ACTA ABP BIOCHIMICA POLONICA

Supplementary Materials Epub: No 2017_1615
Vol. 64, 2017
https://doi.org/10.18388/abp.2017_1615

Regular paper

Selection and analysis of a DNA aptamer binding α-amanitin from *Amanita phalloides**

Klaudia Muszyńska[1#], Dominika Ostrowska[2#], Filip Bartnicki[1], Ewa Kowalska[1], Małgorzata Bodaszewska-Lubaś[1], Paweł Hermanowicz[1], Heinz Faulstich[3] and Wojciech Strzałka[1✉]

[1]Department of Plant Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland; [2]Department of Comparative Biochemistry and Bioanalytics, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland; [3]Bioorganic Research Group, Max Planck Institute for Medical Research, Heidelberg, Germany

Mushroom foraging is very popular in some regions of the world. Sometimes toxic and edible mushrooms are mistaken by mushroom collectors, leading to serious human poisoning. The group of mushrooms highly dangerous for human health includes *Amanita phalloides*. This mushroom produces a toxic octapeptide called α-amanitin which is an inhibitor of nuclear RNA polymerase II. The inhibition of this polymerase results in the abortion of mRNA synthesis. The ingestion of *A. phalloides* causes liver failure due to the fact that most of the toxin is uptaken by hepatocytes. The hospitalization of poisoned patients involves the removal of the toxin from the digestive tract, its dilution in the circulatory system and the administration of therapeutic adjuvants. Since there is no effective antidote against amanitin poisoning, in this study we developed a DNA aptamer exhibiting specific binding to α-amanitin. This aptamer was selected using the SELEX (*Systematic Evolution of Ligands by Exponential Enrichment*) method. Next, its ability of toxin removal from aqueous solution was confirmed by pull-down assay. The aptamer region sufficient for α-amanitin binding was determined. Finally, the dissociation constant of the α-amanitin/DNA aptamer complex was calculated.

Key words: α-amanitin, mushroom poisoning, aptamer, SELEX

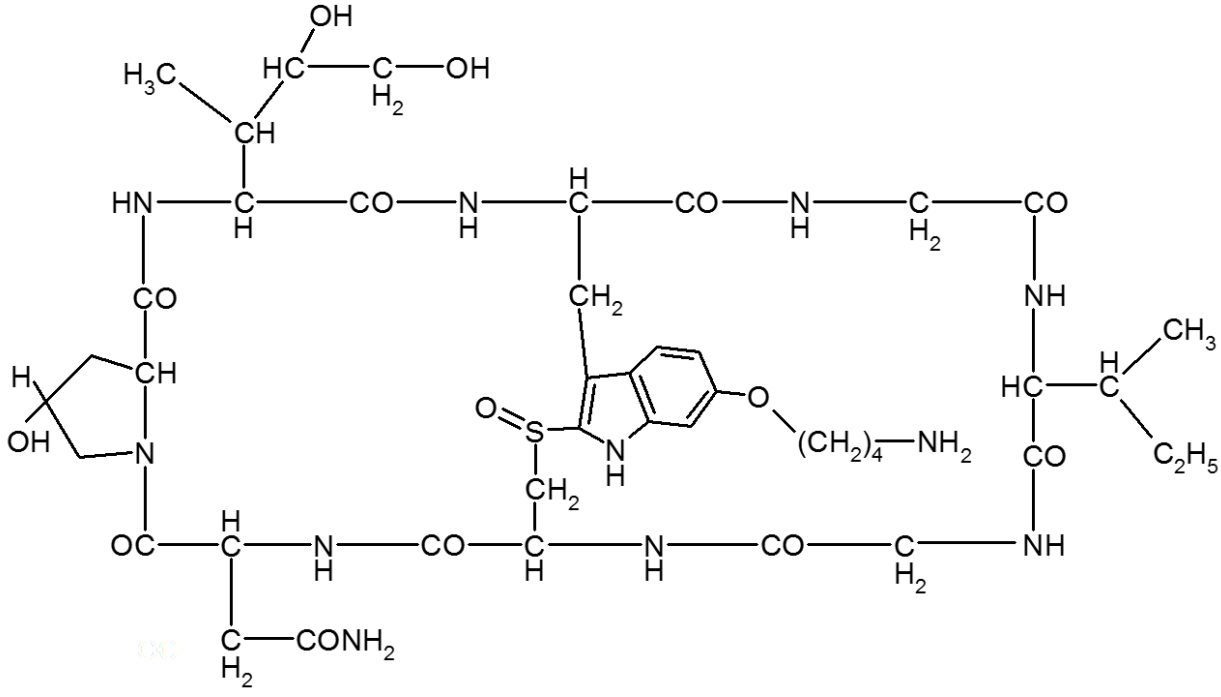
Received: 18 March, 2017; revised: 22 May, 2017; accepted: 22 May, 2017; available on-line: 09 August, 2017

✉e-mail: wojciech.strzalka@uj.edu.pl

#KM and DO equally contributed to experimental work

*Presented at the XLIV Winter School of the Faculty of Biochemistry, Biophysics and Biotechnology of the Jagiellonian University in Cracow, which took place in Zakopane on 14-18 February, 2017

Abbreviations: SELEX, *Systematic Evolution of Ligands by Exponential Enrichment*; HCSA, high capacity streptavidin agarose



Supplementary Fig. 1. Chemically modified α-amanitin used for aptamer selection.