STRESZCZENIE PRACY W JĘZYKU ANGIELSKIM

Title: A study of the interaction of RNA aptamers with the membranes of lipid vesicles

The production of membrane vesicles consisting of a lipid bilayer occurs in bacteria, archaea and eukaryotes. Eukaryotic cells produce a variety of vesicles that carry substances within the cell or are secreted outside the cell. Among these extracellular vesicles (EVsare also exosomes. Exosomes are vesicles, composed of a lipid bilayer and membrane proteins, and their specific lipid composition may influence the formation of domains in the vesicle's membranes called lipid rafts. These membrane rafts are 'small (10–200 nm), heterogeneous, highly dynamic, sterol- and sphingolipid-enriched domains that compartmentalize cellular processes' [Pike 2006]. The presence of these domains in exosome membranes may support vesicle stability. Exosomes mediate cell signalling by transporting molecules produced in cells, such as nucleic acids, proteins or lipids.

Similar groups of RNA have been found in vesicles from a variety of cells, suggesting that there is a common mechanism for selectively introducing RNA into exosomes. In this context, some attempts have been made to explain the process of RNA loading into exosomes. Apart from explanations of this mechanism based on the involvement of protein complexes, there are also other hypotheses of the possible interaction of RNA molecules with vesicle membranes which do not depend on protein mediation. In these processes the following factors are considered: the presence of a raft region in the membrane of multivesicular bodies or the presence of specific nucleotide motifs in the RNA sequence, namely the exosome motifs (CCCU, GGAG, UGAG and UCCU) or raft motifs (CCCU , UCCC, CUCC and UUGU). The aim of this study was to investigate the influence of these factors on RNA interactions with lipid vesicle membranes.

The materials for the study were liposomal vesicles modelling raft and non-raft membranes and exosomes isolated from bovine serum. The RNA material consisted of oligonucleotides obtained in the research of [Janas i in., 2020] as well as specifically designed mutants with introduced nucleotide modifications.

In the database of original oligonucleotides and mutants RNA sequence motifs were found, along with their probable location in the RNA molecules. In the folding of the RNA molecules predicted by the Mfold program, the following secondary structure motifs were identified: a stem, bulges, internal loops, multi-branch loops, hairpin loops and single-stranded regions at the ends of the molecule. Based on the results of the motif searches and the predicted secondary structures, characterization sheets of the analysed RNA aptamers and mutants were created.

The study of the RNA-membrane interaction was based on fluorescence measurements using the Förster resonance energy transfer (FRET) method. Studies of the level of interaction between RNA aptamers with model membranes and exosome membranes, as well as RNA mutants with raft liposomes and exosomes, were carried out. On the basis of the measurements, the values of the dissociation constant (K_D) were obtained for most of the tested RNAs.

A comparison of the K_D values for the interaction of RNA aptamers in complexes with raft and non-raft membranes indicated significant differences in the level of RNA-membrane

affinity depending on the membrane phase. The K_D values were on average almost two times lower for the interactions of aptamers with raft liposomes compared to non-raft liposomes. The results of the K_D measurements for RNA-membrane complexes confirm that the membrane state can regulate RNA membrane affinity. A general trend indicates that raft domains favour the interaction of the studied RNA aptamers with lipid molecules and may be a potential binding site for RNA molecules.

When tested with exosomes, the differences in the affinity of RNAs for exosome membranes appear to be similar to the differences in affinities obtained for the same RNAs in interactions with raft liposomes. The K_D values for the interaction of RNA with exosomes were characterized by a large scatter, therefore this part of the experiment could be repeated in the future.

For the K_D values of the RNA-raft membrane interaction obtained in the studies, analyses of the relationship between K_D variability and the presence of factors such as sequence motifs, structural motifs occurring in the expected folding, as well as RNA chain length, nucleotide composition and the free energy of folding. The analyses may suggest that:

- Some sequence motifs such as CCCU, GGAG or UCCC may promote RNA-membrane interaction. The motif-inserted mutants generally showed an increase in affinity compared to unmodified RNA.
- Although weak, the obtained correlation between the K_D value and the number of hairpins and the correlation between the K_D and the calculated parameter (that indicates the small hairpins), may suggest that the hairpin structural motif favours RNA-membrane affinity.
- The presence of a long single-stranded section at the end of the oligonucleotide molecule may diminish the interaction of the RNA aptamer with membrane rafts.
- It also appears that shortening the loops (deletions) and introducing the motif by substitution increases the affinities. Conversely the enlargement of the loop (insertion) even with the introduction of the motif, does not appear to increase the RNA-membrane affinity.

Based on the conducted research, the following conclusions were formulated:

1. RNA has the ability to directly interact with the region of lipid rafts, and the presence of raft domains in vesicle membranes favours the affinity of the tested RNA aptamers for membranes.
2. The level of interaction of RNA aptamers with the membrane can be influenced by certain nucleotide sequences (RNA motifs), and the influence of these motifs seems to be related to the occurrence of structural motifs.
3. The presence of secondary structure motifs may be important in regulating the level of RNA-membrane affinity. The presence of a hairpin motif (a small hairpin on a stable shaft) may favour RNA-membrane interactions, while the presence of a long single-stranded region at the end of the aptamer molecule probably does not favour RNA-membrane interactions.

In conclusion, the level of affinity of RNA aptamers to vesicle membranes seems to be the result of the coexistence of various factors, among these are the sequence motifs or structural motifs analysed in this work. In the future, it would be interesting to develop a more complex model that could more fully explain the variation in RNA-membrane affinity.