INSTITUTE OF MOLECULAR AND TRANSLATIONAL MEDICINE

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Evaluation Report on the Doctoral Thesis
Modifications of nucleic acids by reactive groups for bioconjugations and cross-linking with lysine containing peptides and proteins
by Mgr. Ivana Ivancová

The presented dissertation thesis is focused on the preparation of DNA and RNA probes with modified nucleotides that contain a reactive squaramate moiety. Such probes were expected to form covalent crosslinks with lysine-rich proteins that naturally occur in a close proximity to DNA or RNA. Such probes may serve as important tools to study interactions between DNA or RNA and proteins and reveal the mechanism of various biological processes.

The actual work consisted in the design and preparation of squaramate-modified deoxyribonucleotides or ribonucleotides and in their enzymatic incorporation into the DNA or RNA molecules of desired length and sequence. These modified nucleic acids then served as a probes to study possible cross-links or conjugate formation.

In the first part of the thesis, a squaramate modified dNTP was prepared and incorporated into modified DNA using PEX and PCR reactions. The probes were subjected to cross-link experiments with lysine or short peptides containing lysine. It was found that reasonable reaction occurs only when a large abundance of the lysine moiety is present. Further experiments with lysine-containing proteins such as BSA or GSTp53-CD showed no reaction while the reaction with histones formed cross-linked products quite easily. This suggests that there is a significant proximity effect.

In the second part, a modified ribonucleotide was prepared in a similar manner as in the first part. The triphosphate was then incorporated into RNA *via in vitro* transcription. Following experiments allowed to label the RNA with sulfo Cy-5-amine which may be generally used for post-synthetic labelling of nucleic acids without a presence of any additional component/activator. Further, a cross-link reactions were studied between the modified RNA and various lysine-rich proteins involved in RNA maintenance such as RNA dependent RNA polymerases (RdRp), nucleoproteins etc. The most efficient crosslinking went between the flaviviral RdRp such as JEV and YFV NS5. In contrast, some of the other selected proteins only afforded traces of a cross-linked product. Last not least, an important part of this study was an investigation of the effects of the modified NTP on the polymerase activity. Curiously, SARS-CoV-2 RdRp was not affected by the triphosphate at all while JEV RdRp reacted with the squaramate modified NTP pretty well. I really appreciate the following proteomic study that revealed at which lysines the cross-linking was taking place.

The thesis has a traditional structure with a highly informative introductory section, followed by specific objectives that provide the rationale for the thesis, then results and discussion, conclusion, experimental section and references. The thesis is written in a relatively clear language and it is easy to follow the main ideas, hypotheses, experimental setup and results. The experimental part is described in detail, with all necessary spectral and physical data given, and the descriptions of the experiments and gels are very easy to navigate through. The experiments are fully consistent with the conclusions of the thesis.

I only have these topics for the discussion:

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1. What was the rationale of selection of various proteins as cross-linking substrates in RNA studies? I mean why specifically proteins from JEV, SARS-CoV-2, YFV, HIV?

2. In the DNA studies it is quite clear, that those proteins that naturally occur in proximity to DNA form cross-links (histones) and other proteins (e.g. BSA) not. On the other hand, it was not as clear in experiments with modified RNA. Is there a possibility to drive some clear conclusion why some of the proteins cross-linked well while others did not? Is it because of the way how exposed or hidden are the lysine residues or is there any other explanation?

At the end I must conclude that the stated objectives of the submitted work have been fully met. This is a very innovative interdisciplinary research, where the adept had to combine a deep knowledge of organic synthesis with many methodologies used in the fields of biochemistry and molecular biology. A significant part of it was published in excellent international scientific journals and the candidate has thus demonstrated that she is capable of independent scientific work.

In conclusion, the presented results are original and of high scientific value, and therefore I recommend the thesis for defense and further proceedings for the degree of PhD.

In Olomouc 9.1.2023

Doc. RNDr. Milan Urban, Ph.D.