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Two SPRi biosensors for the determination of cathepsin S in blood plasma

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ABSTRACT

Cathepsin S is an emerging marker for ovarian cancer. Two 'analytically specific' SPRi biosensors for the determination of Cath S have been developed. The reception part of one of the biosensors consists of the rat monoclonal antibody specific for cathepsin S attached to the gold surface via covalent bonds with cysteamine linker, while the second biosensor consists of the inhibitor LY3000328 attached via hydrophobic interaction with the 1-octadecanothiol linker. Under optimized conditions, in terms of pH and receptor concentration, both biosensors have linear response ranges between LOQ (0.14 ng mL^{-1}) and 2.5 ng mL^{-1}, which is suitable for the determination of Cath S in blood plasma samples of ovarian cancer patients and healthy individuals, after corresponding dilution with 0.15 M PBS buffer.

Precision and recoveries are quite acceptable: below 7% and 98–101% respectively for the biosensor with antibody, and below 12% and 101–103% for the biosensor with inhibitor. The biosensors were validated by the determination of Cath S in series of plasma from ovarian cancer patients and healthy volunteers using both biosensors and ELISA, giving Pearson coefficients close to 1. Plasma Cath S concentration can be used as an ovarian cancer marker, in view of the highly elevated concentrations detected.

1. Introduction

Cathepsin S is a cysteine protease that is strongly expressed in malignant tissues [1]. High Cath S concentrations have been found in the tissue of colon cancer [2], lung cancer [3], breast cancer [4], prostate cancer [5], melanoma [6], thymoma [7] and hepatocellular carcinoma [8]. Therefore, Cath S concentration can be expected to be elevated in the serum or plasma of cancer patients, and it appears to be an emerging cancer marker. Elevated Cath S concentration was found in the serum of hepatocellular carcinoma patients, and this concentration was correlated with tumour size and extrahepatic metastasis [9].

Cath S is one of 11 cysteine cathepsin proteases. It consists of 217 amino acids and has an MW of 23.7 kDa [10,11]. Cath S shows optimal activity at pH values of 6.50–7.50 [12–15]. Reported serum or plasma Cath S concentration data for healthy volunteers are highly divergent, ranging from 2.11 to 5.23 ng mL^{-1} [16] to 39–102 ng mL^{-1} [17]. This value should be determined more precisely, because it will serve as a reference level for Cath S as a potential cancer marker. So far, the only method used for Cath S determination has been the sandwich enzyme-linked immunosorbent assay (ELISA). There is a need for the development of new methods for Cath S determination.

Surface Plasmon Resonance Imaging (SPR*i*) is a relatively new, label-free, quick and inexpensive technique. It combines an SPR method with spatially resolved measurement [18,19]. The basis of determinations using the SPRi method is that it uses a specific interaction between the antibody and the antigen or inhibitor and the enzyme. The SPR signal reacts to mass increase with a change in the wavelength and polarization angle. Then, in the SPRi technique, this signal is converted into an image. The biosensor contains a specific antibody [20,21] or inhibitor [22] immobilized on the surface, which gives an analytical signal through specific reactions with an appropriate analyte. A biosensor can be created *in situ* for use with fluidic measurement [23] or *ex situ* in the form of an array of measuring points for use with stationary dry measurement [24]. SPRi biosensors for the determination of other cathepsins such as cathepsin B, D, E, G and L have been developed [25–28]. The development of a biosensor for the determination of cathepsin S

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supplements this series.

The aim of the present work was to develop a SPRi biosensor for the determination of Cath S in blood plasma or serum. Two potential designs of biosensor were considered, containing an antibody against Cath S or a Cath S-specific inhibitor in the sensing part of the biosensor. The antibody against Cath S can be immobilized via cysteamine linker using the EDS/NHS protocol. The amine group of cysteamine reacts with the carboxyl group of the Fc region of the antibody, and an antigen-binding paratope is located on the top of the immunosensor. Rat monoclonal antibody specific for cathepsin S was selected for this purpose. The inhibitor LY3000328 specific for cathepsin S [29] was chosen for the construction of an alternative biosensor. Due to the absence of a carboxy group in the inhibitor structure, a linker, 1-octadecanothiol, was used, forming hydrophobic interactions with the inhibitor for immobilization. Initial experiments show that both solutions may be successful. Therefore, both of the biosensors were developed. Plasma samples from patients with ovarian cancer and healthy volunteers were selected for validation of the developed methods. Cath S has not previously been determined in the plasma of patients with ovarian cancer.

