Studies on influence of cytochrome c on oxidative damage of 2-thiouridines built in an RNA chain

Nucleic acids (DNA or RNA) are biopolymers, which although present in organisms in only small amounts, yet are very important. For example, DNA is crucial to heredity by carrying genetic information about the characteristics and properties of given organism. Another class constitute transfer ribonucleic acids (tRNA) which bind amino acids and transport them to a ribosome where, during the translation process, they are incorporated into a growing polypeptide chain. Thus, tRNAs play a crucial role in the biosynthesis of proteins. A tRNA molecule is composed of a few dozen nucleotides and some of them are post-transcriptionally modified. These modified nucleosides, such as pseudouridine or thiouridine, tune the translation process. The structure of tRNA is usually visualized as the "cloverleaf structure" (**Fig.1.**) and can be divided into four arms. The most important is the anticodon loop, which recognizes and binds to a complementary triplet of nucleotides (a codon) of mRNA. Modified nucleosides, located in the anticodon loop, are involved in reading of the genetic code. The modified nucleosides located in the first position of the anticodon (a position 34, the wobble position) constitute an important sub-group of nucleosides containing a sulfur atom substituted for the oxygen atom at the C2 position of the uracil residue, ie. 2-thiouridine (S2U) and its derivatives carrying an additional substituent at the C5 position (R5S2U).

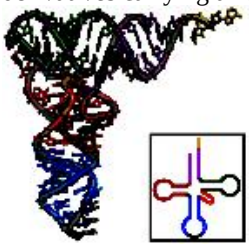

Figure 1. Three-dimensional model of the yeast $tRNA^{Phe}$ and secondary structure of tRNA (frame) with the anticodon loop (color blue).

It has been shown recently that in the oxidative stress (defined as a disturbance in the balance between the production and removal of reactive oxygen species (ROS)), damage of various biomolecules, including RNA, takes place. The sulfur-modified nucleosides may be a primary site for the attack of ROS. Our studies showed that RNAs containing 2-thiouridine (S2U) are particularly susceptible to oxidative desulfuration in the presence of hydrogen peroxide. Interestingly, this process predominantly furnishes the products containing 4-pyrimidinone nucleoside (H2U) and not those with commonly expected uridine. Transformation of S2U into the 4-pyrimidinone riboside may have tremendous biological significance. Due to a different acceptor/donor pattern of hydrogen bonds, H2U losses its ability to interact properly with an opposite adenosine residue in RNA duplexes and may impair the codon-anticodon interactions and the reading of genetic information during translation.

Recently some data have been reported that the whole tRNA molecules and its halves are involved in the programmed cell death (PCD, apoptosis), induced by release of cytochrome c (cyt c) from mitochondria. The release of cyt c, which may be initiated by several factors, including increased concentration of ROS, initiates a series of biochemical events, including formation of an apoptosome, with subsequent cell death. It was also observed that cytosolic tRNA interacts with the released mitochondrial cytochrome c, and this process blocks the formation of apoptosome and prevents the cell death. Moreover, it has been demonstrated that cytochrome c in the presence of H_2O_2 catalyzes guanosine oxidation, and finally makes a covalent complex with the RNA molecule.

Since from the mechanistic point of view desulfuration of S2U and oxidation of guanosine in the presence of H_2O_2 may have something in common, we decided to check whether cytochrome c speeds up the desulfuration process. Indeed, the preliminary results indicate that cytochrome c catalyzes oxidative desulfuration of S2U-RNA under conditions that mimic oxidative stress in the cell (an aqueous solution of H_2O_2). The reaction proceeds at low concentrations of hydrogen peroxide and is much faster then the control reaction in the absence of cytochrome c. It predominantly produced H2U-RNA, but, interestingly, no oxidation of guanosine residues in the RNA chain was detected. For sure, the reaction requires additional detailed research, especially as the observed effects may depend on tiny changes in the intracellular conditions.

The general aim of the project is to analyze the process of oxidative desulfuration of 2-thiouridine (S2U) in a broad spectrum of model molecules (at the nucleoside, oligonucleotide and the entire tRNA levels) under oxidative stress conditions induced by hydrogen peroxide in the presence of cytochrome c. The interactions between cytochrome c and modified RNA oligonucleotides (containing either S2U or H2U), and the formation of a potential covalent tRNA/cyt c complex, which may inhibit apoptosis process, will be studied. I will achieve the goal by conducting comprehensive research on the desulfuration process of modified model molecules using a variety of oxidizing agents and analyze the kind of interactions between cyt c and modified RNA. In these studies I will use methodologies known in synthetic organic chemistry, biochemistry, and molecular biology.

The research will extend the knowledge on structural and functional consequences of oxidative desulfuration of modified nucleosides, especially in the context of participation of cytochrome c in oxidative damage of RNA. The results are expected to help in explaining the interactions of cytochrome c with RNA during the formation of complexes, which are potential inhibitors of apoptosis.