Analytical and Bioanalytical Chemistry (2022) 414:257–263
https://doi.org/10.1007/s00216-021-03772-z

COMMUNICATION

Quantification of active selenols in cells: a selenol-specific recognition europium-switched signal-amplification ICP-MS approach

Jiaxuan Zhang[1] · Yang Zhou[1] · Dongliang Zuo[2] · Limin Yang[1] · Xiaowen Yan[1] · Pingguo Liu[2] · Qiuquan Wang[1]

Received: 16 July 2021 / Revised: 29 October 2021 / Accepted: 3 November 2021 / Published online: 11 December 2021
© Springer-Verlag GmbH Germany, part of Springer Nature 2021

Abstract

Selenium (Se) is a mysterious thus tempting element playing a dual bio-chemical function, mainly through selenol, during life processes. Quantification of the selenols is thus of great significance for understanding the biological roles of Se, but remains a big challenge. Herein we report a selenol-specific recognition-mediated and europium (Eu) signal-switched amplification inductively coupled plasma mass spectrometry (ICP-MS) approach for quantifying the free active selenols (*act*-SeH) in cells. A bifunctional molecule, 2,4-dinitrobenzenesulfonyl-piperidin-4-yl-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic europium (DNBS-DOTA-Eu), was designed and synthesized for the specific recognition and highly sensitive quantification of *act*-SeH via switching Se to more sensitive Eu ICP-MS signals. The limit of detection (LOD, 3σ) of 3.41 pg/mL (22.43 pmol/L), corresponding to the absolute mass LOD of 6.82 ag *act*-SeH per cell, is almost 25 times lower than 83.76 pg/mL (1.06 nmol/L), 167.52 ag, when monitoring ^{80}Se. The results indicate that *act*-SeH in the selenite-precultured cancerous HepG2 and paracancerous HL7702 cells are 0.090 ± 0.002 pg/cell (n = 7) and 0.021 ± 0.006 pg/cell (n = 7), more than 4.28 times higher in HepG2 than in HL7702. Preliminary application of this approach to the cells from real hepatic tissue samples suggested that *act*-SeH has a positive relationship with the degree of hepatic disease. *act*-SeH in cells appears to be a very promising relevant index for understanding the biochemical functions of Se, besides the total Se in cells and blood serum and/or plasma.

Keywords ICP-MS · Selenium · Selenol · Quantification · Cell

Introduction

Selenium (Se) in its right chemical forms with an adequate nutritional status is an essential trace element for various physiological functions in the human body, for example, cellular redox homeostasis and immune regulation [1, 2]. However, arguments on Se biochemical functions have never stopped since it was discovered as a toxic element [3]. Among the various chemical forms of Se [4], selenomethionine (SeMet) is incorporated randomly into proteins at the site of methionine to form Se-containing proteins that act mostly as Se carriers [5], while selenocysteine (Sec), the 21st natural amino acid, is assembled into UGA-coded specific selenoproteins, playing many basic physiological roles during life processes [6]. Sec has a selenohydryl group (selenol, -SeH) instead of a sulfhydryl (thiol, -SH) in its analogue, cysteine (Cys). The weaker chemical bond to hydrogen, along with the increase in the size and polarizability of Se compared to sulfur, results in substantially stronger acidity of -SeH than -SH by 3–4 pK_a units [2]. Thus, Cys is largely in the intact thiol state (-SH)

Published in the topical collection celebrating *ABCs 20th Anniversary*.

Jiaxuan Zhang and Yang Zhou contributed equally to this work.

✉ Limin Yang
lmyang@xmu.edu.cn

✉ Qiuquan Wang
qqwang@xmu.edu.cn

[1] Department of Chemistry & the MOE Key Laboratory of Spectrochemical Analysis and Instrumentation, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen 361005, China

[2] Department of Hepatobiliary Surgery, Fujian Provincial Key Laboratory for Chronic Liver Disease and Hepatocellular Carcinoma, Zhong Shan Hospital Xiamen University, Xiamen 361004, China

at physiological pH, whereas Sec is almost fully dissociated to -Se$^-$, which increases nucleophilicity. Conceivably, the activity and biochemical functions of small Se compounds and selenoproteins are mainly deployed and realized through the selenols. To further assess Se status and understand its biochemical roles in cells, quantification of the total reducible selenols (*t*-SeH), especially the free active selenols (*act*-SeH), is therefore more meaningful compared to the total Se (*t*-Se) in cells.