2. Experimental

2.1. Materials and reagents

Cathepsin S protein and rat monoclonal antibody specific for cathepsin S (R&D Systems, USA), inhibitor LY3000328 specific for cathepsin S, (3R,4S)-4-(4-fluorobenzamido)-6-(4-(oxetan-3-yl)piperazin-1-yl)chroman-3-yl methylcarbamate**)** (APExBIO, USA), cysteamine hydrochloride, N-ethyl- N'-(3-dimethylaminopropyl) carbodiimide (EDC), human albumin (all SIGMA, Steinheim, Germany) and 1-octadecanothiol (ODM), N-hydroxysuccinimide (NHS) (ALDRICH, Munich, Germany) were used. The bases of the biosensors were chips covered with a layer of gold (Sens, Netherlands).

A complete list of materials and reagents is given in Appendix A.1.

2.2. Biological material

The plasma samples used for the tests came from donors from the Regional Blood Donation and Blood Treatment Center in Bialystok, and from patients with ovarian cancer from the Maria Sklodowska-Curie Oncology Center. All samples were provided after obtaining the consent of the Bioethical Commission.

2.3. Chip architecture

Gold chips were manufactured as described in a previous paper [30, 31]. The chip is an array of 108 active places. It has nine round measuring sites, each with 12 free gold surfaces. Using this chip, nine different solutions can be measured simultaneously without mixing the tested samples, and 12 separate SPRI measurements can be performed from a single 0.3 μL droplet of tested solution.

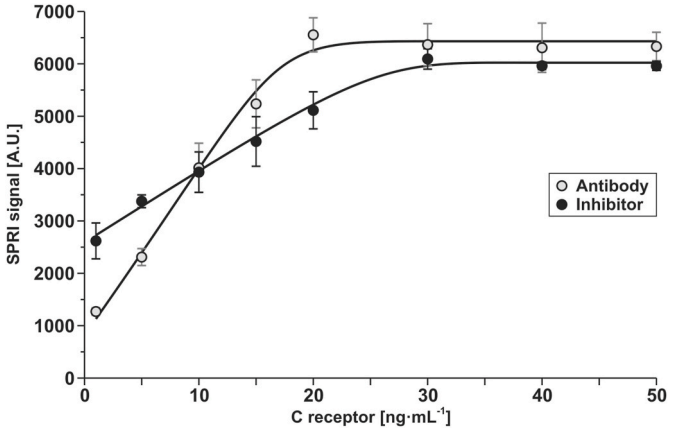

Fig. 1. Surface saturation curves. Concentration of Cath S = 5 ng mL^{-1}, pH = 7.4.

2.4. Procedures

2.4.1. Antibody immobilization

In the first stage of antibody immobilization, the gold chip was covered with cysteamine linker. Antibody immobilization was performed following the well-known EDS/NHS protocol, described in e.g. Ref. [30], with rat monoclonal antibody specific for cathepsin S. A detailed description of the antibody immobilization procedure is given in the Appendix A.2. The prepared biosensor is designed to be used directly for Cath S determination.

2.4.2. Inhibitor immobilization

In the first stage of immobilization of the inhibitor LY3000328, the gold chip was covered with 1-octadecanothiol linker. The linker ODM monolayer was obtained by immersing a gold-covered chip in 20 mM alcoholic ODM solution for 12 h. After this time, the chip was rinsed with absolute ethyl alcohol and redistilled water, and then dried under a stream of argon.

In the next step, the solution of inhibitor LY3000328 was applied to the biosensor's active sites and incubated for 24 h at 37 °C. The chip was washed several times with redistilled water. To eliminate non-specific adsorption, after incubation of the chip with the receptor, BSA solution with a concentration of 1 ng mL^{-1} was applied to the biosensor's active sites. The chip was washed again several times with redistilled water before testing, and dried under a stream of argon. The prepared biosensor is designed to be used directly for Cath S determination.

2.4.3. SPRi measurements

The SPRi device and the testing procedure are presented in a previous article [30]. A description of the SPRi apparatus is given in Appendix A.3. The SPRi signal reflects the quantity of coupled biomolecules within the optimal concentration range. The first stage of the measurements involved selection of the correct SPR angle. This was performed for the chip after immobilization of the receptor layer. The SPR signal was measured at a fixed SPR angle on the basis of registered images. After immobilization of the antibody or inhibitor on the biosensor surface, the first photograph was taken. Then Cath S solution was applied to the biosensor surface for 10 min. After interaction, the biosensor surface was washed with HBS-ES buffer and distilled water and dried under a stream of argon. After this, a second photograph was taken. 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. Evaluation of the SPR images in two-dimensional form and conversion of numerical signals to quantitative signal were performed using the National Institutes of Health (NIH) ImageJ software, version 1.32. Cath S concentration was evaluated using a calibration curve for cathepsin S.

2.4.4. ELISA measurements

The determination of cathepsin S concentration in blood plasma was performed using a human cathepsin S enzyme-linked immunoassay from an ELISA kit (Biomatic Corporation, Canada) according to the manufacturer's instructions.

