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

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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

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

INTRODUCTION

Every year, in European and American countries edible and toxic mushrooms are mistaken by mushroom collectors. This phenomenon is the cause of mushroom poisoning (mycetismus) in human (Smith & Davis, 2016). Among the 5000 known species of mushrooms, around 100 are recognized as poisonous. The data from Polish statistical reports indicate that each year in Poland around 500–1000 mycetismus cases are diagnosed, of which 70% are adults and 30% are children (Ferenc *et al.*, 2009). Toxic mushrooms are classified according to the content of toxic substances, including cyclopeptides, gyromitrin, muscarine, coprine, isoxazoles, orellanine, psilocybin and gastrointestinal irritants. The most dangerous mushroom species are those that produce toxic cyclopeptides. These peptides are responsible for 90–95% of mushroom poisoning fatalities. One of the mushroom producing toxic cyclopeptides is *Amanita phalloides* commonly referred to as death cap. Three groups of toxins, amatoxins, phallotoxins and virotoxins, were found in *A. phalloides*. Amatoxins are a group of bicyclic octapeptides, comprising nine compounds: α-amanitin, β-amanitin, γ-amanitin, δ-amanitin, ε-amanitin, amanin, amaninamide, amanullinic acid and proamanullin (Garcia *et al.*, 2015). The clinical symptoms of amatoxin poisoning are defined as the phalloides syndrome (Smith & Davis, 2016). Mortality caused by *A. phalloides* poisoning reaches approximately 20–30% in adults and exceeds 50% in children (Ferenc *et al.*, 2009).

The clinical course of *A. phalloides* poisoning can be divided into three phases. The initial period is called the quiescent period lasting from 6 to 24 h after ingestion. In that time, the symptoms of intoxification are not specific to mushroom poisoning and can be misdiagnosed as food poisoning or viral gastroenteritis. Among the symptoms are headache, muscle pain, abdominal pain, nausea, vomiting and watery diarrhea, leading to disorders of acid-base and water-electrolyte balance. The second phase of amatoxin poisoning lasts from 24 to 48 h after mushroom ingestion and is characterized by apparent clinical improvement, progressive increase in liver function markers (transaminases AlAT, AspAT) and bilirubin level, indicating liver damage. Moreover, early kidney damage may be biochemically expressed as an increase in serum creatinine and blood ammonia concentration. The last stage of phalloides syndrome occurs around the third day after ingestion. Clinical symptoms of liver failure develop, resulting from progressive organ necrosis. A dramatic increase in transaminase, bilirubin and creatinine levels is observed. Additionally, hyperbilirubinemia, coagulopathy, hypoglycemia and metabolic acidosis occur. In severe cases of poisoning fulminant hepatitis develops and, in 15% of patients, hepatic coma begins. Furthermore, symptoms of neurological disorders may occur resulting from the accumulation of toxic metabolites or multi-organ failure. For this reason, in the period from 6 to 16 days after poisoning, patients can die in the most severe cases (Bonnet & Basson, 2002; Ferenc *et al.*, 2009; Santi *et al.*, 2012; Garcia *et al.*, 2015; Smith & Davis, 2016).

The mechanism of action of amatoxins is fairly well studied. After death cap ingestion and digestion in the intestines, amatoxins are absorbed from the human gastrointestinal tract into the blood circulation. The toxins can be bound by serum proteins and detected in blood