Currently, most of the reported methods for recognition of selenols followed by optical detection are based on the nucleophilic divergence between -SeH and -SH, trying to eliminate the interference from the large amounts of coexisting thiols in a relatively intended acidic medium [7, 8]. These optical, especially fluorescent methods did a good job of imaging the profile and confirming the existence of selenols, but not quantifying the absolute selenols, in addition to the frequently encountered problems of photobleaching and autofluorescence from the sample matrix. Comparatively, atomic mass spectrometry, especially inductively coupled plasma mass spectrometry (ICP-MS), as we know, is good at quantification and speciation of Se using a species-unspecific isotope dilution technique when coupling with a pre-separation technique [9–12]. However, two intrinsic weak points remain: (1) low ionization efficiency of Se (ca. 30%) in ICP [13], resulting in a relatively high detection limit of pg/mL levels, even though the polyatomic ion spectral interferences could be effectively overcome with the aid of additional reaction gas-based collision cell and/or dynamic reaction cell (DRC); and (2) inability to distinguish different Se species, for example, difficulty in differentiating Sec in selenoproteins from SeMet in Se-containing proteins. We hypothesize that selective recognition of selenols plus effective conversion of Se to an element—for example, europium (Eu)—that has nearly 100% ICP ionization efficiency [13] and, importantly, negligible biological background should be a way to meet the selectivity and sensitivity requirements for quantification of *act*-SeH in cells. *act*-SeH might be a promising and relevant index for evaluating the status, especially the roles of Se. We thus designed and synthesized a two-functional molecular probe, in which 2,4-dinitrobenzenesulfonyl (DNBS) serves as a selenol-specific recognition moiety, while a 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid europium complex (DOTA-Eu) moiety can be stoichiometrically released after the -SeH-recognition reaction for converting Se to the more sensitive Eu ICP-MS signals (Fig. 1).

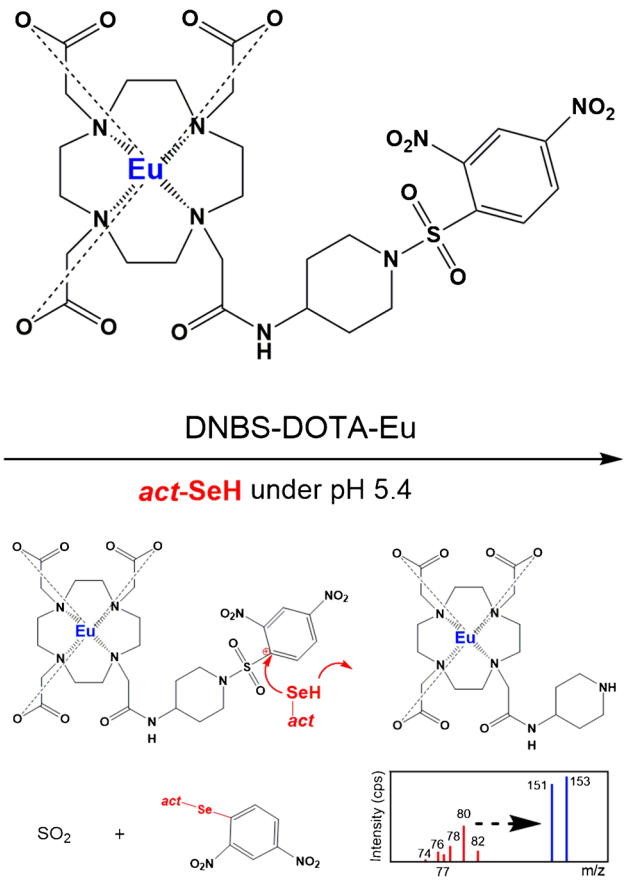

Fig. 1 2,4-Dinitrobenzenesulfonyl-piperidin-DOTA-Eu (DNBS-DOTA-Eu) for recognition and quantification of *act*-SeH in cells by converting Se to more sensitive Eu ICP-MS mass signals

Materials and methods

Design and synthesis of DNBS-DOTA-Eu In order to synthesize the DNBS-DOTA-Eu bifunctional probe (see Electronic Supplementary Material Fig. S1), we started from 2,4-dinitrobenzenesulfonic acid (compound **1**; Sigma-Aldrich, St. Louis, MO, USA), which was dissolved in CH_2Cl_2 (DMC) containing Et_3N (TEA), and then 4-(*N*-Boc-amino) piperidine (**2**; purity > 98%; J&K Scientific Co., Ltd., Beijing, China) in DMC was added dropwise under an ice bath over a period of 10 min. The ice bath was then removed and the mixture was continually stirred for another 6 h at room temperature. The reaction mixture was then diluted with DMC, washed with H_2O, dried over anhydrous Na_2SO_4, and subjected to reduced-pressure distillation (RPD) in sequence to obtain [1-(2,4-dinitrobenzenesulfonyl)-piperidin-4-yl]-carbamic acid *tert*-butyl ester (**3**). It was further purified on a glass chromatographic column (3.0 I.D. × 150 cm in length) of 300-400 mesh silica gel using DMC/ACN (20/1, v/v) as the mobile phase with a yield of 90%, and characterized by nuclear magnetic resonance (NMR) and high-performance liquid chromatography–electrospray ionization quadrupole time-of-flight mass spectrometry (HPLC-ESI-Q-TOF-MS) (Fig. S2). After deprotection of the Boc group of **3** overnight with stirring at room temperature, RPD and silica gel column purification, 1-(2,4-dinitrobenzenesulfonyl)-piperidin-4-yl] amine (**4**, 95% yield) was obtained (Fig. S3). Compound **4**

