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ABSTRACT OF DISSERTATION

New Analogs of Adenine Cofactors as Tools for Studying Biological Processes – Synthesis, Characterization, and Applications

Adenine nucleotides are molecules found in living organisms that perform many key functions in cells. They are, among others, building blocks of nucleic acids (DNA and RNA), regulate metabolic processes occurring in cells, act as energy carriers (ATP), participate in intra- and extracellular signaling, and as cofactors, they serve as electron carriers involved in oxidation-reduction reactions. They also enable many other enzymatic reactions, as cofactors, involving the transfer of biologically important functional groups. Due to their multitude of functions, adenine nucleotides have become the subject of scientific research, which has systematically uncovered and helped to understand the processes taking place in cells. To this end, various biological tools, structurally analogous to adenine nucleotides, have been continuously developed—for example, ATP analogs as tools for studying kinases or SAM analogs to elucidate the functions of methyltransferases. Despite the great successes achieved in this field, many classes of adenine nucleotides and their associated nucleic acid modifications remain poorly (or not at all) explored in terms of designing and synthesizing functional analogs. As a result, studying enzymes and biological processes dependent on these nucleotides is hindered. Examples of such molecules that have caught my attention include nucleotides that modify the 5' ends of RNA and analogs of 3'-phosphoadenosine 5'-phosphosulfate (PAPS), which is a universal cofactor for sulfotransferases. The limited number of molecular tools suitable for studying these two processes motivated me to undertake the research described in my doctoral dissertation.

The aim of the doctoral dissertation was to design and synthesize new analogs of NAD (nicotinamide adenine dinucleotide) and PAPS (3'-phosphoadenosine 5'-phosphosulfate) as tools for the structural and functional study of enzymes that recognize these compounds or their derivatives. These analogs were designed as tools for RNA modification to study the biological functions of NAD-RNA or as universal tools for investigating enzymes from the sulfotransferase group.

This project was intended to consist of two parts: (i) a synthetic part and (ii) biophysical and biochemical studies with selected model enzymes. In the first part, I synthesized adenine cofactor analogs that could be used in the future to study the activity of specific enzymes in vitro and in cellular conditions, study their cellular localization, identify them, or develop methods for finding inhibitors of therapeutic significance. The second part involved the development of biophysical methods for evaluating the synthesized tools and demonstrating example applications. Additionally, the experience gained in chemical synthesis allowed me to expand the research to include side projects, such as the synthesis of adenosine dinucleotide triphosphate analogs for studying plant metabolic pathways or the synthesis of flavin adenine dinucleotide (FAD) analogs, which were used in biological studies with the DXO exonuclease.

The NAD molecule is one of the most important and abundantly occurring adenine cofactors in cells. Its most well-known function is its role in oxidation-reduction processes within cells (as an electron carrier). In 2009, a study published in Nature Chemical Biology demonstrated the presence of an NAD cap structure at the 5' end of bacterial RNA. In subsequent years, this structure was also discovered at the 5' end of RNA in other eukaryotic organisms, including mammals. To date, it has been shown that in bacteria, the presence of a 5'-NAD cap increases RNA stability by reducing its susceptibility to specific pyrophosphatases. In contrast to its role in bacteria, the presence of an NAD cap at the 5' end in eukaryotes seems to destabilize RNA and facilitate its degradation. These studies suggest that the role of the NAD cap varies across different types of organisms, and despite the growing number of new analytical and chemical tools, it remains largely unexplored.

As part of my doctoral research, I designed and synthesized 8 NAD analogs, which were intended to exhibit partial or complete resistance to the action of hydrolyzing enzymes such as NudC, Nudt12, and DXO (enzymes that recognize NAD-RNA in cells). The synthesized compounds successfully incorporated into the 5' end of RNA, and four of them showed resistance to all three enzymes that degrade NAD-RNA. Due to their increased cellular stability, such analogs may serve as valuable tools for structural and functional studies of non-canonical RNA.

The next part of my work focused on creating molecular tools based on the PAPS structure and developing methods for studying sulfotransferases. Sulfotransferases are a group of enzymes involved in sulfur metabolism within cells by catalyzing sulfonylation processes. This process involves transferring a sulfate group from the universal cofactor, PAPS, to acceptors such as small endogenous biomolecules, endogenous macromolecules, or xenobiotics. The catalytic functions of sulfotransferases impact a number of important phenomena, including detoxification, drug metabolism, hormone regulation, molecular recognition in immune processes, and neurotransmitter level control. Impaired activity of this enzyme group has been linked to various diseases, including Parkinson's disease, hemophilia, and cancer.

A key aspect of my research is the fact that despite the important role sulfotransferases play in various organisms, tools based on the universal cofactor PAPS that would enable the application of modern chemical and medical biology methods for studying these enzymes have not yet been developed. One likely reason for the lack of molecular tools based on PAPS is the high instability of the phosphosulfate bond and the resulting difficulties in synthesis. In my dissertation, I addressed this issue by optimizing a three-step synthesis method for such compounds, which I then used to obtain a series of analogs with modifications in the nitrogenous base (N6, C2, and C8 positions), ribose residue (2' position), and phosphosulfate group (various functionalized and isosteric analogs).

To enable systematic investigation of the structure-activity relationships of the obtained compounds, I developed two biophysical and biochemical methods. The first method allowed for the determination of the affinity (dissociation constants, K_D) of these compounds for model sulfotransferases using microscale thermophoresis. The second method enabled real-time monitoring of sulfotransferase activity using ^{19}F NMR and the evaluation of potential inhibitor activity.

All these topics are further developed in the literature and experimental sections of the doctoral dissertation.