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Development of surface plasmon resonance imaging biosensors for detection of ubiquitin carboxyl-terminal hydrolase L1

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ABSTRACT

We have developed a new method for highly selective determination of the ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1) concentration using a surface plasmon resonance imaging (SPRI) technique and two different biosensors. UCH-L1 was captured from a solution by immobilized specific rabbit monoclonal antibody or specific LDN-57444 inhibitor due to formation of receptor–UCH-L1 complex on the biosensor surface. The analytically useful dynamic response range of both biosensors is between 0.1 and 2.5 ng/ml. The detection limit is 0.06 ng/ml for the biosensor with antibody and 0.08 ng/ml for the biosensor with inhibitor. Biosensors based on both antibody and inhibitor were found to be suitable for quantitative determination of the UCH-L1 and exhibit good tolerance to the potential interferents. Both biosensors gave comparable results in the range of 0 to 0.20 ng/ml for plasma samples and 0.30 to 0.49 ng/ml for cerebrospinal fluid samples. To validate the new methods, comparative determination of UCH-L1 by the commercial enzyme-linked immunosorbent assay (ELISA) kit was performed. In general, in terms of UCH-L1 concentration, a good correlation between SPRI and ELISA was found. The developed biosensors can be used successfully for the determination of UCH-L1 in body fluids.

Ubiquitin carboxy-terminal hydrolase L1 (UCH-L1),[1] also known as PGP9.5, is one of the deubiquitinating enzymes. The ubiquitin–proteasome system is responsible for degradation of intracellular proteins. It plays important roles in the elimination of misfolded and damaged toxic proteins produced in response to various cellular stresses. This pathway is responsible for turnover of various classes of proteins that control cell cycle progression, division, development and differentiation of the cells, intracellular signaling, and apoptosis [1]. The central element of this system is the 26S proteasome. It is a multi-subunit proteolytic complex that is the key enzyme system for non-lysosomal protein degradation [2]. Degradation of protein by the ubiquitin–proteasome system consists of two steps. The first step involves the covalent conjugation of ubiquitin to the targeted protein. This process is catalyzed by ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2), and ubiquitin–protein ligase (E3). The next step is degradation of the ubiquitin-tagged protein by the 26S proteasome with the release of free and reusable ubiquitin.

UCH-L1 belongs to the family of peptidases. It is a 223-amino-acid neuronal protein that contains the typical active site triad of cysteine, histidine, and aspartic acid [3,4]. It hydrolyzes small ubiquitin C-terminal adducts and plays a role in regulating the degradation of free ubiquitin monomers. This enzyme hydrolyzes a peptide bond at the C-terminal glycine of ubiquitin [5] and is a mono-ubiquitin stabilizer [6]. UCH-L1 is expressed in the central and peripheral nervous systems and consists of 1 to 5% of the total soluble protein in the brain. Its expression is also observed in neuroendocrine cells and tissues of the reproductive system, although it is localized in most adult tissues [7,8]. UCH-L1 concentration in plasma can also be determined, especially in the case of patients after epileptic seizure [9] or after severe head injury [10,11]. Disturbances in the functioning of UCH-L1 are associated with neurological diseases such as Parkinson's disease and Alzheimer's

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[1] *Abbreviations used:* UCH-L1, ubiquitin carboxy-terminal hydrolase L1; ELISA, enzyme-linked immunosorbent assay; SPRI, surface plasmon resonance imaging; EDC, *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide; ODM, 1-octadecanothiol; NHS, *N*-hydroxysuccinimide; PBS, phosphate-buffered saline; AFM, atomic force microscopy; SD, standard deviations.

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disease [12,13]. The relationship between variable expression of UCH-L1 and a variety of cancers has been reported [14]. It has been implicated in oncogenesis, tumor cell invasion, and metastasis [7,8,15,16]. The literature data suggest that UCH-L1 may play a role as a prognostic marker for tumor growth and progression [17,18]. UCH-L1 may also be a potential therapeutic target for certain types of cancer, and determination of its concentration may be an indicator of response to therapy [14,19]. In general, C-terminal ubiquitin hydrolase L1 expression is assessed [20,21]. In some studies, the UCH-L1 activity and concentration is determined [22]. The most common methods used for UCH-L1 detection are Western blot, immunohistochemistry [8,23], and enzyme-linked immunosorbent assay (ELISA) [9,10]. Immunofluorescence with confocal microscopy [24] and pre-embedding immunoelectron microscopy are also applied [20]. The UCH-L1 polypeptide backbone has been studied by using nuclear magnetic resonance (NMR) spectroscopy [25]. Most of techniques that can be found in the literature are "labeled methods." These methods have some drawbacks. The use of labels may be the reason for protein losing its functional properties (functional proteomics). They are time-consuming and multi-step, and their results are not quantitative (except ELISA). Therefore, a quantitative, label-free, and easy-to-perform assay is required.

Surface plasmon resonance imaging (SPRI), as a label-free technique, may overcome many of these assay limitations [26]. The combination of SPRI technique with the development of sensitive biosensors is a promising tool for the quantitative determination of biologically active species [27–30]. The SPRI method is an optical technique that measures the changes in refractive index caused by molecules bound to the metal surface. The resulting signal depends on the change in the wavelength and the angle of polarization of light. It is directly proportional to the mass change on the surface of the metal [31]. This signal is converted into an image in the SPRI technique. Selectivity of the analytical signal ensures that a biosensor cooperates with the SPRI instrument. The biosensor is usually gold-coated glass with a layer of active biomolecules as a receptor. The SPRI biosensor method uses a very specific interaction between enzyme and inhibitor or antigen and antibody, which provides real-time information and allows for the direct determination of analyte concentration [32].