and 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid mono-*N*-hydroxysuccinimide ester (DOTA-NHS ester) (**5**; purity > 90%; Macrocyclics, Inc., Plano, TX, USA) were respectively dissolved in dimethylformamide. After thorough mixing, diisopropylethylamine (DIPEA; J&K Scientific Co., Ltd., Beijing, China) was added with stirring overnight at room temperature for the conjugation of **4** to DOTA via the reaction between -NH_2 and *N*-hydroxysuccinimide. The resulting product was purified on a preparative chromatography [Shimadzu 10AD HPLC system with an SPD-M10Avp detector and an Elite Hypersil BDS C18 column (10.0 I.D. × 250 mm in length, 5 μm particle size)] using H_2O containing 0.05% TFA (A) and ACN-0.05% TFA (B), with a gradient elution program of B increasing from 5% to 80% over 25 min] and characterized using ESI-Q-TOF-MS (**6**, DNBS-DOTA) with 90% yield and $[M + H]^+ = 717.2177$ (Fig. S4). Finally, $Eu(NO_3)_3$ solution prepared form Eu_2O_3 (purity > 99.9999%, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences) reacted overnight with **6** in Tris-HCl buffer (pH = 6.84) at room temperature for coordinating Eu^{3+} into **6**. The obtained DNBS-DOTA-Eu was purified using the preparative chromatography with 95% yield using H_2O (A) and ACN (B) with a gradient elution program of B ramping from 5% to 80% over 15 min, and characterized by ESI-Q-TOF-MS at m/z 867.0809 of $[M + H]^+$ (Fig. S5).

Cell culture and treatment Hepatic HepG2 and HL7702 cell lines from the cell bank of Shanghai Institute for Biological Sciences were selected as the models of cancerous and paracancerous cells. They were grown respectively with RPMI 1640 (Roswell Park Memorial Institute) and DMEM (Dulbecco's modified Eagle's medium, Gibco, CA, USA) supplemented with 10% fetal bovine serum (FBS; Gibco) and penicillin-streptomycin (Gibco) as well as an appropriate amount of selenite in an atmosphere of 5% CO_2 with constant humidity of 55% at 37 °C. Before starting the quantitative analysis experiments, in order to obtain good growth conditions for HepG2 and HL7702, the amounts of selenite added to the culture media were investigated using CCK-8 cytotoxicity assay (Fig. S6). HepG2 and HL7702 cells were plated with a cell density of 5×10^5 cells per plate and incubated overnight in the culture media with 0–60 μM selenite for HepG2 and 0–20 μM selenite for HL7702, respectively. The CCK-8 solution containing tetrazolium salt WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfobenzene)-2H-tetrazole monosodium salt] (Xiamen Yingwei Biotechnology Co., Ltd.) was added for another 30 min cultivation, and then detected at 450 nm on an EnSpire microplate reader (PerkinElmer, Waltham, MA, USA). The obtained optical density (OD) and cell viability indicated that 10 μM and 0.5 μM selenite were suitable for HepG2 and HL7702 when considering that higher Se and/or selenol content benefits the subsequent quantification. However, in order to compare the total Se (*t*-Se), *t*-SeH and *act*-SeH between HepG2 and HL7702 cells, 0.5 μM selenite was used for both HepG2 and HL7702 cultivation. When the cells grew to about 80% cell confluence, they were subjected to trypsin (0.25%) digestion (Gibco, CA, USA) for further use.

