P-34-013
Development of an expression system for bispecific antibodies

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Production of full-length bispecific antibodies is more challenging than the expression of conventional antibodies due to the double number of heavy and light chain genes that need to be expressed. Typically, this problem is solved using four expression plasmids for transfection. The goal of this work was to create two expression plasmids for the efficient production of full-length bispecific IgG1 antibodies. The sequences encoding the variable domains of the immunoglobulin heavy and light chains were linked to the constant domains of human antibodies. Also, Kozak sequence, leader peptide sequence and restriction endonucleases recognition sites for further cloning were added. Knock-into-hole mutations were introduced into the CH3 domains of heavy chains for correct assembly of the bispecific antibody. We also introduced mutations that reduce binding to Protein A into one of the heavy chains. The aim is to improve the purity of the protein by using gradient elution in affinity chromatography. Between the light and heavy chain genes we inserted an IRES element – regulatory region of eukaryotic mRNA and their viruses, which provides cap-independent, or internal translation initiation for efficient heavy chain translation. The resulting bicistronic sequence was placed under the control of the CMV promoter. We obtained two expression plasmids, each containing the heavy and light chain genes for one part of the bispecific antibody. The bispecific antibody was transiently expressed in CHO cells and then purified from culture supernatant by affinity and size-exclusion chromatography. Expression yield and biochemical parameters of the bispecific antibody were analyzed. This work was supported by a subsidy of the Ministry of Science and Higher Education of the Russian Federation (RFME-F160417X0189). ∗The authors marked with an asterisk equally contributed to the work.

P-34-014
Disulfide polycationic amphiphiles for stimuloresponsive siRNA delivery

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Gene silencing provided by RNA interference mechanism is one of the most promising approaches for the treatment of diseases caused by overexpressed genes. Small interfering RNA (siRNA) delivered to the target cell may complementary binding target mRNA preventing undesirable protein synthesis. Since siRNA is unstable in biological medium, special delivery systems, for example cationic liposomes, are needed for siRNA delivery to the cells. In this work, we synthesised novel polycationic amphiphiles and studied siRNA delivery efficiency mediated by cationic liposomes based on them and zwitter-ionic helper lipid DOPE (1,2-dioleoyl-sn-glycerol-3-phosphoethanolamine). Amphiphiles are provided with disulfide groups making them sensitive to degradation by intracellular reducing agents such as glutathione for a better siRNA release into the cytoplasm. Dynamic light scattering studies were carried out to determine hydrodynamic diameters as well as zeta-potentials of cationic liposomes and their complexes with siRNA. Transfection efficiency was investigated by a delivery of siRNA into transgenic BHK IR780 cells expressing green fluorescent protein. It was demonstrated that both disulfide cationic liposomes provided high green fluorescent protein synthesis inhibition up to 10% as compared with untreated cells. Moreover, results were exceeded those demonstrated by commercial transfectant Lipofectamine 2000. In conclusion, cationic liposomes based on novel disulfide polycationic amphiphiles are promising carriers for delivery of siRNA into eukaryotic cells. This research was supported by Russian Science Foundation (grant 18-73-00270). E.V. Shmendel is a recipient of Presidential fellowship 1199.2018.4. ∗The authors marked with an asterisk equally contributed to the work.

P-34-015
PorU – the ultimate player in protein secretion in type IX bacteria secretion system

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Deciphering mechanisms underlaying protein secretion is of special interest because of its crucial role in bacterial pathogenesis. Here, we present data about type IX secretion system (T9SS) which is present in prominent periodontitis pathogens Porphyromonas gingivalis and Tannerella forsythia. T9SS is found in Gram negative bacteria belonging to phylum Bacteroidetes. In those bacteria the inner membrane transfer is done by the Sec translocon. Further steps of protein secretion (periplasm crossing and outer membrane release) involve around 17 proteins building the T9SS translocon. Presented work focus on the ultimate player engaged in secretion process, the PorU protein. The 130 kDa PorU protein is localized in outer membrane facing the extracellular milieu. The PorU is a sortase- an enzyme that cleaves the C-terminal secretion signal (the C-terminal domain, CTD) and attaches the A-lipopolysaccharide moiety. The deletion of the PorU protein blocks T9SS cargos secretion. This is manifested by the loss of the black pigmentation of P. gingivalis, lack of proteolytic gingipains’ activity, and the accumulation of unprocessed client proteins in periplasm. Moreover, the deletion of PorU increases the expression of other T9SS components. The PorU mutant studies revealed the presence of few conserved essential amino acid residues, and the presence of uncleaved CTD domain in this protein. Finally, the switch of P. gingivalis PorU for T. forsythia ortholog shows that, though the protein is expressed it cannot restore the function. This observation shows that however, the Bacteroidetes protein secretion system is highly conserved, it can possess major differences between species which need to be studied more deeply. This study was supported by the NCN funding: 2014/15/D/NZ6/02546.