2.4.5. Preparation of biological samples

Blood plasma was obtained from blood (2 mL) by separation from hemoglobin through centrifugation (1000 g) for 15 min and three subsequent filtrations. The samples were immediately frozen and stored at −70 °C. For Cath S determination, plasma samples from cancer patients were diluted 10 times with 0.15 M PBS buffer, while control plasma samples were diluted 5 times.

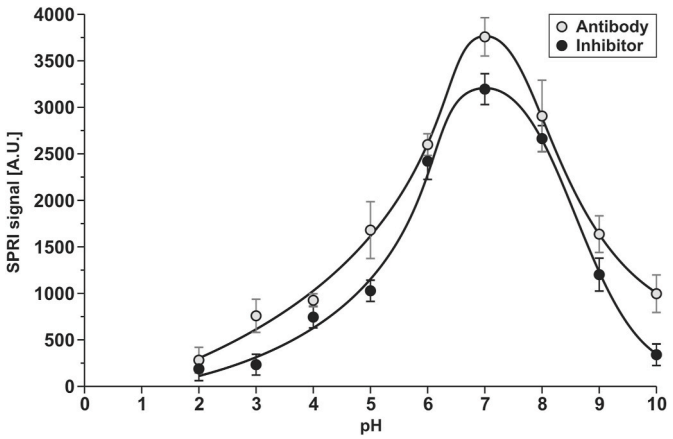

Fig. 2. Influence of pH solution on SPRi signal. Concentration of Cath S = 1 ng mL^{-1}, antibody = 20 ng mL^{-1}, inhibitor = 30 ng mL^{-1}.

2.4.6. Statistical analysis

Statistical analysis was performed using Student's *t*-test. All results are given as means ± confidence limits. A value of $P < 0.05$ was considered significant.

3. Results and discussion

3.1. Optimization of sensing element concentration

Sensing element concentration optimization experiments were performed at constant Cath S concentration and pH, equal to 5 ng mL^{-1} and 7.4 respectively, and in a sensing element concentration range from 1 to 60 ng mL^{-1}. Cath S solutions were prepared in 0.15 M PBS buffer. Experiments were performed as described in 2.4.1 and 2.4.3 with rat monoclonal antibody specific for cathepsin S as the sensing element, and as described in 2.4.2 and 2.4.3 with the inhibitor LY3000328 as the sensing element. The results are shown in Fig. 1.

The SPRi signal grows with an increase in sensing element concentration until saturation of the surface is reached. A further increase in antibody concentration above 20 ng mL^{-1} and inhibitor concentration above 30 ng mL^{-1} causes no further increase in the signal. Therefore, 20 ng mL^{-1} for the antibody and 30 ng mL^{-1} for the inhibitor were selected as optimal concentrations.

3.2. Influence of pH on SPRi signal

The influence of pH on the SPRi signal of Cath S for both biosensors was studied in a range from 2 to 10 in 0.15 M PBS buffer at a constant Cath S concentration of 1.0 ng mL^{-1}, and with an antibody concentration of 20.0 ng mL^{-1} or an inhibitor concentration of 30 ng mL^{-1}. The results are shown in Fig. 2.

Both biosensors exhibit a maximum of the SPRi signal for Cath S at pH 7.4.

3.3. Analytical response of biosensors to Cath S concentration. Calibration curves

The analytical response of both biosensors to changes in Cath S concentration was measured within a range of Cath S concentration between 0.05 and 4.0 ng mL^{-1}. Experiments were performed at pH 7.4 with an antibody concentration of 20 ng mL^{-1} or an inhibitor concentration of 30 ng mL^{-1}. Experiments using the biosensor with antibody were performed as described in 2.4.1 and 2.4.3, and with the inhibitor as described in 2.4.2 and 2.4.3.

The results are shown in Fig. 3. Gradual formation of successive layers on the biosensors was observed using an atomic force microscope. These images are given in Appendix A.4: Fig. A.4.1 for the biosensor with antibody and Fig. A.4.2 for the biosensor with inhibitor.

Both calibration curves exhibit a typical r-type shape with plateau above 2.5 ng mL^{-1} and an approximately linear initial section. The formation of a plateau is due to saturation of receptors on the biosensor's surface. These linear sections are suitable for analytical purposes. Concentrations above 2.5 ng mL^{-1} can be determined after dilution with 0.15 M PBS buffer. The limit of detection (3SD) is equal to 0.04 ng mL^{-1} for the biosensor with antibody and 0.13 ng mL^{-1} for the biosensor with inhibitor, while the limit of quantification (10SD) is equal to 0.14 ng mL^{-1} for the biosensor with antibody and 0.39 ng mL^{-1} for the biosensor with inhibitor. Thus, the dynamic response range of both biosensors is almost the same, lying approximately between 0.14 and 2.5 ng mL^{-1}, and can be extended by suitable dilution of the samples with 0.15 M PBS buffer.