up to 36 h after poisoning (Ferenc *et al*., 2009). Next, amatoxins are rapidly eliminated from the blood and distributed to the liver and kidneys within 48 h (Garcia *et al*., 2015). The toxin uptake by the liver is carried out by an organic anion-transporting polypeptide (OATP1B3) located on the sinusoidal membrane of hepatocytes. Amatoxins are not metabolized in hepatic cells. A small amount can leave the liver with bile, however afterwards they are reabsorbed via the enterohepatic circulation, which prolongs hepatic cell exposure to the toxins (Garcia *et al*., 2015). Several amanitin toxicity mechanisms have been described. The main one is based on strong non-covalent binding of amanitin to RNA polymerase II (RNAP II). This interaction blocks the activity of RNAP II which leads to the inhibition of mRNA synthesis (Bushnell *et al*., 2002; Kaplan *et al*., 2008). Finally, the decrease in mRNA levels results in arrested protein synthesis and cell death. Additionally, it is postulated that amanitin accumulation plays a major role in oxidative stress of hepatic cells. Amatoxins may induce superoxide dismutase activity and decrease catalase activity, consequently contributing to lipid peroxidation and accumulation of malondialdehyde products (oxidative stress markers, products of polyunsaturated fatty acids peroxidation in the cells, generated by free radicals). The oxidative stress of hepatic cells may contribute to their massive necrosis and severe hepatic failure. Amatoxins are eliminated from the organism mainly in urine and maximal toxin excretion occurs in the first 72 hours after ingestion. Therefore, kidneys are the second target organ for amanitin toxicity. The exposure of kidney cells to amatoxins can result in acute tubular necrosis leading to kidney failure (Garcia *et al*., 2015).

The treatment of the phalloides syndrome is difficult due to the non-specific symptoms of the first phase and the clinical course of poisoning. Taking into account that a specific antidote against amatoxins is not available, the treatment measures applied to amanitin-poisoned patients are various and include for example: prevention of poison absorption, enhanced elimination of amatoxins, use of antioxidants and hydration, stabilization of vital functions, prevention of complications (Enjalbert *et al*., 2002; Garcia *et al*., 2015). Therefore, the elaboration of a specific α- and β-amanitin absorber could significantly contribute to the development of a new treatment option for *A. phalloides* poisoning. Due to the lack of effective therapeutics neutralizing amatoxin activity, in this study we tested the possible application of DNA aptamers as a tool for the treatment of amatoxin poisoning.

DNA aptamers are short (usually 40 to 120 nucleotides) single-stranded DNA molecules with a unique sequence-defined structure. They can recognize different targets including metal ions, peptides, proteins or even cells (Pei *et al*., 2014). The binding between aptamer and target can be mediated for example by electrostatic interaction, hydrogen bonds, or interaction between aromatic rings (Hermann & Patel, 2000). DNA aptamers can be identified by an *in vitro* selection method called SELEX (Systematic Evolution of Ligands by Exponential Enrichment) (Ellington & Szostak, 1990; Tuerk & Gold, 1990). In SELEX the target molecule, immobilized on a solid carrier, is incubated with a mixture of random ssDNA sequences called a DNA library. Next, unbound ssDNA molecules are washed out. The aptamers bound to the target are amplified and prepared for the next round of selection. Usually from 5 to 20 rounds of selection are needed. The SELEX method allows to find aptamers which can bind the target with high specificity and affinity. In this study, we developed and characterized a DNA aptamer binding α-amanitin.

MATERIAL AND METHODS

Peptides and oligonucleotides. Modified α-amanitin (Supplementary Fig. 1 at www.actabp.pl) was used as a target in the aptamer selection process. Unmodified α-amanitin and primers were purchased from Sigma-Aldrich (Germany). All oligonucleotides were synthesized by IBA GmbH (Germany).

Computational analysis. The models of aptamer secondary structure and ΔG values were calculated using the mfold web server (Zuker, 2003). The calculation was performed for the following conditions: temperature 25°C, 157 mM NaCl and 5 mM $MgCl_2$.

Determination of α-amanitin concentration. α-amanitin concentration was estimated using the Buhlmann Laboratories AC Amanitin ELISA Kit (Switzerland) according to the manufacturer's protocol. Serial dilutions of α-amanitin were made in AS buffer (137 mM NaCl, 12.3 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, 5 mM $MgCl_2$, 0.01% (v/v) Tween 20; pH 7.5).