Quantification of *t*-Se, *act*-SeH and *t*-SeH in cells Before quantification, the cells were washed five times with PBS (pH 7.4) to completely remove the excess selenite. For quantifying *t*-Se in cells, the cells (1.0×10^6) were first digested using concentrated HNO_3 and diluted to 2% HNO_3 solution, and then *t*-Se was directly determined using the PerkinElmer NexION 2000 ICP-MS (PerkinElmer, MA, USA) equipped with a concentric pneumatic nebulizer, a cyclonic spray chamber and DRC (CH_4 reaction gas) for monitoring ^{80}Se. The operational parameters were as follows: nebulizer Ar gas, 0.88 L/min; auxiliary Ar gas, 1.0 L/min; plasma Ar gas, 15 L/min; RF power, 1200 W; lens voltage, 7.2 V. Parameters including Ar nebulizer gas flow and lens voltage were optimized daily to obtain the best sensitivity with a tuning solution containing Be, Ce, Fe, In, Li, Mg, Pb and U in 1% HNO_3 (1 μg/L each), and the sampling depth was also optimized as 13 mm to guarantee the lowest oxide ratio (valued by Ce/CeO) as < 0.025. For quantifying *act*-SeH defined as free active -SeH-containing molecules, the cells (1.0×10^6) were subjected to RIPA lysis [RIPA contains 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS and 1% PMSF (phenylmethylsulfonyl fluoride) protease inhibitor purchased from Beijing Solarbio Science & Technology Co., Ltd.] in boiling water, and then lysed ultrasonically on ice. After centrifugation (16,000×*g*, 15 min), the supernatant obtained is cell extract. Afterwards, the optimized 20 nM bifunctional DNBS-DOTA-Eu (Fig. S7) was added to the cell extract buffered with PBS at pH 5.4 for recognizing the *act*-SeH and releasing DOTA-Eu for ^{151}Eu species-unspecific isotope dilution (SUID) ICP-MS quantification [$^{151}Eu(NO_3)_3$ solution (10 ng/mL corresponding to 65.79 nmol/L) was prepared from ^{153}Eu-enriched Eu_2O_3 (purity > 99.9%) purchased from Cambridge Isotope Laboratories, Andover, MA, USA] after HPLC separation of the released DOTA-Eu and the excess unreacted DNBS-DOTA-Eu with a Waters Alliance e2695 chromatography system equipped with an Agilent C18 column (1.0 I.D. × 150 mm, 3.5 μm particle size) with gradient elution from 5% to 80% ACN over 30 min at a flow rate of 0.05 mL/min. The enriched ^{151}Eu isotope solution of 65.79 nmol/L was supplemented through a T-joint at a flow rate of 0.25 mL/min for the ^{151}Eu-SUID-ICP-MS quantification. It should be noted that all these procedures were performed in a glove box under a N_2 atmosphere, and all the reagents were deoxygenated before use in order to avoid possible oxidation. For quantifying the *t*-SeH defined as the sum of all the reducible -Se-Se- and

-Se-S- forms including *act*-SeH, 100 times equivalent dithiothreitol (DTT) was used before selenol recognition.

Results and discussion

Specific recognition and sensitive determination are two crucial factors in the quantification of *act*-SeH in cells. We thus selected DNBS, which has a strong electrophilic carbon atom on the two electron-withdrawing nitryls and one sulfonyl-substituted benzene for recognizing *act*-SeH based on its higher nucleophilicity than the thiols [2]. It was then linked to DOTA-Eu via the amidation reaction between the -COOH of DOTA and the -NH_2 of piperidin-4-yl amine to obtain DNBS-DOTA-Eu (Fig. S1). DOTA-Eu was released after the selenol's nucleophilic substitution reaction, and separated using HPLC from the excess unreacted DNBS-DOTA-Eu for a more sensitive readout of the *act*-SeH recognized on ICP-MS. In this way, a limit of detection (LOD, 3σ) of 3.41 pg Eu /mL (22.43 pmol/L) was obtained, almost 25 times lower than that of 83.76 pg Se/mL (1.06 nmol/L) (Fig. S8). Considering that a sampling flow rate of 0.3 mL/min was adapted for the cell solution of 1.0×10^6 cells/mL in this study, 2 nL of the cell solution containing two cells during the ion plume duration time (about 400 μs) of a single cell in ICP was infused into the ICP-MS [14]. We therefore estimated the absolute mass LOD per cell via the assumption that ICP-MS determines the signals from the single-cell duration time, with an absolute LOD of 6.82 ag Eu and 167.52 ag Se per cell. Subsequently, the selectivity of DNBS-DOTA-Eu was first evaluated using Sec and a Sec-containing SLUGT peptide and their thiol counterparts of Cys and SLCGT. The results obtained suggested that Sec and SLUGT can react with DNBS-DOTA-Eu, forming Sec-DNB [m/z at 335.9054 ($M+H^+$)] and SLUGT-DNB [694.0920 $(M+H)^+$] adducts (Fig. 2a-c) at pH 5.4 buffered by 20 mM PBS, while Cys and SLCGT do not (Fig. S9). Considering the high amounts of the coexisting thiols in cells, especially GSH at the micromolar level, we investigated the reaction of GSH with DNBS-DOTA-Eu. With an increase in the mole ratio between SLUGT and GSH from 1:5 to 1:1000, GSH did not react with DNBS-DOTA-Eu