The aim of this work was to develop an SPRI method for the highly selective determination of UCH-L1 and to demonstrate the sensor applicability for the determination of the protein concentration in biological samples. Two approaches to the biosensor construction were considered. Rabbit monoclonal antibody specific for human UCH-L1 was selected for one biosensor construction, and the UCH-L1 inhibitor LDN-57444 was used for the other biosensor construction.

LDN-57444 is a potent, reversible, competitive inhibitor of UCH-L1 with an IC_{50} of 0.88 μM. It shows 28-fold greater selectivity relative to UCH-L3 (another UCH family member) [33]. The final aim was to optimize the analytical parameters for both SPRI biosensors. It is worth stressing that the aim of this work was development of two analytical methods with the application of the same measuring technique, namely SPRI. Each of these methods should use different biosensor construction as well as a different manner of UCH-L1 entrapment from an analyzed solution. For comparative evaluation of the two developed methods, an ELISA test was performed.

Materials and methods

Materials

Recombinant human UCH-L1 protein (M = 24–27 kDa) as a standard was used. Rabbit monoclonal mouse IgG_{2A} antibody (M = 160 kDa) specific for human UCH-L1 and LDN-57444 inhibitor of UCH-L1 (M = 397.64 Da) (R&D Systems, USA), glycoprotein GPIIb/IIIa and cathepsin B from human liver (Calbiochem, Merck, Warsaw, Poland), cysteamine hydrochloride, *N*-ethyl-*N*'-(3-dimethylaminopropyl)carbodiimide (EDC), and human albumin (all from Sigma, Steinheim, Germany), 1-octadecanothiol (ODM) and *N*-hydroxysuccinimide (NHS) (Aldrich, Munich, Germany), and photopolymer ELPEMER SD 2054 and hydrophobic protective paint SD 2368 UV SG-DG (Peters, Kempen, Germany) were used, and absolute ethanol, acetic acid, hydrochloric acid, sodium hydroxide, sodium chloride, sodium carbonate, sodium phosphate, potassium phosphate, sodium acetate, potassium chloride, magnesium chloride (all from POCh, Gliwice, Poland), and HBS–ES buffer (pH 7.4) (0.01 M Hepes, 0.15 M sodium chloride, 0.005% Tween 20, and 3 mM ethylenediaminetetraacetic acid [EDTA]), acetic buffer (pH 3.79–5.57), phosphate-buffered saline (PBS, pH 7.4), phosphate buffer (pH 7.17–8.04), and carbonate buffer (pH 8.50–9.86) (all from Biomed, Lublin, Poland) were used as received. An ELISA kit (USCN Life Science, People's Republic of China) was used. Aqueous solutions were prepared with MilliQ water (Simplicity, Millipore). Argon N 5.0 with a content Ar ⩾ 99.999% was used (Air Liquide Polska, Poland). Q1

Biological samples

Blood samples taken from healthy adult donors were supplied by the Blood Donor Centre (Bialystok, Poland). Cerebrospinal fluid samples were taken from the Department of Pediatric Surgery at the Medical University of Bialystok. Cerebrospinal fluid was obtained from healthy patients who did not have active inflammation processes within the central nervous system and who had no signs of head injury. A general laboratory study of the fluid was conducted, and the normal values of the protein and cytosis were confirmed.

All of the samples were provided after obtaining the consent of the local bioethical commission for the study of biological material collected.

Procedures

Chip preparation

Gold chips were manufactured as described in previous articles [34,35]. The gold surface of the chip was covered with photopolymer and hydrophobic paint as described previously [36]. The chip has nine places with 12 free gold surfaces. (see Fig. 1A). Using this chip, nine different solutions can be measured simultaneously without mixing the tested samples, and 12 single SPRI measurements can be performed from one solution. Atomic force microscopy (AFM) enables observation of the surface of the biosensor during its preparation. AFM measurements were performed to confirm the creation of subsequent layers. All measurements were done in ambient conditions.

Antibody immobilization

The chips were immersed in 20 mM cysteamine ethanolic solution for at least 2 h at room temperature. Next, chips were rinsed with ethanol and water and dried under a stream of argon. The chips prepared in this manner can be stored and used for subsequent measurements.

The antibody solution in a PBS buffer was activated with NHS (250 mM) and EDC (250 mM). Activation of the antibody was done by adding the mixture of NHS and EDC (1:1) in a carbonate buffer solution (pH 8.5) into the antibody solution and with vigorous stirring for 5 min at room temperature. Surface carboxylic acid groups in the UCH-L1 antibodies were converted to the activated NHS esters using the water-soluble coupling reagent EDC.

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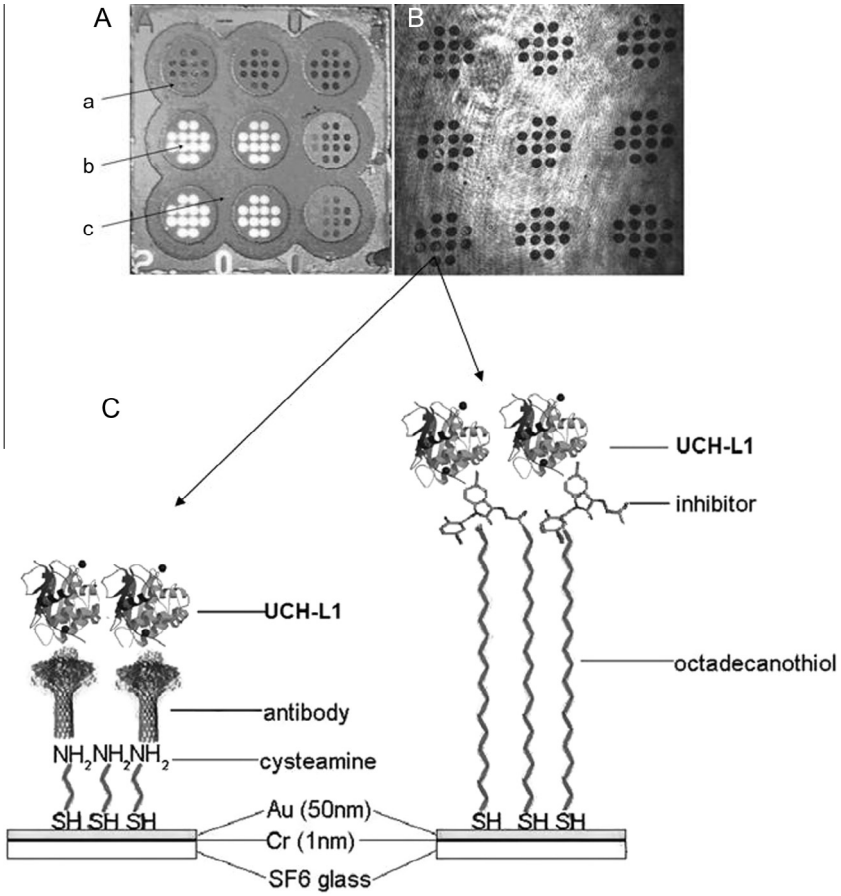