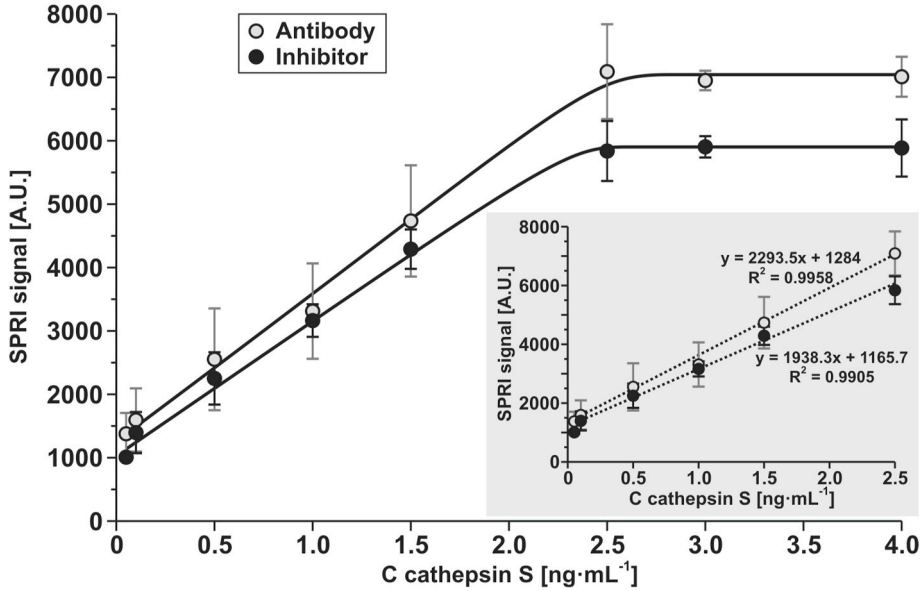

Fig. 3. Calibration curves. Concentration of antibody = 20 ng mL^{-1}, inhibitor = 30 ng mL^{-1}, pH = 7.4.

Table 1
Precision and accuracy of methods for determination of Cath S concentration (n = 36).

	ANTIBODY				INHIBITOR			
C_{added} [ng mL^{-1}]	C_{found} [ng mL^{-1}]	SD [ng mL^{-1}]	Recovery [%]	RSD [%]	C_{found} [ng mL^{-1}]	SD [ng mL^{-1}]	Recovery [%]	RSD [%]
0.10	0.11	0.04	111	36.60	0.12	0.08	120	66.66
1.00	0.98	0.07	98	7.14	1.03	0.12	103	11.65
2.50	2.52	0.04	101	1.58	2.51	0.07	101	2.78

Table 2
Analytical specificity of the developed biosensors.

			ANTIBODY		INHIBITOR	
Potential interferent	C_{CatS} vs. $C_{interferent}$	Added CC_{athS} [ng mL^{-1}]	Found CC_{atS} [ng mL^{-1}]	Recovery [%]	Found CC_{atS} [ng mL^{-1}]	Recovery [%]
Cathepsin B	[1:1]	0.50	0.51	102	0.52	104
	[1:10]		0.51	102	0.51	102
Cathepsin D	[1:1]		0.51	102	0.51	102
	[1:10]		0.49	98	0.52	104
Cathepsin E	[1:1]		0.50	100	0.51	102
	[1:10]		0.52	104	0.51	102
Cathepsin G	[1:1]		0.50	100	0.50	100
	[1:10]		0.50	100	0.51	102
Cathepsin L	[1:1]		0.51	102	0.52	104
	[1:10]		0.50	100	0.51	102
Human albumin	[1:1]		0.51	102	0.51	102
	[1:10]		0.51	102	0.51	102
	[1:100]		0.52	104	0.52	104

3.4. Precision and accuracy of the method for determination of Cath S concentration

Precision and accuracy (represented by recovery of the spike) were tested for three Cath S concentrations: 1.0 ng mL^{-1} (located in the middle of the calibration graph), 2.5 ng mL^{-1} (representing the highest concentration within the linearity range) and 0.1 ng mL^{-1} (located slightly below LOQ but above LOD). Experiments were performed under the optimized conditions defined above. The number of measurements was 3 × 12 for each concentration. The results are shown in Table 1.