P-34-016
The relationship between proton ATPase and Dcu transport system during glucose fermentation at pH 6.5

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During anaerobic growth E. coli can use C₄-dicarboxylate (succinate, fumarate, malate etc.) as energy and electron sources. In this study total and DCCD inhibited ATPase activity was investigated in E. coli BW25113 wild type and dcuACB, dcuACBD and dcuD mutants encoding different C₄-dicarboxylate carriers. These
carriers transport succinate ions with H\(^+\) via symport. It is well known that H\(^+\) can be transported mainly through the proton F\(_2\)F\(_2\):ATPase or different carriers/transporters. ATPase activity of membrane vesicles was determined by measuring the amount of inorganic phosphate (P\(_i\)) produced in the reaction of membrane vesicles with ATP in the assay mixture. Total ATPase activity in wild type was 172.98 nM P\(_i\)/min/µg protein. In dcu mutants the ATPase activity was ~1.5–1.9 times lower compared to wild type. The highest proton ATPase activity was detected in E. coli wild type. In the other cases proton F\(_2\)F\(_2\):ATPase activity was lowered by ~86%, 83% and 62%, respectively in dcuD, dcuACBD and dcuACB mutants. In dcuD mutant K\(^+\) ions stimulated the total ATPase activity ~2 fold compared to wild type. Moreover, when succinate was added the total ATPase activity was lowered ~1.3 fold in dcuACB and ~1.4 fold higher in dcuD. Interestingly, K\(^+\) ions stimulated total ATPase activity ~1.3 fold in dcuD and inhibited ~1.2 fold in dcuACB. In addition, DCCD inhibited ATPase activity in the study was increased ~1.2 fold in dcuD and decreased in succinate assays ~1.8 fold compared to wild type. The presence of succinate in the mixture decreased the DCCD inhibited ATPase activity ~2.8, 1.3, and 3.4 fold respectively in dcuD, dcuACBD and dcuACB in presence of K\(^+\) ions. It might be concluded that dcu transport system is connected directly with ATPase. DcuD has the main role for proton transport across the membrane. *The authors marked with an asterisk equally contributed to the work.

P-34-017

The role of Escherichia coli hydrogenase 3 subunits in hydrogen production during fermentation of high glucose concentration at different pHs

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E. coli Hyd-3 is known as the main producer of molecular hydrogen (H\(_2\)) during glucose fermentation. However, it was shown in this study that not all subunits are responsible for H\(_2\) formation to occur and can change their function depending on glucose concentration and pH values. Hyd-3 works to produce H\(_2\) during 8 g/L (high) glucose fermentation at pH 5.5. Moreover, it seems that glucose concentration in the membrane plays a role in hydrogen formation and pH 7.5. As the overall pH increases, hydrogen production is reduced. In this study, the highest hydrogen production was detected in the presence of Hyd-3 and Hyd-4. However, when succinate was added, the total hydrogenase activity was increased ~1.3 fold in dcuACB and ~1.4 fold higher in dcuD. Interestingly, K\(^+\) ions stimulated total ATPase activity ~1.3 fold in dcuD and inhibited ~1.2 fold in dcuACB. In addition, DCCD inhibited ATPase activity in the study was increased ~1.2 fold in dcuD and decreased in succinate assays ~1.8 fold compared to wild type. The presence of succinate in the mixture decreased the DCCD inhibited ATPase activity ~2.8, 1.3, and 3.4 fold respectively in dcuD, dcuACBD and dcuACB in presence of K\(^+\) ions. It might be concluded that dcu transport system is connected directly with ATPase. DcuD has the main role for proton transport across the membrane. *The authors marked with an asterisk equally contributed to the work.