*Arachis hypogaea*	Seed carboxylesterase	Negative
15	*Daucus carota*	Carrot carboxylesterase	Negative
16	*Zea mays*	Tender seeds carboxyl esterase	Negative
17	*Cucumis sativus*	Raw fruit carboxyl esterase	Negative

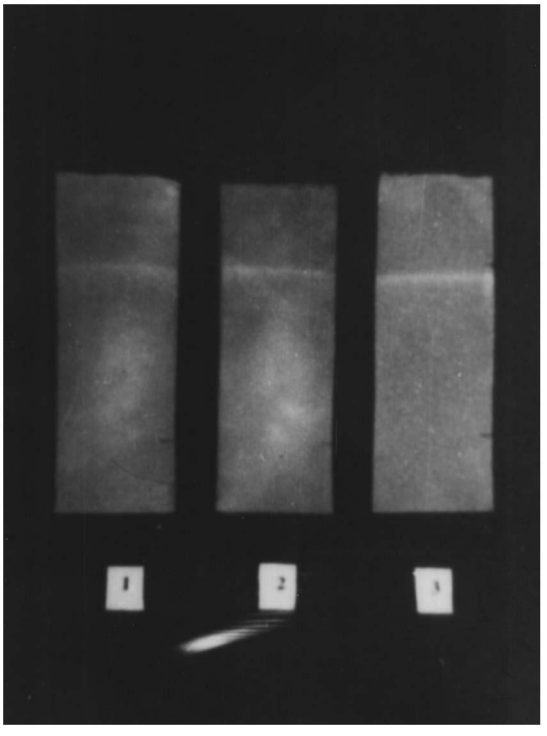

Fig. 3. Detection of selenium dioxide by chromatographic *Thevetia peruviana* seed carboxyl esterase inhibition from fortified soil sample. (1) Technical grade selenium dioxide, 20 μg; (2) detection of selenium dioxide from fortified soil, about 200 mg/kg; (3) water extract of soil devoid of selenium dioxide and organophosphorus pesticide (methyl parathion).

lised by carboxyl esterase to 1-naphthol which couples with the diazonium salt fast blue B. A magenta colour indicates the enzymatic reaction [24–26]. The method is simple, sensitive, not laborious, and might find application in the qualitative detection of trace amounts of selenium.

References

[1] G. Anna, K. Mariann, G. Erika, G. Odon, B. Gyorgy, Egeszsegtudomany 41 (1997) 166.
[2] J.G. Lipman, S.A. Waksman, Science 1 (1923) 57.
[3] O.A. Beath, J.H. Draize, H.F. Eppson, C.S. Gibert, O.C. McCreary, J. Am. Pharm. Assoc. 23 (1934) 94.
[4] O.A. Beath, C.S. Gilbert, H.F. Eppson, Am. J. Bot. 28 (1941) 887.
[5] O.A. Beath, A.F. Hagner, C.S. Gilbert, Geol. Surv. Wyoming 36 (1946) 1.
[6] J.S. Joffe, Pedology 1 (1990) 124.
[7] H.G. Byers, US Dept. Agric. Tech. 482 (1935) 1.
[8] H.G. Byers, US Dept. Agric. Tech. 530 (1936) 1.
[9] S.P. Canton, W.D. Van Derveer, Environ. Toxicol. Chem. 16 (6) (1997) 1255.
[10] D. Wang, G. Alfthan, A. Aro, A.S. Knvutila, T. Hammar, Agric. Ecosyst. Environ. 54 (1-2) (1995) 137.
[11] J.P. Diaz, M. Navarro, H. Lopez, M.C. Lopez, Sci. Total Environ. 186 (1996) 231.
[12] T.P. McCarthy, J. Chromatogr. 225 (1981) 9.
[13] R. Hasunuma, P. Ogawa, P. Tadao, D. Ishii, K. Jukichi, Y. Kawanishi, J. Chromatogr. 537 (1991) 397.

[14] C. Sarzanini, O. Abollino, E. Mentasti, V. Porta, Chromatographia 30 (1990) 293.
[15] W. Funk, V. Dammann, T. Couturier, J. Schiller, L. Voelker, J. High. Resolut. Chromatogr. 9 (1986) 224.
[16] H.M. Stahr, J. Kinker, D. Nicholson, W. Hyde, J. Liq. Chromatogr. 5 (1982) 1191.
[17] B. Debski, A. Krynski, T.P. Zasski, Ann. Warraw. Agric. Univ. SGGW AR Vet. Med. 17 (1993) 33.
[18] L.J. Throop, Anal. Chem. 32 (1960) 1807.
[19] C.C.Y. Chan, R.S. Sadana, Anal. Chim. Acta 270 (1) (1992) 231.
[20] J. Aaseth, E. Glattre, H. Rrey, H. Frey, Sixth Int. Trace Elem. Symp. 3 (1989) 911.
[21] Y. Prameela Devi, N.V. Nanda Kumar, J. Assoc. Off. Anal. Chem. 64 (1981) 1301.
[22] C.C. Cassil, Residue Rev. 1 (1962) 37.
[23] S. Udayabhaskar, N.V. Nanda Kumar, Proc. Symp. Toxicol. Defence Sci. 1 (1980) 12.
[24] N.V. Nanda Kumar, K. Visweswaraiah, S.K. Majumder, J. Agric. Biol. Chem. 40 (1976) 431.
[25] N.V. Nanda Kumar, K. Visweswaraiah, S.K. Majumder, J. Assoc. Off. Anal. Chem. 59 (1976) 641.
[26] S. Udayabhaskar, N.V. Nanda Kumar, J. Assoc. Off. Anal. Chem. 64 (1981) 1312.