Peptide immobilization and aptamer selection. Modified α-amanitin used in SELEX was immobilized to cyanogen bromide-activated-sepharose 4B from Sigma-Aldrich (Germany) according to the producer's protocol. The resin with immobilized α-amanitin was kept in 1 M NaCl with 0.05% (w/v) sodium azide at 4°C until use. In the first selection round 25 μg of synthetic ssDNA library (5'-CATGCTTCCCCAGGGAGATG$(N)_{40}$GAGG AACATGCGTCGCAAAC-3') resuspended in AS buffer were incubated with immobilized α-amanitin for O/N at 4°C with continuous mixing. From the second to the seventh selection round 1 μg of ssDNA was used. After incubation, the beads were washed with AS buffer and used as a template for PCR amplification. PCR was performed using a mixture composed of 1xPCR buffer (10 mM Tris−HCl, 50 mM KCl, 0.08% (v/v) Nonidet P40, 2.5 mM $MgCl_2$, pH 8.8), 500 μM dNTPs, 1 μM forward (5'-CATGCTTCCCCAGGGAGATG-3') and reverse (5'-phosphate-GTTTGCGACGCATGTTCCTC-3') primer, 1.5 units of Taq polymerase (Thermo Scientific) and an aptamer template. The reaction conditions were 94°C for 5 min, followed by 24 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s, final amplification 72°C for 5 min, performed in a gradient T100 thermocycler (Bio-Rad, Germany). Next, the PCR product was reamplified and precipitated with isopropanol. To prepare ssDNA for the next round of selection the PCR product was digested with exonuclease Lambda (Thermo Scientific) (Avci-Adali *et al*., 2010) according to the protocol supplied by the producer. DNA aptamers after the fifth round of SELEX were ligated into the pTZ57R/T vector (Thermo Scientific) according to the producer's protocol. DNA sequencing was performed by Genomed (Poland).

Quantitative Real-Time PCR. The binding of the aptamer pools or single aptamers to α-amanitin was evaluated using quantitative PCR as described previously (Kowalska *et al*., 2014; Bartnicki *et al*., 2015; Bartnicki, 2017). The beads with the immobilized target were incubated with 35 ng of ssDNA in AS buffer as described in the SELEX procedure section. The qPCR reaction mixture contained: 1×HS-PCR Master Mix SYBR (A&A Biotechnology, Poland), 0.5 μM forward, 0.5 μM reverse primers and as a template: i) beads with α-amanitin after SELEX, or ii) 0.35 ng of reference DNA. The reaction

conditions were: 95°C for 10 min, followed by 35 cycles of 95°C for 15 s, 53°C for 30 s, and 72°C for 30 s. The obtained results were referred to calibration curves based on the used DNA library, and appropriate negative controls were also included. The binding of tested aptamer pools or single aptamers to the target molecule was expressed as: aptamer quantity bound to beads/aptamer quantity present in the reference sample. Next, to compare the change in binding level between tested aptamer pools, the enrichment parameter was calculated as the ratio: tested aptamer pool binding/initial DNA aptamer library binding to the target. For single aptamers enrichment parameter was calculated as the ratio: tested aptamer binding/reference aptamer binding to the target.

Pull down assay. High capacity streptavidin-agarose resin (HCSA, Thermo Fisher Scientific) was washed in water and AS buffer. Next, 60 nmoles of 5'-biotinylated tested or reference (5'-CATGCTTCCCCAGGGAGATG $(ACTG)_{10}$GAGGAACATGCGTCGCAAAC-3') aptamer were incubated with 40 μL of 50% (w/v) HCSA in 400 μL AS buffer for 1 h at RT with continuous mixing. Aptamer immobilization was confirmed by spectrophotometric measurement at 260 nm. Next, the resin was washed with AS buffer and incubated at RT with 200 μM biotin (Sigma Aldrich) dissolved in AS buffer to block the remaining free streptavidin. After 30 min the resin was washed again with AS buffer and incubated with 60 μL of α-amanitin dissolved in AS buffer (concentration 50 ng/mL). After 1 h incubation the content of α-amanitin in the supernatant sample was analyzed using the Buhlmann Laboratories AC Amanitin ELISA Kit (Switzerland) according to the protocol delivered by the manufacturer.