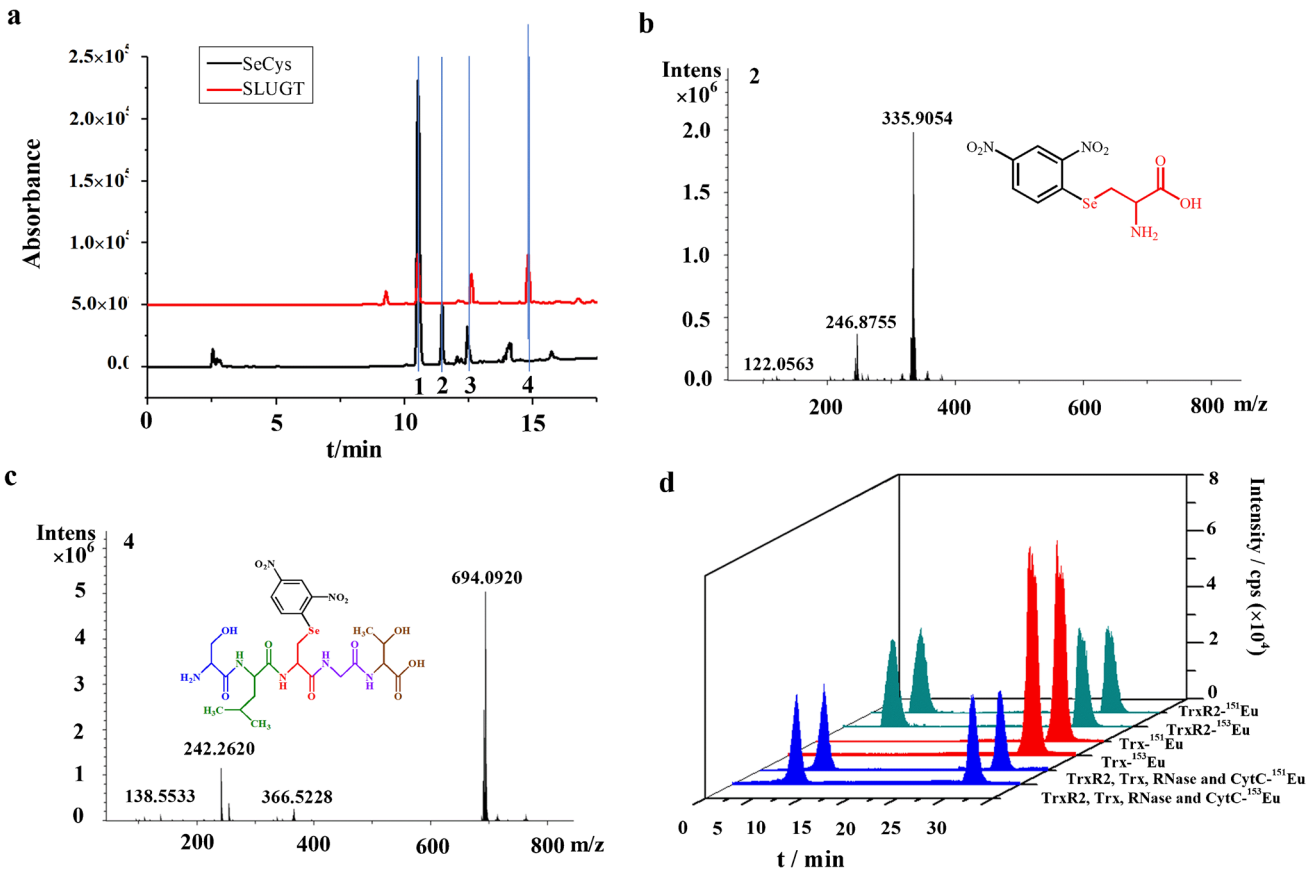

Fig. 2 HPLC chromatograms of the reaction mixture of DNBS-DOTA-Eu with Sec and SLUGT (**a**). Peak 1 is DTT, peak 2 Sec-DNB (**b**), peak 3 DNBS-DOTA-Eu and peak 4 SLUGT-DNB (**c**). Selectivity of DNBS-DOTA-Eu (20 nM) towards TrxR2, Trx and the mixture of Trx, RNase, CytC and TrxR2 of 10 nM each at pH 5.4 buffered by 20 mM PBS. The first peak is DOTA-Eu released after TrxR2 reaction with DNBS-DOTA-Eu (**d**). A gradient elution program of ACN ramping from 5% to 80% over 15 min was used (**a**), while a gradient elution program of ACN ramping from 5% to 80% over 30 min was used (**d**)