Fig.1. (A) Picture of chip (a: photopolymer; b: free gold surface; c: hydrophobic paint). (B) Image of chip obtained by CCD (charge-coupled device) camera. (C) Schematic illustration of the sensor active part.

Subsequent reaction between the antibody–NHS esters and amines on the SPR chip surface led to antibody immobilization by stable amide linkages.

Next, 3 μl of this solution was placed on each of nine active places with the amine-modified surface and incubated at 37 °C for 1 h [37]. Creating antibody–antigen complex is contingent on matching the antigen binding site to an epitope of antibody. It is caused by van der Waals forces, electrostatic forces, hydrophobic interactions, and hydrogen bonds. Valence bond of the enzyme is determined by the number of antigenic determinants that can bind a single molecule of antibody. So, prepared biosensor should be used directly for the analysis.

Inhibitor immobilization

The chips were then immersed in 2.7 mM ODM ethanolic solution for at least 24 h at room temperature. After that, the chips were rinsed with ethanol and water and dried under a stream of argon. So, prepared chips can be stored and used for subsequent measurements. The inhibitor UCH-L1 solution was placed on the thiol-modified surface and incubated at room temperature for 24 h. At this time, the immobilization process of inhibitor by hydrophobic interactions was done. LDN 57444 is a reversible and competitive inhibitor and binds in the active site of enzymes. This inhibitor is similar in structure to the substrates of their targets and attaches to UCH-L1 by noncovalent interaction such as hydrogen bonds (H-bound acceptor). Biosensor prepared in this way should be used directly for the analysis.

SPRI measurements

SPRI measurements for protein array were performed as described in previous articles, and a schematic diagram of the apparatus is given in Ref. [29]. The SPRI signal is proportional to the amount of coupled biomolecules within the optimal concentration range. The first stage of the measurements was to select the correct SPR angle. This was done for the chip after the immobilization of the receptor layer. Signal SPR was measured at a fixed SPR angle on the basis of registered images. The first image after immobilization of the antibody or inhibitor was taken. Then, the other image after interaction with UCH-L1 was taken. The SPRI signal was obtained by subtraction of the signal after and before interaction with a biomolecule for each spot separately. The contrast values obtained for all pixels across a particular sample single spot were integrated. Then, the SPRI signal was integrated over the spot area. National Institutes of Health (NIH) ImageJ version 1.32 software was used to evaluate the SPRI images in two-dimensional form and to convert numerical signal to quantitative signal (AU).

ELISA measurements

The determination of UCH-L1 concentration in blood plasma and cerebrospinal fluid was performed using a human UCH-L1 enzyme-linked immunoassay of the ELISA kit (USCN Life Science) according to the manufacturer's instructions.

Preparation of biological samples

Blood (2 ml) was centrifuged (1000g) for more than 15 min and filtered three times for blood plasma separation from hemoglobin.

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Cerebrospinal fluid (1.0 ml) was centrifuged (5000*g*) for more than 10 min. The supernatant was used for analysis. The samples were frozen and stored at −70 °C. The prepared plasma of blood or cerebrospinal fluid was transferred onto the sensor surface for 10 min at room temperature. After interaction, the surface of the biosensor was washed two times with HBS–ES buffer and six times with distilled water. The concentration was evaluated using a UCH-L1 calibration curve.

Statistical analysis

Student's *t* test was used for statistical analysis. All results are given as the means ± confidence limits. A value of $P < 0.05$ was considered significant.

Results and discussion

Optimization of receptor concentration

The purpose of this investigation was to find the best conditions for UCH-L1 concentration determination.

Optimization of antibody concentration

The experiment was performed within an antibody concentration range of 1 to 15 μg/ml at a constant UCH-L1 concentration (2 ng/ml). Nine antibody solutions of increasing concentration were used (1, 2, 4, 6, 8, 9, 10, 12, and 15 μg/ml). The antibody solutions were transferred onto the chip surface modified by cysteamine. Next, the chip was treated with the UCH-L1 solution for 10 min, rinsed with HBS–ES buffer and water (at least 10 times), and dried, and then the SPRI measurement was performed. The results are given in Fig. 2. The obtained curves are of the Langmuir isotherm type. The plateau of the signal is observed for antibody concentrations above 10.0 μg/ml (see Fig. 2, curve a).