Dissociation constant determination. To determine the dissociation constant of the α-amanitin/Ama1 aptamer complex the pull-down assay was employed. First, the HCSA resin was washed in water followed by AS buffer. Next, 40 μL of 50% HSCA, resuspended in 400 μL of AS buffer, were incubated at RT with the appropriate amount of Ama1 aptamer to obtain 0; 1.25; 3.75; 5.0; 7.5; 10.0; 16.25; 22.5 or 45 μM final concentration of ssDNA immobilized on the beads. After 1 h complete aptamer immobilization was confirmed by spectrophotometric measurement at 260 nm. To block the remaining free streptavidin the resin was first washed with AS buffer and incubated at RT with 200 μM biotin (Sigma Aldrich) dissolved in AS buffer. After 30 min the resin was washed again with AS buffer. Subsequently, the resin was placed in 60 μL of AS buffer supplemented with α-amanitin (concentration 12 ng/mL) and incubated for 1 h at RT with continuous mixing. To determine the amount of α-amanitin complexed with the DNA aptamer the Buhlmann Laboratories AC Amanitin ELISA Kit (Switzerland) was used. Using the equilibrium concentration of Ama1 aptamer/α-amanitin as a function of free Ama1 aptamer (total Ama1 aptamer), the dissociation constant (Kd) of the analyzed complex was calculated with the help of GraphPad Prism software.

Statistical analysis. Standard deviations were calculated based on data from three independent experiments.

RESULTS

Identification of α-amanitin binding DNA aptamers

The qPCR analysis of the anti-α-amanitin aptamer selection progress indicated that the aptamer pool after the fifth SELEX round was characterized by the highest value of the enrichment parameter (Fig. 1). Moreover, the values of the enrichment parameter calculated for the aptamer pools selected after cycles VI and VII gradually decreased in comparison to cycle V, thus ssDNA molecules from these selection rounds were not used in later experiments. Based on qPCR analysis ssDNA molecules from the fifth SELEX round were cloned and sequenced. Among the analyzed clones 15 different sequences were identified. Subsequently, the enrichment parameter for all 15 ssDNA molecules was determined using the qPCR technique (Fig. 2). This analysis demonstrated that out of the 15 tested molecules only 4 aptamers (A2-2, A2-3, A2-11, A2-19; Fig. 3) showed significantly higher binding to modified α-amanitin in comparison to reference ssDNA (Fig. 2), representing one of the many possible sequences of the ssDNA library, characterized by non-specific binding to α-amanitin. It

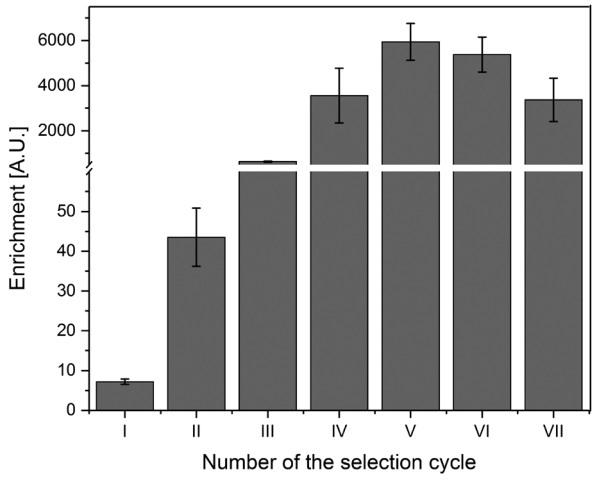

Figure 1. qPCR analysis of selected DNA aptamer pool binding to α-amanitin.
The enrichment of aptamer pools after I-VII selection rounds was expressed as the ratio: binding of tested aptamer pool/binding of ssDNA library to α-amanitin. Results are the mean of three measurements. Error bars represent the standard deviation.

