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Searching for biologically active peptide fragments of hACE2 interacting with the S1 protein of the SARS-CoV-2 virus

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Introduction

Over four years after the COVID-19 pandemic outbreak, we still lack a perfect diagnostic tool for detecting the SARS-CoV-2 virus infection. While the rt-qPCR gained great popularity and became the analytical gold standard, it also has notable drawbacks such as high costs connected with long-term procedures, and the necessity to perform the analysis by well-trained personnel in biosafety level 3 (BSL-3) laboratories. Furthermore, the results are highly dependent on the type and method of sample collection and storage, symptom duration and severity, thus influencing the variable viral load and leading to an increasing number of false-positive results [1]. As an alternative, the rapid test indicating the presence of the virus's spike protein or specific antibodies in the form of LFI tests was used. Nevertheless, they are characterised by lower sensitivity and are typically suitable only for symptomatic individuals, limiting their effectiveness as a broad screening tool.

In response to these challenges, researchers have increasingly focused on developing new types of biomedical tests indicating COVID-19 disease. One promising option is the use of biosensors as small, reliable and portable devices providing real-time results. The receptor layer, typically composed of biological molecules, can ensure high selectivity and specificity with small sample volumes and without the need to isolate the target analyte from the mixture [2]. The covalent immobilization of the biological component offers additionally higher resistance to external factors and allows even for sensor regeneration.

However, the use of protein structures is associated with problematic and time-consuming biosensor development. Their synthesis, isolation and purification are expensive. Additionally, proteins are also prone to enzymatic degradation, thus affecting their stability. A different approach could involve using peptide fragments that maintain the biological activity instead of using the whole protein. Peptides are characterised by higher durability for pH and temperature changes and stability in sterilization and storage conditions. Their smaller size reduces the cost of the synthesis and provides an excellent opportunity for structure modification, enhancing desired properties. What's more, their self-assembling properties can result in forming monolayers with better orientation levels, ensuring also higher density surface, fast binding kinetics and lower risk of aggregation during the interaction, compared with proteins [3]. Fragments mimicking the activity of the receptor would recreate the outer sphere of the protein and be involved in protein-protein or protein-peptide interaction. One of the key receptors, responsible for binding spike protein of the SARS-CoV and SARS-CoV-2 viruses is human angiotensin-converting enzyme type 2 (hACE2).

Result and Discussion

Research conducted in the Institute of Organic Chemistry at the Lodz University of Technology focuses on the development of biosensors indicating the presence of the SARS-CoV-2 virus physiological fluids (e.g. blood, urine, saliva particles, exhaled air) or in the air using hACE2 peptide fragments. The procedure established in our Institute enables the selection of fragments with potential biological activity based on the Dot-blot methodology and involves multiple steps [4].

The initial phase considered the automated SPOT synthesis of overlapping peptide fragments covering the primary structure of the hACE2 on cellulose matrixes with the use of 1,3,5-triazine derivatives (DMT/NMM/$TosO^-$) [5]. Next, the incubation of the received 399-element library with HRP-labelled S1 protein subunit of the SARS-CoV-2 virus and further enzymatic oxidation of 4-chloro-1-naphtol into coloured 4-chloro-1-naphtone allowed the selection of fragments interacting with spike protein (Figure 1).

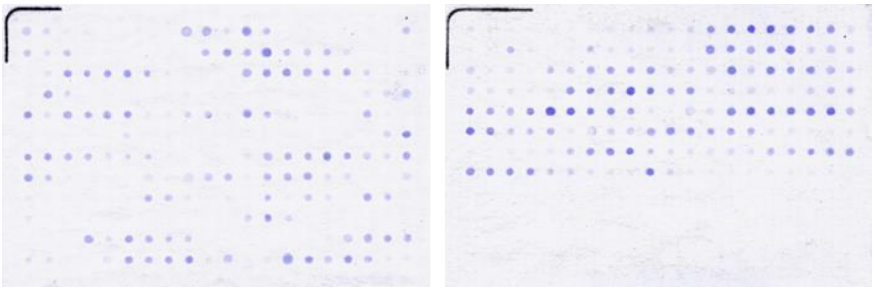

Fig. 1. *Scans of the cellulose matrix with immobilized decapeptides of hACE2 protein after the incubation with HRP-labelled S1 protein subunit of the SARS-CoV-2 virus. The intensity of each coloured complex represents the strength of the peptide-protein interaction.*

Based on the intensity of the received complexes, measured on a 256-grey scale, 32 structures with different lengths of peptide chains were chosen, including 16 fragments strongly interacting with the spike protein and 16 peptides with moderate affinity (Table 1).