Recoveries and precision for the 1.00 and 2.50 ng mL^{-1} spikes of Cath S are quite acceptable for the biosensor with antibody and worse for biosensor with inhibitor. For the 0.10 spike, located between LOD and LOQ, recoveries and precision are rather poor, showing that the LOQ is well-determined. Precision for the biosensor with inhibitor as a receptor is slightly inferior to that for the biosensor with antibody, although it is still acceptable.

3.5. Specificity of biosensor responses

'Analytical specificity' is a crucial factor for the successful application of developed biosensors, in view of the fact that blood serum or plasma contains hundreds of similar proteins and glycoproteins, and only Cath S should be determined in this mixture. The term 'specificity' has dual meaning, and in diagnostics may refer to the proportion of false negative results. To avoid such confusion, the terms 'analytical specificity' and 'diagnostic specificity' will be used. Human albumin and cathepsins B, D, E, G, L were used as potential interferents. Albumin is present in serum or plasma in high excess. The results are shown in Table 2. All recoveries are acceptable, showing the good analytical specificity of the developed biosensors.

No influence of albumin or cathepsins on the results for determined Cath S concentration was observed. Thus, the analytical specificity of both biosensors was confirmed.

Additional confirmation of the analytical specificity and acceptable precision and recovery of both developed biosensors was provided by experiments with blood plasma, which contains all of the potential interferents. Such experiments should also reveal a potential matrix effect, i.e. worse results than in the model investigation. Spikes of Cath S were added to two different plasma samples from healthy volunteers. The results for the biosensor with antibody are shown in Table 3, and those for the biosensor with inhibitor in Table 4. Surprisingly, the precision and recoveries of both biosensors are extremely good and superior to those obtained in the model experiments with spikes in 0.15 M PBS buffer. Thus, no negative matrix effect was observed. However, the main conclusion is that both biosensors exhibit analytical specificity, because any interference from the plasma matrix would significantly change the results.

Table 3
Recovery and precision of biosensor with antibody in real blood plasma sample.

ANTIBODY				
C0 [ng mL^{-1}]	C_{added} [ng mL^{-1}]	C_{found} [ng mL^{-1}]	Recovery [%]	RSD [%]
1.17	2.5	3.77 ± 0.13	102.7	3.4
1.14		3.66 ± 0.01	100.5	0.3
1.18		3.70 ± 0.03	100.5	0.8
1.12		3.63 ± 0.09	100.3	2.5
1.17		3.68 ± 0.01	100.3	0.3

C0 – concentration before standard addition.

Table 4
Recovery and precision of biosensor with inhibitor in real blood plasma sample.

INHIBITOR				
C_0 [ng mL^{-1}]	C_{added} [ng mL^{-1}]	C_{found} [ng mL^{-1}]	Recovery [%]	RSD [%]
1.94	2.5	4.45 ± 0.02	100.2	0.4
1.95		4.48 ± 0.03	100.7	0.7
1.91		4.44 ± 0.04	100.7	0.9
1.94		4.47 ± 0.04	100.7	0.9
1.91		4.42 ± 0.01	100.2	0.2
1.94		4.46 ± 0.05	100.5	1.1

C0 – concentration before standard addition.

3.6. Validation of developed biosensors. Cathepsin S determination in real samples

Validation of the developed biosensors was performed with nine

Table 5
Validation of developed biosensors. Comparison of results for blood plasma samples from patients with ovarian cancer and from healthy volunteers, as determined by biosensors with antibody or inhibitor as receptors, and by ELISA as the reference method.

Plasma from patients with ovarian cancer			
Number of sample	SPRi		ELISA
	ANTIBODY	**INHIBITOR**	
	Concentration [ng mL^{-1}]		
1A	16.89 ± 0.16	16.03 ± 0.44	16.95 ± 0.92
2A	13.01 ± 0.18	12.65 ± 0.31	13.78 ± 0.78
3A	9.64 ± 0.17	10.97 ± 0.34	3.94 ± 1.06
4A	17.79 ± 0.56	17.01 ± 0.41	17.86 ± 0.77
5A	10.57 ± 0.58	9.99 ± 0.13	10.88 ± 0.46
6A	7.73 ± 0.57	8.94 ± 0.45	8.29 ± 0.05
7A	13.97 ± 0.51	13.67 ± 0.40	13.87 ± 0.49
8A	8.93 ± 0.25	9.63 ± 0.35	10.09 ± 0.09
9A	15.71 ± 0.40	15.78 ± 0.20	10.23 ± 0.03
Plasma from healthy volunteers			
Number of sample	SPRi		ELISA
	ANTIBODY	INHIBITOR	
	Concentration [ng mL^{-1}]		
1	0.39 ± 0.03	0.41 ± 0.03	0.29 ± 0.03
2	0.93 ± 0.01	0.85 ± 0.02	1.06 ± 0.08
3	2.58 ± 0.03	2.49 ± 0.04	2.07 ± 0.04
4	1.95 ± 0.03	1.06 ± 0.03	9.13 ± 1.44
5	0.48 ± 0.03	0.45 ± 0.04	0.37 ± 0.06
6	1.16 ± 0.03	1.93 ± 0.02	1.16 ± 0.08
7	2.44 ± 0.04	2.94 ± 0.04	2.38 ± 0.39
8	2.38 ± 0.02	2.40 ± 0.03	4.17 ± 0.06
9	0.40 ± 0.02	0.43 ± 0.05	0.28 ± 0.04