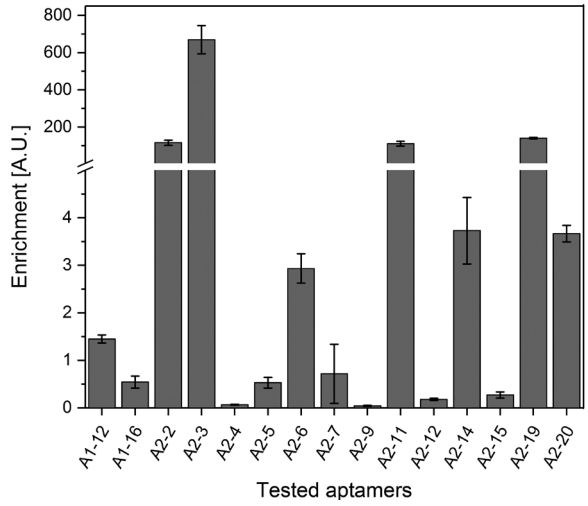

Figure 2. qPCR analysis of selected DNA aptamer binding to α-amanitin.
The enrichment of selected aptamers was expressed as the ratio: binding of tested aptamer/binding of reference aptamer to α-amanitin. Results are the mean of three measurements. Error bars represent the standard deviation.

A2-2 *CATGCTTCCCCAGGGAGATG*CCGGGCGGGTGTGGTGGGTTTTGGGCTTCTTACTGCGTTG*GAGGAACATGCGTCGCAAC*
A2-3 *CATGCTTCCCCAGGGAGATG*GAGGTCTTTTTGGTTGTTGGTGGGGGAATCTTTTGGTATT*GAGGAACATGCGTCGCAAAC*
A2-11 *CATGCTTCCCCAGGGAGATG*GAGGTCTTTTTGGCTGTTGGTGGGGGAATCTTTTGGTATT*GAGGAACATGCGTCGCAAAC*
A2-19 *CATGCTTCCCCAGGGAGATG*GTCACGGGTTGGGAGGGGGGGTAGGTGACCATTTGCCGAT*GAGGAACATGCGTCGCAAAC*

Figure 3. Sequences of DNA aptamers with significantly higher binding to modified α-amanitin in comparison to reference ssDNA. Underlining indicates constant regions of selected aptamers used for primer annealing.

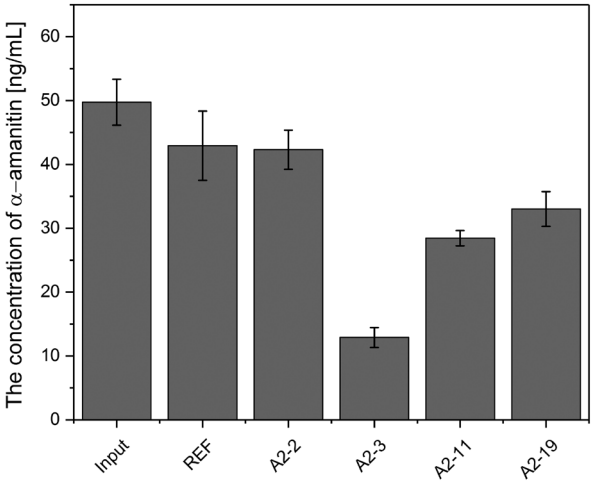

Figure 4. Screening for the DNA aptamer characterized by the best efficiency of α-amanitin removal from model solution. 5′-biotinylated reference (REF) or tested aptamer was immobilized on HCSA resin. Next, it was incubated with a solution containing 50 ng/mL of α-amanitin. After incubation, the aptamer was separated from the solution and the concentration of α-amanitin in the supernatant was determined using the Buhlmann Laboratories AC Amanitin ELISA Kit. Results are the mean of three measurements. Error bars represent the standard deviation.

