Chemically-modified aptamers generation to recognize relevant bacterial antigens and antibiotic resistance proteins

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The aim of this study was to develop a panel of aptamers with chemically modified side chains designed to specifically recognize and bind clinically relevant bacterial surface markers and antibiotic-resistance enzymes, such as beta-lactamases via SELEX procedure using Pure Biologics' patent-covered PureApta™ proprietary selection platform.

Introduction

Targeted treatment of bacterial infectious diseases and the prevention of the spread of multi-drug resistant pathogens remains a clinical challenge, therefore it is essential to search for new molecules to fight these pathogens, e.g., aptamers that could recognize selected bacterial antigens and act as effector molecules or their carriers [1].

SELEX process using PureApta™ platform

The aptamer selection campaigns were carried out using PureApta™ selection platform based on the SELEX procedure, where all deoxythymidines in the ssDNA library were replaced with an unnatural hydrophobic uridine derivative obtained in click chemistry method (Figs. 1 and 2) by incorporation via primer extension reaction.

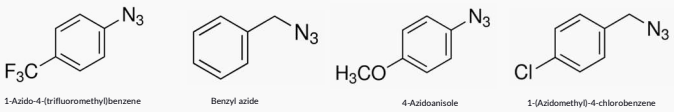

Figure 1. Azides used in Click chemistry reaction.

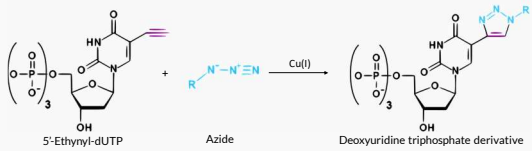

Figure 2. Click chemistry reaction adapted to obtain uridine derivatives with hydrophobic moieties.

Aptamers affinity towards bacterial targets

The selections resulted in the identification of highly specific aptamers against five antigens, including β-lactamase enzymes: OXA-48, KPC-2 and IMP-1, responsible for the most prominent antibiotic resistance mechanisms in opportunistic bacteria, and against protein G and protein A, surface proteins on Streptococcal and Staphylococcal bacterial cells, respectively (Tab. 1).

Table 1. Characteristics of aptamers (76 nucleotides in length) obtained in SELEX procedure towards bacterial proteins using ssDNA libraries containing chemically modified side chains.

Target protein	Aptamer name	Chemical modification	K_D
OXA-48	MA.S9.1	*1-Azido-4-(trifluoromethyl)benzene*	16 ± 2.1 pM
KPC-2	MA.S13.4	*Benzyl azide*	160.0 ± 40.0 pM
	MA.S13.6		47.0 ± 0.5 pM
IMP-1	MA.S15.1	*4-Azidoanisole*	3.0 ± 2.0 nM
	MA.S15.2		1.0 ± 0.1 nM
	MA.S15.3		1.6 ± 0.5 nM
	MA.S15.4		2.0 ± 2.0 nM
	MA.S15.5		12.8 ± 0.2 nM
	MA.S15.6		4.3 ± 0.3 nM
Protein G	MA.S5.5	*1-(Azidomethyl)-4-chlorobenzene*	1.0 ± 0.3 nM
	MA.S5.8		3.3 ± 0.8 nM
	MA.S5.9		2.2 ± 0.3 nM
Protein A	MA.S11.3	*2'-Fluoro-2'-deoxyguanosine, 4-Azidoanisole*	0.3 ± 0.2 nM

Affinity of the truncated aptamers

The shortest and best binding aptamers: MA.S9.1.8-53 (46 nt), MA.S13.4.1-63 (63 nt) and MA.S11.3.1-61 (61 nt) were identified for proteins: OXA-48, KPC-2 and protein A, respectively. Their K_D parameters were in pico- to nanomolar range (Fig 3. A, B and C) and did not differ much from the original, full-length aptamers (Tab. 1).

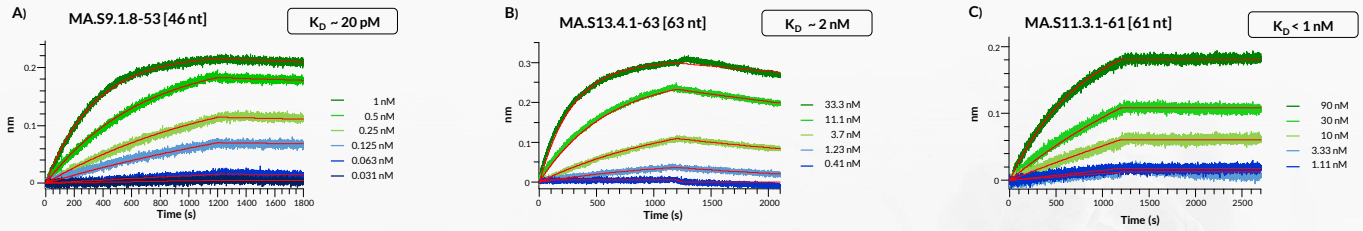

Figure 3. Affinity measurements of truncated aptamers A) MA.S9.8-53, B) MA.S13.4.1-63 and C) MA.S11.3.1-61 towards: OXA-48, KPC-2 and protein A, respectively, using BLI technology. 1:1 kinetic binding model was applied in a series of dose-response experiments.

ELONA with protein A positive and negative cells

For the MA.S11.3.1-61 aptamer, affinity tests for bacterial cells were performed, where its binding specificity to protein A positive cells was demonstrated, in contrast to protein A negative cells (Fig. 4).

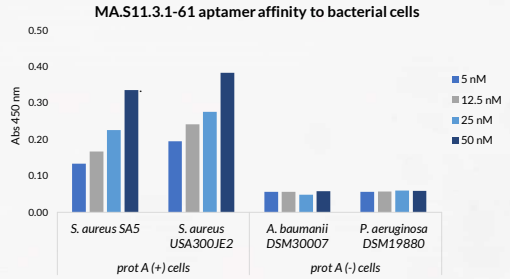

Figure 4. Bacterial cells binding assay on protein A positive and negative cells using MA.S11.3.1-61 aptamer.

Conclusions

High specificity and affinity of chemically modified aptamers, obtained using PureApta™ platform towards bacterial molecular targets, provides promising candidates for future use in the diagnostics and treatment of bacterial infections.

Acknowledgements

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References

[1] Took C.L., Hinchliffe P., Bragginton E.C. et al. 2019. ß-lactamases and ß-lactamase inhibitors in the 21st century. JMB 431, 3472-3500.

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