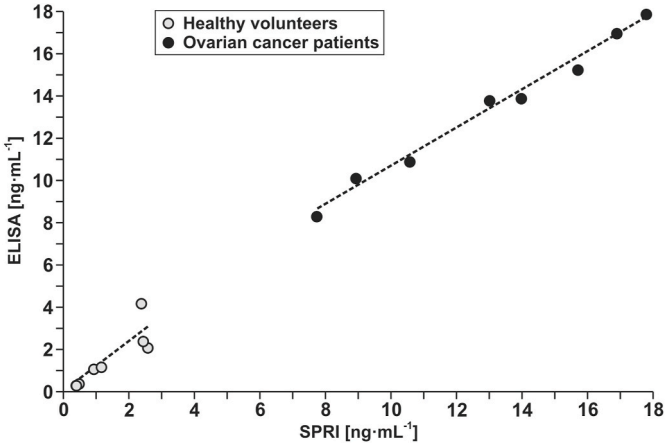

Fig. 4. Correlation of results for the SPRi biosensor with antibody as a sensing element and ELISA.

plasma samples from ovarian cancer patients and nine plasma samples from healthy volunteers, using ELISA as the reference method for Cath S determination. Samples of plasma were diluted with 0.15 M PBS buffer to fit the linear range of the biosensors. Taking into consideration that the developed biosensors use significantly different sensing parts, three independent methods were compared. The results are shown in Table 5.

Seven plasma samples for ovarian cancer patients and six plasma samples from healthy volunteers exhibit general agreement of the results obtained by the three methods. In four additional cases the results for both biosensors are in agreement, but the ELISA results are not. However, two of these ELISA results are obviously erroneous: one is too low (3.94) for ovarian cancer samples and the second (9.13) is too high for a healthy volunteer sample. Concluding, both biosensors have been validated. This conclusion is supported by analysis of the correlation of the results from the developed biosensor with antibody and the ELISA results, as shown in Fig. 4.

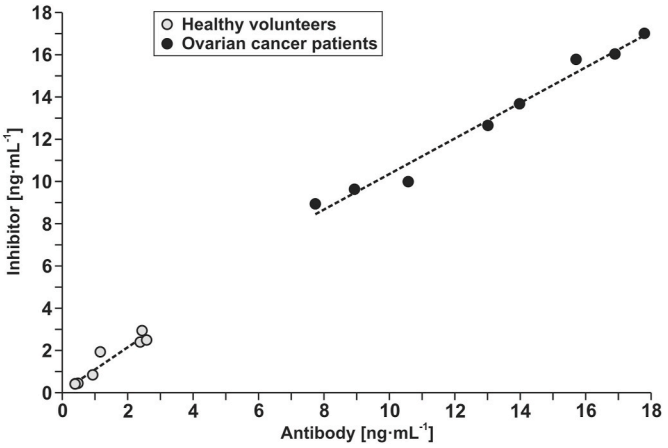

Fig. 5. Correlation of results for the developed biosensors.

The Pearson factor is equal to 0.996. A Pearson correlation coefficient close to 1 indicates agreement between the two compared methods.

Equally significant is the analysis of the correlation of results from both developed biosensors. This is shown in Fig. 5.

The Pearson factor is equal to 0.997. A Pearson correlation coefficient close to 1 indicates agreement between the two developed biosensors. Thus, both the biosensor with antibody and the biosensor with inhibitor are successfully validated.

An additional conclusion from the results given in Table 5 is that Cath S concentration in plasma samples from ovarian cancer patients is several times higher than in the plasma of healthy volunteers. Thus, plasma Cath S is an emerging marker of ovarian cancer. The results also contribute to the determination of the normal level of Cath S in a healthy population; they support the data of [16] (2–5 ng mL^{-1}) and contradict the data of [17] (2012) (40–100 ng mL^{-1}).

4. Conclusions

Two analytically specific SPRi biosensors for the determination of Cath S have been developed, having different structures in their reception parts. Under optimized conditions, both biosensors have linear response ranges suitable for the determination of Cath S in blood plasma samples of ovarian cancer patients and healthy individuals, as well as acceptable precision and recoveries. The biosensors were validated by the determination of Cath S in series of plasma from ovarian cancer patients and healthy volunteers using both biosensors and ELISA, obtaining Pearson coefficients close to 1. Plasma Cath S concentration can be used as an ovarian cancer marker.