until 1000 higher concentration of GSH over SLUGT was reached, with less than 5% at pH 5.4 (Fig. S10). Moreover, because the almost three orders of magnitude faster reaction rate of Sec than Cys [15], such a small contribution from GSH could be further reduced by reducing the reaction time to less than 5 min, suggesting that the interference of coexisting GSH could be neglected, even though the thiol concentration in cells might be over 1000 times greater. In addition, the selectivity of DNBS-DOTA-Eu towards biomacromolecules was studied. Among the selected thioredoxin reductase 2 (TrxR2 containing 1 Sec and 13 Cys) and its thiol counterpart thioredoxin (Trx containing no Sec and 5 Cys), as well as other protein ribonuclease (RNase 8 Cys) and cytochrome C (CytC 2 Cys), only the selenoprotein TrxR2 reacted with DNBS-DOTA-Eu at pH 5.4, whether it existed alone or together with Trx, RNase and CytC, when DDT (100:1) was used to reduce the proteins, while the other proteins tested did not react with DNBS-DOTA-Eu although they had Cys residues in their amino acid sequence (Fig. 2d). Taken together, all these results suggest that DNBS-DOTA-Eu can selectively recognize the selenols of both small molecules and biomacromolecules.

Prior to the quantification of *act*-Sec in human liver cancer HepG2 and paracancerous hepatic HL7702 cells, *t*-Se was directly determined in the selenite-precultured HepG2 and HL7702 cells (1.0×10^6 each) using ICP-MS when monitoring $^{80}Se^+$ under DRC mode using CH_4 as a reaction gas. The results obtained (Fig. 3a) indicated that the average *t*-Se in the HepG2 of 3.40 ± 0.09 pg/cell ($n = 7$) is 3.47 times higher than 0.98 ± 0.01 pg/cell ($n = 7$) in HL7702. Subsequently, *act*-SeH was quantified via the determination of Eu using ^{151}Eu-SUID-ICP-MS thanks to that one selenol reacts selectively with one DNBS-DOTA-Eu releasing one DOTA-Eu, means that the LOD of Eu is the LOD of *act*-SeH. The results indicated that the average *act*-SeH in the HepG2 of 0.090 ± 0.002 pg/cell ($n = 7$) is 4.28 times higher than 0.021 ± 0.006 pg/cell ($n = 7$) HL7702. In order to verify the accuracy and recovery of this developed approach, we used standard selenoprotein TrxR2 to go through the sample preparation, recognition reaction, separation, and quantification procedures, the recovery of TrxR2 was between 96.92% and 99.31% with the average value of 98.71% and relative standard deviation (RSD) of 3.7% ($n = 7$) (Fig. S11). These results indicated that the necessary deoxygenation of all the reagents before use and performing the experiments in a glove box under a N_2 atmosphere do matter to avoid possible oxidation and to keep the selenols unchanged. Furthermore, before the recognition reaction with DNBS-DOTA-Eu, the cell extracts were subjected to DDT reduction to convert all the Se in the possible forms of -Se-Se- and -Se-S- into the *act*-SeH; together with the *act*-SeH, such total reducible *t*-SeH were quantified to be on average 0.28 ± 0.01 pg/cell ($n = 7$) in the HepG2 and 0.07 ± 0.02 pg/cell ($n = 7$) HL7702, implying that *t*-SeH in the HepG2 is also nearly four times higher than that in HL7702. The *act*-SeH accounts for 2.65% of *t*-Se and 32.1% of *t*-SeH in the HepG2, while accounting for 2.1% and 30.0% in HL7702. Moreover, the *t*-SeH accounts for 8.24% of the *t*-Se in the HepG2 and 7.14% in HL7702 (Fig. 3b). All these results suggest that the amounts of *act*-SeH, *t*-SeH and *t*-Se, as well as the percentages of *act*-SeH in *t*-SeH, *act*-SeH in *t*-Se and *t*-SeH in *t*-Se, follow the same general trends of higher amounts in cancerous HepG2 than paracancerous HL7702, in accord with the consensus that cancerous cells are in an uncontrollable growth state, being smart enough to utilize Se to escape the normal apoptosis or programmed death of cells [16]. It is worth pointing out that *act*-SeH is the most sensitive for differentiating cancerous and healthy cells even though it accounts for only about 1/50th of *t*-Se and one third of *t*-SeH. Finally, we preliminarily applied this approach to the quantification of *act*-SeH, *t*-SeH and *t*-Se in cells from five hepatic samples including one metastatic carcinoma and two moderately differentiated and two hyperplastic tissue samples from Xiamen University affiliated Zhongshan Hospital. The obtained results (Table 1) indicated that all the *act*-SeH, *t*-SeH and *t*-SeH quantified in the five samples follow the order of metastatic carcinoma cells > moderately differentiated cells > hyperplastic cells. It should be noted that