Optimization of inhibitor concentration

To select LDN-57444 inhibitor concentration, the investigation was performed at a constant UCH-L1 concentration (2 ng/ml). A series of nine inhibitor solutions of increasing concentration was used (0.05, 0.1, 0.5, 0.8, 1.0, 2.0, 5.0, 8.0, and 10.0 μg/ml). The inhibitor solutions were transferred onto the chip, which had previously been modified with ODM. The chip was then treated with the UCH-L1 solution for 10 min. After the interaction, the chip was rinsed with HBS–ES buffer and water (at least 10 times) and dried, and then the SPRI measurement was performed. The results are given in Fig. 2.The plateau of the signal is observed for inhibitor concentrations above 2.0 μg/ml (see Fig. 2, curve b). The concentration of antibody equal to 10.0 μg/ml and the concentration of inhibitor equal to 2.0 μg/ml were selected as the optimal concentrations for further investigation.

Influence of pH solution on interaction process

The influence of the solution pH on the SPRI signal was studied for nine different pH values within the range of 5.0 to 9.5. The experiments were performed at the optimal concentration of antibody (10.0 μg/ml) or inhibitor (2.0 μg/ml) and constant UCH-L1 concentration (2.0 ng/ml). The measurements were carried out at a constant ionic strength solution (0.16 mol/L).

The results are also shown in Fig. 2. To compare the results obtained with both biosensors, both dependences are shown in Fig. 3. For both biosensors, the maximum of the SPRI signal is in a similar range. However, a slight difference in the maximum is observed. The maximum of the SPRI signal is in the range of pH 7.0 to 7.4 for the biosensor with the antibody and pH 7.4 to 8.0 for the biosensor with inhibitor. In the case of both biosensors, the value of pH 7.4 was selected as optimal for further investigation.

In further studies a PBS solution was used, with a constant ionic strength (0.16 mol/L) and ionic composition similar to human body fluids. The difference in the position of maximum range for the biosensors may be caused by another mechanism of the interaction between antibody and UCH-L1 or between inhibitor and UCH-L1.

Analytical response of biosensors to UCH-L1 concentration calibration curves

The response of the analytical SPRI signal of both biosensors for UCH-L1 concentration was measured within an UCH-L1 concentration range of 0.1 to 5.0 ng/ml. The chip surface of the biosensor with antibody was covered with a monolayer of cysteamine and a layer of immobilized antibody formed at constant antibody concentration (10.0 μg/ml). The chip surface of the biosensor with inhibitor was covered with a monolayer of ODM and immobilized constant inhibitor concentration (2.0 μg/ml). The experiments were performed at pH 7.4. Interaction time was 10 min for both biosensors. The obtained calibration curves are shown in Fig. 4.

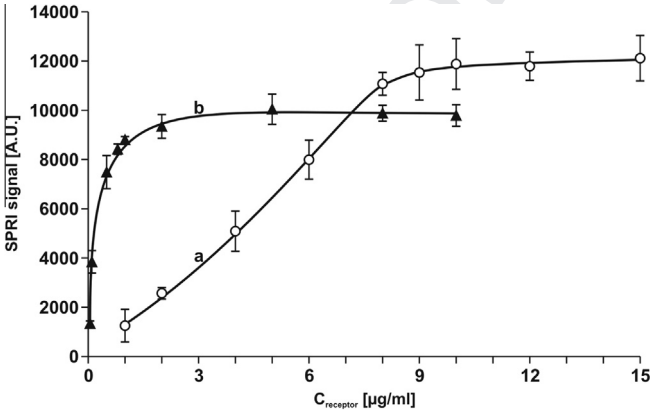

Fig.2. Dependence of SPRI signal (arbitrary units, A.U.) of receptor–UCH-L1 complex on antibody concentration (a) and on UCH-L1 inhibitor (b). UCH-L1 concentration: 2 ng/ml. Initial pH of UCH-L1 solution: 7.4. Error bars were calculated for 12 independent measurements for every concentration at a 95% confidence level.

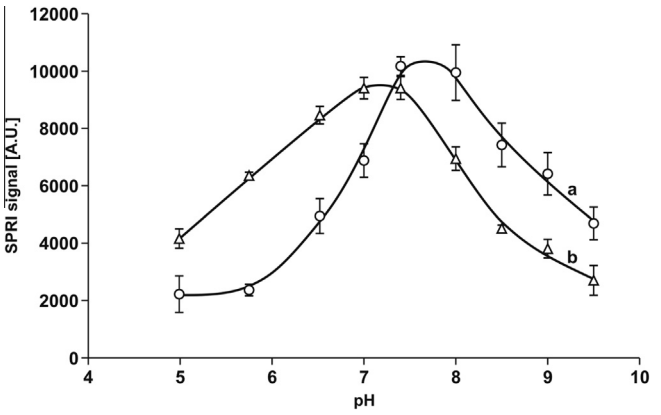

Fig.3. Dependence of SPRI signal (arbitrary units, A.U.) of the biosensor with antibody (a) and the biosensor with inhibitor (b) on pH. Antibody concentration (a): 10.0 μg/ml. Inhibitor concentration (b): 2.0 μg/ml. UCH-L1 concentration: 2.0 ng/ml. Error bars were calculated for 12 independent measurements for every concentration at a 95% confidence level.

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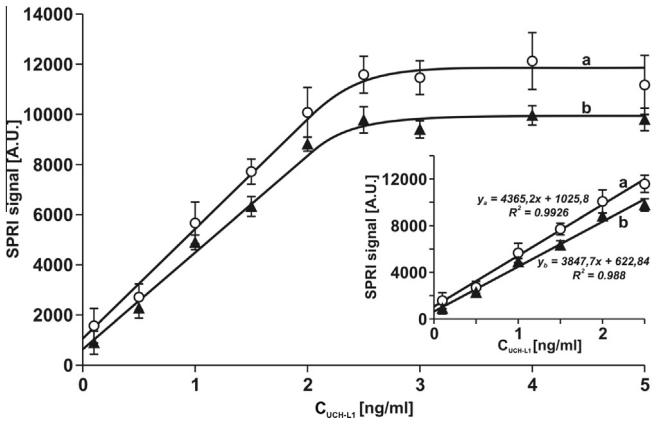

Fig.4. Dependence of SPRI signal (arbitrary units, A.U.) on UCH-L1 protein concentration for the biosensor with antibody (a) and the biosensor with inhibitor (b). Linear ranges are shown in the inset. Antibody concentration (a): 10.0 μg/ml. Inhibitor concentration (b): 2.0 μg/ml. Initial pH value of solutions: 7.4. Error bars were calculated for 12 independent measurements for every concentration at a 95% confidence level.