CRediT author statement

Oldak L and Sankiewicz A: Data curation, Investigation, Formal analysis, Writing - original draft. Żelazowska-Rutkowska B.and Cylwik B. - ELISA measurements, Validation, Z. Lukaszewski: Writing - review & editing. Skoczylas M: Statistical processing of the results, E. Gorodkiewicz: Conceptualization, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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References

[1] B. Wang, J. Sun, S. Kitamoto, M. Yang, A. Grubb, H.A. Chapman, R. Kalluri, G.-P. Shi, Cathepsin S controls angiogenesis and tumor growth via matrix-derived angiogenic factors, J. Biol. Chem. 281 (2006) 6020–6029.
[2] A. Gormley, The role of Cathepsin S as a marker of prognosis and predictor of chemotherapy benefit in adjuvant CRC: a pilot study, Br, J. Canc. 105 (2011) 1487–1494.
[3] J. Kos, A. Sekirnik, G. Kopitar, N. Cimerman, K. Kayser, A. Stremmer, W. Fiehn, B. Werle, Cathepsin S in tumours, regional lymph nodes and sera of patients with lung cancer: relation to prognosis, Br, J. Canc. 85 (2001) 1193–1200.
[4] J. Gautam, Y.K. Bae, J.A. Kim, Up-regulation of cathepsin S expression by HSP90 and 5-HT7 receptor-dependent serotonin signalling correlates with triple negativity of human breast cancer, Breast Canc. Res. Treat. 161 (2017) 29–40.
[5] P.L. Fernandez, X. Farre, A. Nadal, E. Fernandez, N. Peiro, B.F. Sloane, G.P. Shi, H. A. Chapman, E. Campo, A. Cardesa, Expression of cathepsins B and S in the progression of prostate carcinoma, Int. J. Canc. 95 (2001) 51–55.
[6] L. Paraoan, D. Gray, P. Hiscott, M. Garcia-Finana, B. Lane, B. Damato, I. Grierson, Cathepsin S and its inhibitor cystatin C: imbalance in uveal melanoma, Front. Biosci. (Landmark Ed) 14 (2009) 2504–2513.
[7] S. Kiuchi, U. Tomaru, A. Ishizu, M. Imagawa, T. Kiuchi, S. Iwasaki, A. Suzuki, N. Otsuka, T. Deguchi, T. Shimizu, K. Marukawa, Y. Matsuno, M. Kasahara, Expression of cathepsins V and S in thymic epithelial tumors, Hum. Pathol. 60 (2017) 66–74.
[8] J. Xu, Cathepsin S is aberrantly overexpressed in human hepatocellular carcinoma, Mol. Med. Rep. 2 (2009) 713–718.
[9] Y.G. Yang, B.Y. Zhang, L. Liu, Y.Q. Bai, M.B. Cao, Clinical significance of serum Cathepsin S in patients with hepatocellular carcinoma, Shijie Huaren Xiaohua Zazhi 21 (2013) 3742–3746.
[10] B. Wiederanders, D. Broemme, H. Kirschke, N. Kalkkinen, A. Rinne, T. Paquette, P. Toothman, Primary structure of bovine cathepsin S. Comparison to cathepsins L, H, B and papain, FEBS, Letters 286 (1991) 189–192.
[11] W.-L. Liu, D. Liu, K. Cheng, Y.-J. Liu, S. Xiang, P.-D. Chi, X.-H. Liu, N. Xue, Y.-Z. Lai, L. Guo, G. Zhang, Evaluating the diagnostic and prognostic value of circulating cathepsin S in gastric cancer, Oncotarget 7 (2016) 28124–28138.
[12] S. Gupta, R.K. Singh, S. Dastidar, A. Ray, Cysteine cathepsin S as an immunomodulatory target: present and future trends, Expert Opin. Ther. Targets 12 (2008) 291–299.
[13] O. Vasiljeva, M. Dolinar, J.R. Pungercar, V. Turk, B. Turk, Recombinant human procathepsin S is capable of autolytic processing at neutral pH in the presence of glucosaminoglycans, FEBS Lett. 579 (2005) 1285–1290.
[14] H. Kirschke, B. Wiederanders, Cathepsin S and related lysosomal endopeptidases, Methods Enzymol. 244 (1994) 500–511.
[15] P.J. Wolters, W.W. Raymond, J.L. Blount, G.H. Caughey, Regulated expression, processing, and secretion of dog mast cell dipeptidyl peptidase I, J. Biol. Chem. 273 (1998) 15514–15520, https://doi.org/10.1074/jbc.273.25.15514.