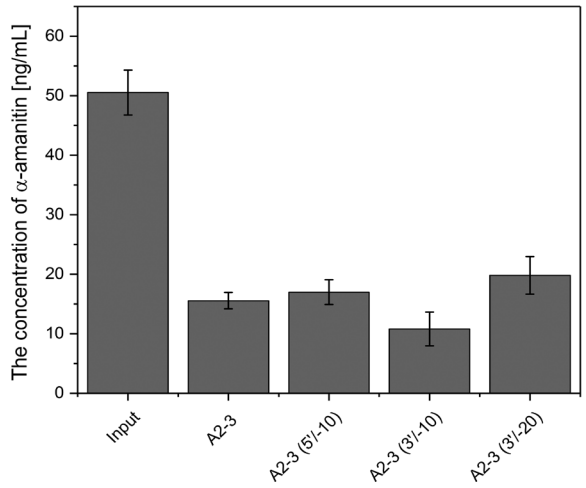

Figure 5. Identification of the A2-3 aptamer region necessary for optimal binding of α-amanitin. Full length A2-3 aptamer and its variants shortened either from the 5′- or the 3′- end: A2-3 (5′/-10), A2-3 (3′/-10), A2-3 (3′/-20) were tested. 5′-biotinylated aptamers were immobilized on HCSA resin. Next, α-amanitin solution was added. After incubation, the resin with analyzed aptamers was removed and the concentration of α-amanitin remaining in the solution was determined. Results are the mean of three measurements. Error bars represent the standard deviation.

cannot be excluded that the single nucleotide difference between the A2-3 and A2-11 aptamers was created by Taq polymerase during DNA amplification. The enrichment parameter calculated for these 4 aptamers was up to several hundred times higher than for the other tested molecules. Moreover, the analysis of A2-3 and A2-11 aptamers demonstrated that a single nucleotide alteration between these two sequences (Fig. 3) was reflected in an almost 4.5-fold higher enrichment parameter value in favor of the A2-3 aptamer (Fig. 2).

Biochemical analysis of the A2-3 aptamer

In the first experiment the pull-down assay was employed to test the ability of the selected aptamers to clean the model solution (AS buffer) from unmodified α-amanitin naturally occurring in death cap. Our results clearly indicated that the ssDNA molecule denoted as A2-3 was characterized by the most efficient binding of α-amanitin from solution (Fig. 4). Therefore, the A2-3 aptamer was selected for further biochemical studies. In the next experiment, the A2-3 aptamer region essential for α-amanitin binding was identified, again with the help of the pull-down assay. In comparison to the full-length aptamer a variant lacking 10 nucleotides from the 3’ end was more efficient in α-amanitin binding (Fig. 5). This result suggests that nucleotides at positions 71–80 of the analyzed molecule could cause a steric hindrance which prevented an optimal aptamer/α-amanitin fit. Further shortening of the analyzed aptamer from the 3’ end, as well as the removal of even 10 nucleotides from the 5’ end of the full-length aptamer resulted in a partial loss of its affinity to α-amanitin. Concluding, the region from 1 to 70 nucleotides of the original full length A2-3 molecule, later called Ama1 aptamer, was indispensable for optimal α-amanitin binding. To predict the putative secondary structure of the Ama1 aptamer the mfold web server (Zuker, 2003) was used. Two structures were predicted. Structure 1 with ΔG=–3.51 kcal/mol and structure 2 with ΔG=–3.1 kcal/mol (Fig. 6A, 6B, respectively). Finally, to determine the dissociation constant (Kd) value of the Ama1 aptamer/α-amanitin complex the pull-down assay was again employed (Fig. 7). α-amanitin was incubated with increasing concentrations of Ama1 aptamer immobilized on HCSA resin. Next, the percentage of α-amanitin present in the complex with Ama1 aptamer was calculated on the basis of the difference between the input α-amanitin concentration used in the experiment and the concentration of α-amanitin which remained in solution after incubation with the Ama1 aptamer. The K_d value of the Ama1 aptamer/α-amanitin complex was 5.026±0.69 μM.