The calibration curves represent a Langmuir isotherm type with a dynamic response range of 0.1 to 2.0 ng/ml, which is useful for analytical purposes. The plateau of the curve corresponds to saturation of the sensor active points. The linearity of the curve within the initial section is due to the fact that the Langmuir isotherm turns into the linear Henry isotherm for a low degree of coverage of the surface.

The detection limit, calculated on 3 standard deviations (SD) of the blank (PBS buffer) basis, is equal to 0.06 ng/ml for the biosensor with antibody and 0.08 ng/ml for the biosensor with inhibitor. The limit of quantification (10 SD) is equal to 0.19 ng/ml for the biosensor with antibody and 0.29 ng/ml for the biosensor with inhibitor. Linear range between 0.1 and 2.0 ng/ml can be used for determination of UCH-L1 concentration.

According to a manufacturer's data, the calibration curve of the ELISA kit is within the range of 0.156 to 10.00 ng/ml. The minimum detectable dose of UCH-L1 typically is less than 0.053 ng/ml.

Selectivity of method

To check whether the interaction between UCH-L1 and antibody or inhibitor is selective and the particular sensor does not react with the other proteins, the chip with immobilized antibody or inhibitor was treated with mixtures of UCH-L1 and potential interferents. Human albumin, glycoprotein GPIIb/IIIa, and cathepsin B as potential interferents were used. Albumin is commonly present in body fluids and tissues in a large amount, glycoprotein IIb/IIIa as UCH-L1 belongs to a group of adhesion proteins, and cathepsin B and UCH-L1 are members of the protease family. Therefore, they are good proteins to test biosensor specificity. Various excesses of albumin, glycoprotein GPIIb/IIIa, and cathepsin B in mixture with UCH-L1 (2.0 ng/ml) within a range of 1:1 to 1:10,000 were examined. The results are shown in Table 1. No influence of albumin, glycoprotein GPIIb/IIIa, or cathepsin B on the results of determined UCH-L1 concentration, even at a 1000-fold excess of concentration of interfering proteins, was observed. Thus, high selectivity of both biosensors was confirmed.

Precision and accuracy of method for determination of UCH-L1 concentration

Precision of the method for standard UCH-L1 solution was tested under the optimal conditions. The pH of solutions was 7.4 for both biosensors. The antibody concentration was 10.0 μg/ml, and the inhibitor concentration was 2.0 μg/ml. The precision was tested for the UCH-L1 concentrations 0.5, 1.5, and 2.0 ng/ml. Measurements were performed using 3×12 measuring points (three places with 12 free gold points). The results are shown in Table 2. In general, the effect of relatively poor precision of a single measurement is compensated by the large number of repetitions because the sensor is an array of 12 analyzed spots. The precision of average values, as well as confidence limits, is acceptable. Therefore, recoveries of spikes are good and appear in the range of 99 to 104% for the biosensor with antibody as receptor and 100 to 101% for the biosensor with inhibitor as receptor.

Recovery of the developed methods was checked by the addition of 0.2 ng/ml standard solution of UCH-L1 into nine different samples of human cerebrospinal fluid. The recoveries for both biosensors are within the range of 95 to 105%. The differences of determined concentrations were not statistically significant at $P < 0.05$.

Determination of UCH-L1 concentration in biological samples

To verify the suitability of the developed methods for the analysis of natural samples, nine human blood plasma and nine cerebrospinal fluid samples from healthy volunteers were analyzed. Blood plasma of healthy humans is known to contain especially low levels of UCH-L1, and it was decided to be used as a negative control. Cerebrospinal fluid is abundant in UCH-L1 protein, and thus it served as a positive control [10,38]. The results of measurements were evaluated on the basis of a calibration curve (Fig. 3). To validate the developed method, comparative determination of UCHL-1 concentration by the commercial ELISA kit was performed. The results are shown in Table 3 for blood plasma samples and in Table 4 for cerebrospinal fluid samples.

Table 1
Influence of human albumin, glycoprotein IIb/IIIa, and cathepsin B on protein UCH-L1 concentration determination using antibody and inhibitor.

Protein	C_{UCH-L1}:$C_{protein}$	Found C_{UCH-L1} (ng/ml)		Recovery (%)	
		Antibody	Inhibitor	Antibody	Inhibitor
Albumin	1:1	2.05 ± 0.25	2.04 ± 0.20	102	102
	1:100	1.88 ± 0.08	2.01 ± 0.17	94	100
	1:1000	2.02 ± 0.27	2.01 ± 0.08	101	100
Glycoprotein GPIIb/IIIa	1:1	1.86 ± 0.20	2.00 ± 0.15	93	100
	1:100	2.01 ± 0.10	2.09 ± 0.13	100	104
	1:1000	2.02 ± 0.25	2.03 ± 0.10	101	102
Cathepsin B	1:1	2.10 ± 0.16	2.10 ± 0.12	105	105
	1:100	2.12 ± 0.20	2.00 ± 0.11	106	100
	1:1000	2.10 ± 0.14	2.05 ± 0.09	105	102

Note. The concentration of protein UCH-L1 used was 2.0 ng/ml.

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Table 2
Precision of UCH-L1 concentration measurement of protein.