Table 1. *Peptide fragments of hACE2 protein selected during the Dot-blot with the use of the HRP-labelled S1 protein subunit of the SARS-CoV-2 virus.*

No.	Locant	Peptide fragment	No.	Locant	Peptide fragment
1.	505-526	H-HVSNDYSFIRYYTRTLYQFQFQ-OH	9.	755-776	H-VGIVILIFTGIRDRKKKNKARS-OH
2.	667-692	H-EEDVRVANLKPRISFNFFVTAPKNVS-OH	10.	385-394	H-YAAQPFLLRN-OH
3.	779-788	H-NPYASIDISK-OH	11.	699-718	H-EVEKAIRMSRSRINDAFRLN-OH
4.	545-562	H-SNSTEAGQKLFNMLRLGK-OH	12.	265-282	H-HLLGDMWGRFWTNLYSLT-OH
5.	641-666	H-YLFRSSVAYAMRQYFLKVKNQMILFG-OH	13.	239-256	H-HLHAYVRAKLMNAYPSYI-OH
6.	611-632	H-SPYADQSIKVRISLKSALGDKA-OH	14.	103-122	H-NGSSVLSEDKSKRLNTILNT-OH
7.	733-746	H-PPNQPPVSIWLIVF-OH	15.	467-484	H-EIPKDQWMKKWWEMKREI-OH
8.	587-606	H-YFEPLFTWLKDQNKNSFVGW-OH	16.	123-132	H-MSTIYSTGKV-OH

The further step concerned the resynthesis of chosen fragments using the SPPS method and Fmoc/t-Bu strategy. For some of the fragments, characterized by poor solubility, high hydrophobicity or tendency of aggregation, the process involved automated synthesis with an induction-heating system and "catch-and-release" purification. For this purpose, the coupling of the oxime-based linker to the *N*-terminal amine group of the desired peptide structure was performed. The key benefit of using the linker developed by Zitterbart's team is the rapid, selective immobilization ("catch") of the product to an aldehyde-modified agarose resin. The oxime ligation for the catch step is very robust allowing a wide pH range, the use of organic solvents (ie. dimethylsulfoxide or hexafluoroisopropanol) and chaotropic reagents, thus facilitating the synthesis and purification of "problematic" fragments. After immobilization, any impurities, including by-products, salts and truncated peptides can be washed out. The later traceless cleavage ("release") of the product from solid support is possible through the reduction of an aryl azide to aniline and following acid-catalysed 1,6-elimination of the linker (Figure 2). Moreover, the linker is stable (in its azide form) to acidic environment necessary for peptide cleavage [6].

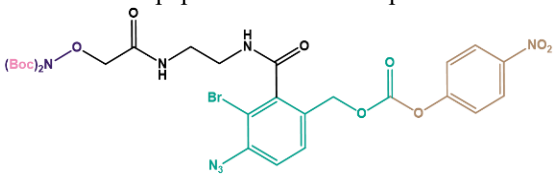

Fig. 2. *The Boc-protected structure of an oxime-based linker. Black colour represents a catch tag (Boc protected), green colours shows a cleavable unit with aryl azide and, brown – leaving group [6].*

The use of the above peptide purification method allowed for obtaining 6 peptide fragments of the hACE2 protein with purities above 90% (Table 2).

Table 2. *Peptides synthesized and purified using an induction-heating system and "catch-and-release" method.*

No.	Locant	Peptide fragment	PEC purity
1.	611-623	H-SPYADQSIKVRIS-OH	97,73 %
2.	755-767	H-VGIVILIFTGIRD-OH	95,21 %
3.	385-394	H-YAAQPFLLRN-OH	95,29 %
4.	699-718	H-EVEKAIRMSRSRINDAFRLN-OH	98,85 % *
5.	265-275	H-HLLGDMWGRFW-OH	94,92 % *
6.	111-122	H-DKSKRLNTILNT-OH	96,84 %

* *represents purities including the oxidation of Met residue.*

The last step of the research will include the confirmation of peptides' biological activity towards the SARS-CoV-2 virus particles in MST analysis.

Conclusions

The rapid advancement of natural and technical sciences has led to the development of innovative analytical tools that may find their application in medical diagnostics. Despite the limitations of current diagnostic methods for the SARS-CoV-2 virus identification, promising alternatives are being developed, including biodetecting devices utilizing hACE2 as the receptor structure. The procedure presented in this work shows an alternative approach

and may facilitate the process of searching for biologically active peptides, as well as their subsequent synthesis and purification for biomedical application. However, extensive testing would be required to validate the utility and accuracy of such prepared devices.

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