STRESZCZENIE W JĘZYKU ANGIELSKIM

Nucleotides play many important roles in living organisms. Synthetic nucleotide analogues exert often anticancer or antiviral properties. Also they can act as biological tool to monitor processes in cell. They serve as monomeric units of the nucleic acid including messenger RNA (mRNA), which is crucial in gene expression process. mRNA carries codes from the DNA in the nucleus to the sites of protein synthesis in the cytoplasm. On the 5' end of eukaryotic mRNA cap structure is presented. Cap regulates crucial processes such as mRNA maturation, nuclear export, initiation of translation and degradation. 5'cap analogues influence mRNA stability but also modification within 3' end (poly(A) tail) may improve mRNA properties. Modified mRNA is widely studied and may be used in gene therapy. Therefore detection, identification, and quantitation of natural nucleotides, nucleic acids and their analogues combined with deciphering their metabolic pathways are instrumental in understanding natural metabolic processes as well as disease diagnostic and discover their therapeutic properties.

First part of this project focused on the qualitative analysis of nucleotides by tandem mass spectrometry (or MS/MS) – a technique that enables controlled fragmentation of molecules. The goal was to apply mass spectrometry to understand fragmentation pathways of nucleotides, to elaborate general rules of fragmentation, and propose possible fragmentation pathways. To this end, over 150 nucleotides including natural nucleotides and their base-, ribose-, and phosphate-modified analogues, among them several compounds of therapeutic interest, were investigated. The careful analysis of the fragmentation spectra enabled distinguishing between structurally related isomers and isobars and determine both the type and the position of the modification. The results of MS/MS were collected in a database available in public domain (http://mstide-db.com) – arguably the first MS database for this class of compounds. The database might be used in studying drug and prodrug metabolism, try to discover new natural nucleotide modifications and biomarkers, as well as perform chemical synthesis of nucleotide analogues and to develop quantitative LC-MS/MS methods. The results obtained during first stage of the project were used to develop LC-MS/MS method to analyse nucleotides fluorescence probes, dinucleotides and mRNA.

The methods enabled analysis of mRNA cap and cap analogues degradation in cell extracts provided insights into cap metabolism, and thereby, mRNA turnover. Moreover selective inhibitors of particular cap degradation steps were used. The inhibitors were developed in our group to investigate the interdependence of various cap degradation pathways and identify metabolites with key biological importance. In the next stage of this project an LC-MS/MS method for quantitative assessment of incorporation of ATP analogues modified at the α-phosphate into transcripts by RNA polymerases was developed and improved. The results showed that polymerases evince similar incorporation efficiency towards ATP and its analogues however they recognise only one stereoisomer. Furthermore incorporation of ATP analogues is stereospecific so absolute configuration of the product is strictly defined what can be used to create new biological tools. Also, mRNA with modified poly(A) tail was obtained. Experiments showed that phosphodiester modifications in mRNA poly(A) tail prevent deadenylation without compromising protein expression.

The methods for analysis nucleotides, dinucleotides and mRNA contribute to discovery of properties of new compounds, their biological and therapeutic potential, and may help in the future to design more stable mRNA-based therapeutics.