	Number of measurements	C_{UCH-L1} added (ng/ml)	C_{UCH-L1} found (ng/ml)	Recovery (%)	Confidence limit (95%) (ng/ml)
Biosensor with antibody	36	0.50	0.52 ± 0.09	104	0.06
		1.50	1.52 ± 0.16	101	0.12
		2.00	1.98 ± 0.17	99	0.13
Biosensor with inhibitor	36	0.50	0.50 ± 0.16	100	0.06
		1.50	1.50 ± 0.32	100	0.13
		2.00	2.02 ± 0.34	101	0.14

Table 3
Concentration of UCH-L1 protein in human blood plasma measured by method of SPRI and ELISA.

Sample number	Protein UCH-L1 concentration (ng/ml)		
	SPRI		ELISA
	Antibody biosensor	Inhibitor biosensor	
1	0.19 ± 0.02	0.18 ± 0.03	0.32 ± 0.06
2	0.18 ± 0.03	0.19 ± 0.02	0.21 ± 0.03
3	0.14 ± 0.02	0.16 ± 0.01	0.19 ± 0.03
4	0.13 ± 0.01	0.11 ± 0.01	0.17 ± 0.02
5	0.00 ± 0.00	0.00 ± 0.00	0.06 ± 0.02
6	0.20 ± 0.03	0.19 ± 0.02	0.20 ± 0.02
7	0.07 ± 0.01	0.03 ± 0.01	0.09 ± 0.03
8	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00
9	0.14 ± 0.03	0.13 ± 0.01	0.18 ± 0.03
Mean value ± confidence limit	0.12 ± 0.05	0.11 ± 0.05	0.16 ± 0.06
Range	0.00–0.20	0.00–0.19	0.00–0.32
Literature range	0.00–0.19 [10,38]		

The comparison of individual results for separated samples obtained by the SPRI technique with the use of two different biosensors—antibody and inhibitor—indicates that they are very close. The results for human plasma vary from 0.00 to 0.20 ng/ml for the biosensor with antibody and from 0.00 to 0.19 ng/ml for the biosensor with inhibitor. The results for cerebrospinal fluid vary from 0.30 to 0.48 ng/ml for the biosensor with antibody and from 0.30 to 0.49 ng/ml for the biosensor with inhibitor. In general, the same ranges for both biosensors were obtained. It is worth stressing that each biosensor represents a different method for determination, taking into account differences in the mechanism of UCH-L1 entrapment from solution. This significantly enhances the credibility of the obtained results. The slight deviations may be caused by differences in the mechanism of interaction of UCH-L1 with the receptor layer.

ELISA is the most common method for quantitative detection of biomarker in biological samples. The comparison of the ELISA test and the two developed SPRI biosensors for assessing concentration of UCH-L1 value was performed. The results obtained from ELISA are slightly higher than those obtained using the two different SPRI methods. However, the ranges of values for ELISA (0.00–0.32 ng/ml in human plasma and 0.25–0.63 ng/ml in cerebrospinal fluid) and for SPRI (0.00–0.20 ng/ml in human plasma and 0.30–0.49 ng/ml in cerebrospinal fluid) are not significantly different and are comparable to literature data [10,38]. The individual characteristics of respondents could influence the concentration of UCH-L1 in body fluids.

The two developed biosensors jointly with SPRI measurement provided alternative approaches to measure UCH-L1 content in humans. SPRI measurements require very low quantities of reagents and samples (2–3 μl) in comparison with ELISA (usually 100 μl). This method does not require labeling or addition of a second reagent and does not require multiple washing steps. It allows measurements of UCH-L1 concentration with sufficient precision and high specificity of the assay.

In comparing the dynamic response ranges of the SPRI (0.1–2 ng/ml) and ELISA (0.156–10 ng/ml) methods, it is clear that the range of ELISA is wider. However, the data based on literature

Table 4
Concentration of UCH-L1 in human cerebrospinal fluid measured by method of SPRI and ELISA.

Sample number	Protein UCH-L1 concentration (ng/ml)		
	SPRI		ELISA
	Antibody biosensor	Inhibitor biosensor	
1	0.31 ± 0.01	0.32 ± 0.02	0.43 ± 0.04
2	0.39 ± ± 0.03	0.35 ± 0.02	0.63 ± 0.06
3	0.39 ± 0.02	0.40 ± 0.03	0.45 ± 0.02
4	0.42 ± 0.01	0.39 ± 0.01	0.41 ± 0.03
5	0.49 ± 0.04	0.45 ± 0.02	0.61 ± 0.05
6	0.32 ± 0.02	0.30 ± 0.02	0.39 ± 0.02
7	0.30 ± 0.01	0.30 ± 0.03	0.25 ± 0.01
8	0.48 ± 0.05	0.49 ± 0.02	0.52 ± 0.05
9	0.35 ± 0.02	0.37 ± 0.01	0.44 ± 0.03
Mean value ± confidence limit	0.38 ± 0.05	0.37 ± 0.04	0.46 ± 0.08
Range	0.30–0.48	0.30–0.49	0.25–0.63
Literature range	0.23–0.72 [10,38]		

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and the data of this research indicate that it can be concluded that for the samples from healthy individuals, the dynamic range for SPRI is quite sufficient. In the case of samples coming from different kinds of lesions, the appropriate dilutions must be used both in the case of the method with antibody and in the case of the method with inhibitor.

Due to the fact that different surface modifications and bioanalytical procedures for ELISA and SPRI are used, additional experimental parameters must be taken into account. This may limit the actual analytical comparison of these techniques. In general, in terms of UCH-L1 levels, a quite good correlation between SPRI and ELISA was found.

However, the developed SPRI biosensors, in comparison with ELISA, proved to be rapid, valuable, and reproducible tools for the quantitative determination of UCH-L1 in biological samples. The ease of sample preparation, simplicity of analytical procedures of SPRI, and analysis in real time are key features that could make this technology applicable in laboratory diagnostics.