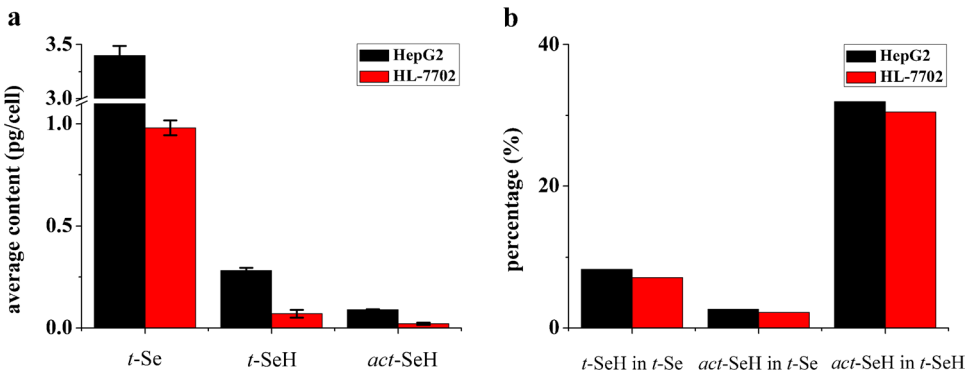

Fig. 3 *t*-Se, *t*-SeH and *act*-SeH in HepG2 and HL7702 cells ($n = 7$) (**a**) and the percentages of *act*-SeH in *t*-Se, *t*-SeH in *t*-Se, and *act*-SeH in *t*-SeH (**b**)

Table 1 Average content of *t*-Se, *t*-SeH, *act*-SeH and percentage of *act*-SeH in *t*-SeH, *act*-SeH in *t*-Se and *t*-SeH in *t*-Se in the cells from the five hepatic samples (5.0×10^5 cells used for each case)

Sample		Average content (pg/cell, $n = 7$)			Percentage (%)					
		t-Se	*t*-SeH	*act*-SeH	*act*-SeH/*t*-SeH		*act*-SeH/*t*-Se		*t*-SeH/*t*-Se	
1	Metastatic carcinoma cells	63.68 ± 0.058	1.22 ± 0.014	0.286 ± 0.012	23.44		0.45		1.92	
2	Moderately differentiated cells	42.53 ± 0.024	0.79 ± 0.012	0.193 ± 0.003	23.34	24.43	0.42	0.45	1.78	1.86
3	Moderately differentiated cells	52.15 ± 0.048	0.89 ± 0.010	0.198 ± 0.016		22.25		0.38		1.71
4	Hyperplastic cells	34.88 ± 0.033	0.57 ± 0.006	0.131 ± 0.002	20.62	22.98	0.31	0.38	1.46	1.63
5	Hyperplastic cells	17.92 ± 0.033	0.23 ± 0.013	0.042 ± 0.002		18.26		0.23		1.28

only five real samples were determined although the general trend in the percentages of *act*-SeH and *t*-SeH in *t*-Se as well as *act*-SeH in *t*-SeH increase with the increase in the degree of disease. Additional samples will likely be determined to have statistical significance in the future.

Conclusions

We developed an HPLC-ICP-MS method for quantifying *act*-SeH in cells for the first time. Selectivity was achieved by the newly designed and synthesized DNBS-DOTA-Eu bifunctional molecular probe, while the sensitivity was improved nearly 25-fold by switching the determination of Se itself to Eu through the quantitative substitution reaction, satisfying the sensitivity requirement of *act*-SeH quantification in cells. More importantly, this novel approach does not need the conventional proteolytic digestion and necessary chemical derivatization of Sec, avoiding possible Sec loss or transformation. Although only the information on global *act*-SeH that might be either bound to or associated with small and/or selenoprotein molecules can be obtained by our method at this time, the *act*-SeH value in cells is more sensitive, providing more relevant insights when elucidating the biochemical roles of Se than the *t*-Se, because the roles of Se during the life process are mainly performed by selenols. The results obtained regarding *act*-SeH and *t*-SeH as well as *t*-Se in the model cell lines and real hepatic cell samples indicated that they are generally higher not only in hepatic cancerous cells than in paracancerous cells, but also in metastatic carcinoma cells than hyperplastic cells. Just when people believed the popular notion that Se might have a role in cancer prevention, many studies found that some selenoproteins—for example, Gpx2 and TrxR1— are overexpressed in cancer cells [16, 17]. Clearly, the roles of Se in the processes occurring before and after tumorigenesis appear to be different. More intensive and extensive investigations on Se cancer prevention or promotion effects are waiting to be conducted. We hope our approach developed here may aid such biochemical research. Moreover, one more opportunity provided by our method that needs to be explored is finding the molecule-specific selenols via DNBS-mediated substitution reaction using soft-ionization MS/MS, being more pertinent to understanding the biological functions of Se at the molecular level. Such studies are ongoing in our laboratory.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00216-021-03772-z.