[16] M. Sponder, C. Minichsdorfer, I.-A. Campean, M. Emich, M. Fritzer-Szekeres, B. Litschauer, J. Strametz-Juranek, Long-term endurance training increases serum cathepsin S levels in healthy female subjects, Ir. J. Med. Sci. 187 (2018) 845–851, https://doi.org/10.1007/s11845-017-1693-x.
[17] J.M. Cox, J.S. Troutt, M.D. Knierman, R.W. Siegel, Y.-W. Qian, B.L. Ackermann, R. J. Konrad, Determination of cathepsin S abundance and activity in human plasma and implications for clinical investigation, Anal. Biochem. 430 (2012) 130–137.
[18] T. Flannery, D. Gibson, M. Mirakhur, S. McQuaid, C. Greenan, A.Trimble, B. Walker, D. McCormick, P.G. Johnstone, the clinical significance of cathepsin S expression in human astrocytomas, Am. J. Pathol. 163 (2003) 175–182, https://doi.org/10.1016/S0002-9440(10)63641-3.
[19] B. Werle, A. Staib, B. Jülke, W. Ebert, P. Zladoidsky, A. Sekiernik, J. Kos, E. Spiess, Fluorometric microassays for the determination of cathepsin L and cathepsin S activities in tissue extracts, Biol. Chem. 380 (1999) 1109–1116, https://doi.org/10.1515/BC.1999.138.
[20] E. Helmerhorst, D.J. Chandler, M. Nussio, C.D. Mamotte, Real-time and label-free biosensing of molecular interaction by surface plasmon resonance: a laboratory medicine perspective, Clin. Biochem. Rev. 33 (2012) 161–173.
[21] A. Tokarzewicz, L. Romanowicz, I. Sveklo, E. Gorodkiewicz, The development of a matrix metalloproteinase-1 biosensor based on the surface plasmon resonance imaging technique, Anal, Methods 8 (2016) 6428–6435.
[22] A. Tokarzewicz, L. Romanowicz, E. Matuszczak, A. Hermanowicz, E. Gorodkiewicz, SPRI biosensors for quantitative determination of matrix metalloproteinase-2, Anal. Methods 9 (2017) 2407–2414.
[23] J.W. Chung, R. Bernhardt, J.C. Pyuna, Additive assay of cancer marker CA 19-9 by SPR biosensor, Sensor. Actuator. B 118 (2006) 28–32.
[24] E. Gorodkiewicz, The surface plasmon resonance imaging sensor for papain based on immobilized cystatin, protein pept, Lettres 14 (2007) 443–445.
[25] Jakimiec E. Gorodkiewicz, T. Guszcz, W. Roszkowska, R. Kozlowski, Cathepsin D serum and urine concentration in superficial and invasive transitional bladder cancer as determined by surface plasmon resonance imaging, Oncol Lett. 8 (2014) 1323–1327, https://doi.org/10.3892/ol.2014.2250.
[26] E. Gorodkiewicz, E. Regulska, SPR imaging biosensor for aspartyl cathepsins: sensor development and application for biological material, Protein Pept. Lett. 17 (2010) 1148–1154.
[27] E. Gorodkiewicz, M. Sieńczyk, E. Regulska, R. Grzywa, E. Pietrusewicz, A. Lesner, Z. Lukaszewski, Surface plasmon resonance imaging biosensor for cathepsin G based on a potent inhibitor: development and applications, Anal. Biochem. 423 (2012) 218–223, https://doi.org/10.1016/j.ab.2012.01.033.
[28] P. Laudański, E. Gorodkiewicz, B. Ramotowska, R. Charkiewicz, M. Kuzmicki, J. Szamatowicz, Determination of cathepsins B, D and G concentration in eutopicproliferative endometrium of women with endometriosis by surface plasmon resonance imaging (SPRI) technique, Eur. J. Obstetr. Gynecol. Reprod. Biol. 169 (2013) 80–83.
[29] P.K. Jadhav, et al., Discovery of cathepsin S inhibitor LY3000328 for the treatment of abdominal aortic aneurysm, ACS Med. Chem. Lett. 5 (2014) 1138–1142.
[30] A. Sankiewicz, Z. Lukaszewski, K. Trojanowska, E. Gorodkiewicz, Determination of collagen type IV by Surface Plasmon Resonance Imaging using a specific biosensor, Anal. Biochem. 515 (2016) 40–46.
[31] A. Sankiewicz, L. Romanowicz, P. Laudanski, B. Zelazowska-Rutkowska, B. Puzan, B. Cylwik, E. Gorodkiewicz, SPR imaging biosensor for determination of laminin-5 as a potential cancer marker in biological material, Anal. Bioanal. Chem. 408 (2016) 5269–5276.