

Research Plan – Bálint Hajdu

The goal of our experiments is to design new types of artificial nucleases that can specifically recognize, bind and cleave DNA for various purposes. Zinc finger proteins are modular specific DNA binders that can be designed to recognize any desired DNA base sequence. As such, they can control the function of genes, but they also can be turned into specific DNA manipulating agents. A hydrolytic method by means of which we can specifically cleave proteins using nickel(II) ions results in square planar metal complex at the N-terminus of the C-terminal cleavage product. The latter complex is able to cleave DNA in the presence of oxidants. Based on these facts, the combination of the two experiments will, according to our expectations, lead to the development of in situ prepared specific nucleases. For this, we need to establish optimal conditions of the specific cleavage of the zinc finger proteins both in the absence and presence of DNA. During my research stay in Warsaw, in the laboratory of Professor Wojciech Bal from the 11th of July to the 5th of August I performed experiments on this topic.

1. Previously I elaborated a test system and the precise protocol to study the specific cleavage of the 1MEY# zinc finger protein - which is modified to contain only one nickel cleavage site available - by nickel(II) ions in the presence of DNA, according to the project plan for the first year of the collaboration. The results of Circular Dichroism, Mass Spectrometry HPLC and gel electrophoresis proved that the reaction took place, and was sequence specific between the protein and the metal ion.
2. During my stay I analysed some further properties of the proteins before and after the hydrolysis so that we could calculate secondary structures and determine the temperature and time dependence of the stability of the structures as well. These experiments highlighted that both the protein-metal complexes and the original proteins are stable in various conditions.
3. As the second step of our project specific plasmid DNA - protein metal complex experiments have been performed as well in various conditions to understand the yet unknown details of this reaction, and the stability of DNA-protein complex. The current results showed that the reaction only takes place between the specific DNA and the protein metal complex. I established a protocol during my stay which provided us reproducible data during the experiments with DNA so there is no obstacle to further studies in this project.
4. It has to be emphasized, that in a case of success of our research the results will lead to a platform, that will allow us to construct a library of ANs based on the already available

knowledge of the zinc finger design. The resulting ANs will be able to introduce a specific cleavage at any chosen DNA target sequence. An enzyme with such property may find extensive applications i.e. in molecular biology research, or in various biotechnological processes. Moreover, such ANs may be used for the targeted disruption of genes e.g. in cancer cells. This raises the prospect of applications in diverse fields.