Acknowledgements We thank the financial supports from the National Natural Science Foundation of China (21874112, 21535007 and 22193053), the Foundation for Innovative Research Groups of the National Natural Science Foundation of China (21521004) and Program for Changjiang Scholars and Innovative Research Team in University (PCSIRT, IRT13036) as well as Medical and Health Key project of Xiamen (3502Z20191106).

Declarations

Five hepatic samples including one metastatic carcinoma and two moderately differentiated and two hyperplastic tissues were provided by Xiamen University affiliated Zhongshan Hospital and permitted by Medical Ethics Council. Informed consent was given by the patients donating the cell sample for analysis.

Conflict of interest The authors declare that they have no competing interests. Qiuquan Wang is editor of *Analytical and Bioanalytical Chemistry* but was not involved in the peer review of this paper.

References

1. Rayman MP. Selenium and human health. Lancet. 2012;379:1256–68.
2. Reich HJ, Hondal RJ. Why nature chose selenium. ACS Chem Biol. 2016;11:821–41.
3. Trofast J. Berzelius' discovery of selenium. Chemistry International. 2011;33:16–9.
4. LeBlanc KL, Mester Z. Compilation of selenium metabolite data in selenized yeasts. Metallomics. 2021;13:mfab031.
5. Schrauzer GN. Selenomethionine: A review of its nutritional significance, metabolism and toxicity. J Nutr. 2000;130:1653–6.
6. Atkins JF, Gesteland RF. The twenty-first amino acid. Nature. 2000;407:463–4.
7. Maeda H, Katayama K, Matsuno H, Uno T. 3′-(2,4-Dinitrobenzenesulfonyl)-2′,7′- dimethylfluorescein as a fluorescent probe for selenols. Angew Chem Int Ed. 2006;45:1810–3.

8. Zhang BX, Ge CP, Yao J, Liu YP, Xie HC, Fang JG. Selective selenol fluorescent probes: design, synthesis, structural determinants, and biological applications. J Am Chem Soc. 2015;137:757–69.
9. Xu M, Yang LM, Wang QQ. Quantification of selenium-tagged proteins in human plasma using species-unspecific isotope dilution ICP-DRC-qMS coupled on-line with anion exchange chromatography. J Anal At Spectrom. 2008;23:1545–9.
10. Chen BB, Hu B, He M, Huang Q, Zhang Y, Zhang X. Speciation of selenium in cells by HPLC-ICP-MS after (on-chip) magnetic solid phase extraction. J Anal At Spectrom. 2013;28:334–43.
11. Bamonti L, Theiner S, Rohr-Udilova N, Keppler BK, Koellensperger G. Accurate high throughput quantification of selenium in biological samples – the potential of combining isotope dilution ICP-tandem mass spectrometry with flow injection. J Anal At Spectrom. 2016;31:2227–32.
12. Bierla K, Lobinski R, Szpunar J. Determination of proteinaceous selenocysteine in selenized yeast. Int J Mol Sci. 2018;19:543.
13. Houk RS. Mass spectrometry of inductively coupled plasmas. Anal Chem. 1986;58:97A–105A.
14. Zhou Y, Chen ZQ, Zeng JX, Zhang JX, Yu DX, Zhang B, Yang LM, Yan XW, Wang QQ. Direct infusion ICP-qMS of lined-up single-cell using an oil-free passive microfluidic system. Anal Chem. 2020;92:5286–93.
15. Hondal RJ, Nilsson BL, Raines RT. Selenocysteine in Native Chemical Ligation and Expressed Protein Ligation. J. Am. Chem. Soc. 2001;123:5140–1.
16. Hatfield DL, Yoo MH, Carlson BA, Gladyshev VN. Selenoproteins that function in cancer prevention and promotion. Biochim Biophys Acta. 2009;1790:1541–5.
17. Roman M, Jitaru P, Barbante C. Selenium biochemistry and its role for human health. Metallomics. 2014;6:25–54.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.