Conclusions

Two array biosensors cooperating with the SPRI technique for the selective determination of ubiquitin carboxy-terminal hydrolase L1 concentration have been developed. One sensor works on the basis of a highly selective interaction between immobilized antibody and UCH-L1 protein in solution, whereas the other sensor uses a selective interaction between immobilized inhibitor and UCH-L1 in solution. Under the optimized conditions, both biosensors exhibit specificity to UCH-L1. The precision and accuracy of the developed method is well-suited to the enzyme determination. To validate the proposed methods, a comparison of the results obtained with the two SPRI methods and the ELISA test was performed. Both SPRI methods and ELISA gave similar results. The advantages of SPRI biosensors could make this method applicable in laboratories for quantitative determinations.

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References

[1] R.Z. Orlowski, The role of the ubiquitin–proteasome pathway in apoptosis, Cell Death Differ. 6 (1999) 303–313.
[2] A. Ciechanover, The ubiquitin–proteasome pathway: on protein death and cell life, EMBO J. 17 (1998) 7151–7160.
[3] K.D. Wilkinson, K. Lee, S. Deshpande, P. Duerksen-Hughes, J.M. Boss, J. Pohl, The neuron-specific protein PGP 9.5 is a ubiquitin carboxyl-terminal hydrolase, Science 246 (1989) 670–673.
[4] C.N. Larsen, J.S. Price, K.D. Wilkinson, Substrate binding and catalysis by ubiquitin C-terminal hydrolases: identification of two active site residues, Biochemistry 35 (1996) 6735–6744.
[5] C. Das, Q.Q. Hoang, C.A. Kreinbring, S.J. Luchansky, R.K. Meray, S.S. Ray, P.T. Lansbury, D. Ringe, G.A. Persko, Structural basis for conformational plasticity of the Parkinson's disease-associated ubiquitin hydrolase UCH-L1, Proc. Natl. Acad. Sci. U.S.A. 103 (2006) 4675–4680.
[6] H. Osaka, Y.L. Wang, K. Takada, S. Takizawa, R. Setsuie, H. Li, Y. Sato, K. Nishikawa, Y.J. Sun, M. Sakurai, T. Harada, Y. Hara, I. Kimura, S. Chiba, K. Namikawa, H. Kiyama, M. Noda, S. Aoki, K. Wada, Ubiquitin carboxy-terminal hydrolase L1 binds to and stabilizes monoubiquitin in neuron, Hum. Mol. Genet. 12 (2003) 1945–1958.
[7] S. Hussain, Y. Zhang, P.J. Galardy, DUBs and cancer: the role of deubiquitinating enzymes as oncogenes, non-oncogenes, and tumor suppressors, Cell Cycle 8 (2009) 1688–1697.
[8] K.S. Orr, Z. Shi, W.M. Brown, K.A. O'Hagan, T.R. Lappin, P. Maxwell, M.J. Percy, Potential prognostic marker ubiquitin carboxyl-terminal hydrolase-L1 does not predict patient survival in non-small cell lung carcinoma, J. Exp. Clin. Cancer Res. 30 (2011) 79.
[9] S. Mondello, A. Linnet, A. Buki, S. Robicsek, A. Gabrielli, J. Tepas, L. Papa, G.M. Brophy, F. Tortella, R.L. Hayes, K.K. Wang, Clinical utility of serum levels of ubiquitin C-terminal hydrolase as a biomarker for severe traumatic brain injury, Neurosurgery 70 (2012) 666–675.
[10] G.M. Brophy, S. Mondello, L. Papa, S.A. Robicsek, A. Gabrielli, J. Tepas III, A. Buki, C. Robertson, F.C. Tortella, R.L. Hayes, K.K. Wang, Biokinetic analysis of ubiquitin C-terminal hydrolase-L1 (UCH-L1) in severe traumatic brain injury patient biofluids, J. Neurotrauma 28 (2011) 861–870.
[11] R.P.R. Berger, R.L.R. Hayes, R.R. Richichi, S.R.S. Beers, K.K. Wang, Serum concentrations of ubiquitin C-terminal hydrolase-L1 and αII-spectrin breakdown product 145 kDa correlate with outcome after pediatric TBI, J. Neurotrauma 29 (2012) 162–167.
[12] J. Choi, A.I. Levey, S.T. Weintraub, H.D. Rees, M. Gearing, L.S. Chin, L. Li, Oxidative modifications and down-regulation of ubiquitin carboxyl-terminal hydrolase L1 associated with idiopathic Parkinson's and Alzheimer's diseases, J. Biol. Chem. 279 (2004) 13256–13264.
[13] M. Facheris, K.J. Strain, T.G. Lesnick, M. de Andrade, J.H. Bower, J.E. Ahlskog, J.M. Cunningham, S. Lincoln, M.J. Farrer, W.A. Rocca, D.M. Maraganore, UCHL1 is associated with Parkinson's disease: a case-unaffected sibling and case-unrelated control study, Neurosci. Lett. 381 (2005) 131–134.
[14] J.H. Kennedy, L.S. Chin, L. Li, Ubiquitin C-terminal hydrolase L1 in tumorigenesis, Biochem. Res. Int. (2012) 123706, http://dx.doi.org/10.1155/2012/123706.
[15] Y. Fang, D. Fu, X.Z. Shen, The potential role of ubiquitin C-terminal hydrolases in oncogenesis, Biochim. Biophys. Acta 2010 (1806) 1–6.
[16] H.J. Kim, Y.M. Kim, S. Lim, Y.K. Nam, J. Jeong, K.J. Lee, Ubiquitin C-terminal hydrolase-L1 is a key regulator of tumor cell invasion and metastasis, Oncogene 28 (2008) 117–127.
