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INFLUENZA VIRUS SEGMENT 5 vRNA SECONDARY STRUCTURE AS THE BASIS FOR ASO DESIGN

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Influenza is an important research subject, as it poses an enormous threat to humanity. Influenza virus A type has a segmented genome, comprised of eight single-stranded RNA segments. The research object herein was segment 5 vRNA (vRNA5) of the A/Vietnam/1203/2004 (H5N1) strain. It is 1565 nt long and encodes a nucleoprotein which plays a key role in the influenza virus replication. Chemical mapping experiments were performed with CMCT, DMS, kethoxal, and NMIA, and the secondary structure was predicted with the RNAstructure software. The secondary structure of vRNA5 contains three domains: domain I (regions 1-69 nt and 1285-1565 nt), domain II (70-797 nt) and domain III (798-1284 nt) with a highly structured region 1065-1281 nt and a stable hairpin (1074-1115 nt). In order to determine accessibility of the presented structure in terms of oligonucleotide binding, the isoenergetic microarrays were used. The hybridization experiments were performed at 37°C in two buffers (containing either sodium or potassium). The RNase H hydrolysis experiments allowed to confirm the location of binding sites for selected isoenergetic microarray probes. The determined structural motifs might have an important role in the virus replication cycle, in such processes as interaction with viral or cellular proteins, RNA packaging to virions, and vRNA transport in the cell. The total of 16 antisense oligonucleotides (ASO) were designed on the basis of the presented vRNA5 secondary structure. The ASOs antiviral activity was tested in MDCK – HA line. The best antisense oligonucleotide caused 98% inhibition of sciIAV A/California/07/2009 replication.

Biography

Paula Michalak has been graduated from Poznan University of Life Sciences as MSc, specialisation includes Medical Biotechnology. She is PhD student at Institute of Bioorganic Chemistry, Poznan, Poland. Presently she is working on Influenza A virus vRNA Secondary Structure

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