[17] B. Seliger, A. Fedorushchenko, W. Brenner, A. Ackermann, D. Atkins, S. Hanash, R. Lichtenfels, Ubiquitin COOH-terminal hydrolase 1: a biomarker of renal cell carcinoma associated with enhanced tumor cell proliferation and migration, Clin. Cancer Res. 13 (2007) 27E–37E.
[18] T. Yamazaki, K. Hibi, T. Takase, E. Tezel, H. Nakayama, Y. Kasai, K. Ito, S. Akiyama, T. Nagasaka, A. Nakao, PGP9.5 as a marker for invasive colorectal cancer, Clin. Cancer Res. 8 (2002) 192–195.
[19] E. Tezel, K. Hibi, T. Nagasaka, A. Nakao, PGP9.5 as a prognostic factor in pancreatic cancer, Clin. Cancer Res. 6 (2000) 4764–4767.
[20] Y. Liu, H. Wu, J. Wu, S. Wang, Y. Liu, Z. Zhao, X. Zhang, R. Li, M. Guo, Z. Zhang, Detection of UCH-L1 expression by pre-embedding immunoelectron microscopy with colloidal gold labeling in diseased glomeruli, Ultrastruct. Pathol. 32 (2008) 5–9.
[21] A. Bheda, J. Shackelford, J.S. Pagano, Expression and functional studies of ubiquitin C-terminal hydrolase L1 regulated genes, PLoS ONE 4 (2009) e6764.
[22] A.E. Cartier, S.N. Djakovic, A. Salehi, S.M. Wilson, E. Masliah, G.N. Patrick, Regulation of synaptic structure by ubiquitin C-terminal hydrolase L1, J. Neurosci. 29 (2009) 7857–7868.
[23] T. Xiang, L. Li, X. Yin, C. Yuan, C. Tan, X. Su, L. Xiong, T.C. Putti, M. Oberst, K. Kelly, G. Ren, Q. Tao, The ubiquitin peptidase UCHL1 induces G0/G1 cell cycle arrest and apoptosis through stabilizing p53 and is frequently silenced in breast cancer, PLoS ONE 7 (2012) e29783.
[24] T. Frisan, G. Coppotelli, R. Dryselius, M.G. Masucci, Ubiquitin C-terminal hydrolase-L1 interacts with adhesion complexes and promotes cell migration, survival, and anchorage independent growth, FASEB J. 26 (2012) 5060–5070.
[25] F.I. Andersson, S.E. Jackson, S.D. Hsu, Backbone assignments of the 26 kDa neuron-specific ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1), Biomol. NMR Assign. 4 (2010) 41–43.
[26] E. Helmerhorst, D.J. Chandler, M. Nussio, C.D. Mamotte, Real-time and label-free bio-sensing of molecular interaction by surface plasmon resonance: a laboratory medicine perspective, Clin. Biochem. Rev. 33 (2012) 161–173.
[27] H.J. Lee, D. Nedelkov, R.M. Corn, Surface plasmon resonance imaging measurements of antibody arrays for the multiplexed detection of low molecular weight protein biomarkers, Anal. Chem. 78 (2006) 6504–6510.
[28] E. Gorodkiewicz, H. Ostrowska, A. Sankiewicz, SPR imaging biosensor for the 20S proteasome: sensor development and application to measurement of proteasomes in human blood plasma, Microchim. Acta 175 (2011) 177–184.
[29] E. Gorodkiewicz, A. Sankiewicz, P. Laudański, Surface plasmon resonance imaging biosensors for aromatase based on a potent inhibitor and a specific antibody: sensor development and application for biological material, Cent. Eur. J. Chem. 12 (2014) 557–587.
[30] J. Ladd, A.D. Taylor, M. Piliarik, J. Homola, S. Jiang, Label-free detection of cancer biomarker candidates using surface plasmon resonance imaging, Anal. Bioanal. Chem. 393 (2009) 1157–1163.
[31] J. Homola, S. Yee, G. Gauglitz, Surface plasmon resonance sensors: review, Sens. Actuat. B 54 (1999) 3–15.
[32] G. Steiner, Surface plasmon resonance imaging, Anal. Bioanal. Chem. 379 (2004) 328–331.
[33] Y. Liu, H.A. Lashuel, S. Choi, X. Xing, A. Case, J. Ni, L.A. Yeh, G.D. Cuny, R.L. Stein, P.T. Lansbury Jr., Discovery of inhibitors that elucidate the role of UCH-L1 activity in the H1299 lung cancer cell line, Chem. Biol. 10 (2003) 837–846.
[34] E. Gorodkiewicz, Surface plasmon resonance imaging sensor for cathepsin determination based in immobilized cystatin, Protein Pept. Lett. 16 (2009) 1379–1385.
[35] E. Gorodkiewicz, M. Sieńczyk, E. Regulska, R. Grzywa, E. Pietrusewicz, A. Lesner, Z. Łukaszewski, Surface plasmon resonance imaging biosensor for cathepsin G based on a potent inhibitor: development and applications, Anal. Biochem. 423 (2012) 218–223.

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[36] E. Gorodkiewicz, E. Regulska, SPR imaging biosensor for aspartyl cathepsins: sensor development and application for biological material, Protein Pept. Lett. 17 (2010) 1148–1154.
[37] B. Johnsson, S. Lofas, G. Lindquist, Immobilization of proteins to a carboxymethyldextran-modified gold surface for biospecific interaction analysis in surface plasmon resonance sensors, Anal. Biochem. 198 (1991) 268–277.
[38] S. Mondello, J. Palmio, J. Streeter, R.L. Hayes, J. Peltola, A. Jeromin, Ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) is increased in cerebrospinal fluid and plasma of patients after epileptic seizure, BMC Neurol. 12 (2012) 85.

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