DISCUSSION

The goal of *A. phalloides* poisoning treatment is to neutralize toxins circulating in the organism. There is no specific pharmacological treatment for intoxication. This is interesting taking into account the fact that commercial antibodies against α-amanitin are available and are not implemented for the clinical treatment of the phal-

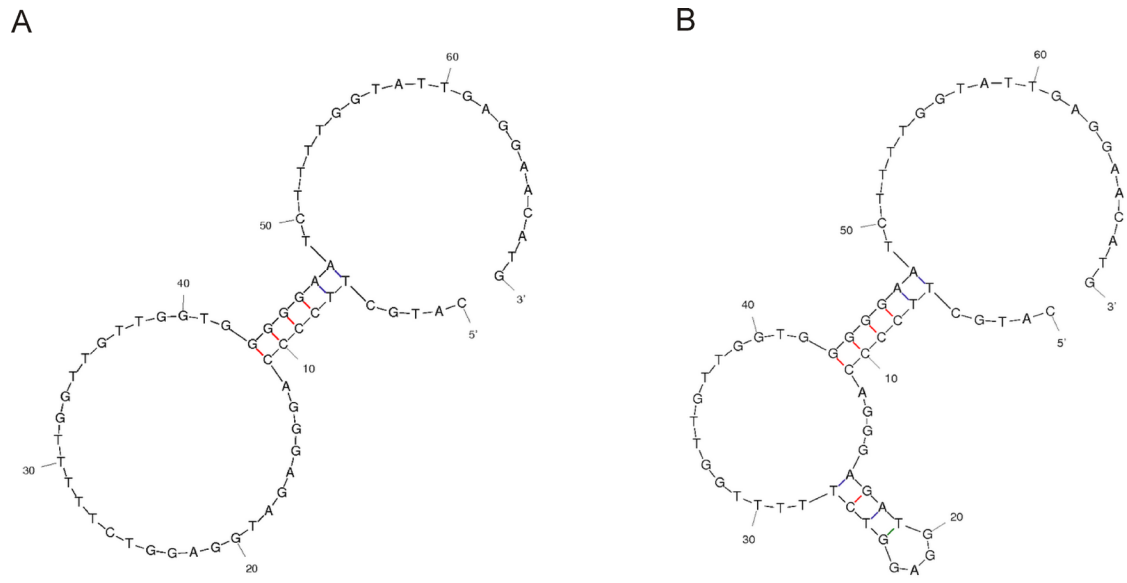

Figure 6. Putative secondary structures of Ama1 DNA aptamer. The structures were predicted using the mfold web server. (A) structure 1, (B) structure 2.

loides syndrome. One of the key elements of patient hospitalization are toxin removal from the digestive tract and circulatory system and combating symptoms. In the treatment of *A. phalloides* poisoning patients the Molecular Adsorbent Recirculating System (MARS), Therapeutic Plasma Exchange (TPE), as well as activated charcoal (Bergis *et al.*, 2012; Wang *et al.*, 2014) are used. Moreover, the administration of high-dose penicillin, cimetidine, silibinin (Kostek *et al.*, 2012; Ward *et al.*, 2013) or vitamin C (Poucheret *et al.*, 2010) can be also implemented. The lack of specific anti-α-amanitin antidote still inspires new research aiming to identify alternative substances that could neutralize effects of amatoxins poisoning more efficiently. For example, polymyxin B was shown to effectively reduce the mortality of mice caused by α-amanitin (Garcia *et al.*, 2015). Moreover, the results of another study suggests that *Cynara scolymus* leaf extract had a healing effect on α-amanitin induced hepatic toxicity (Kaymaz *et al.*, 2016). Interestingly, recently the information about the development of an α- and β-amanitin-binding adsorbent was published (Tan *et al.*, 2016).

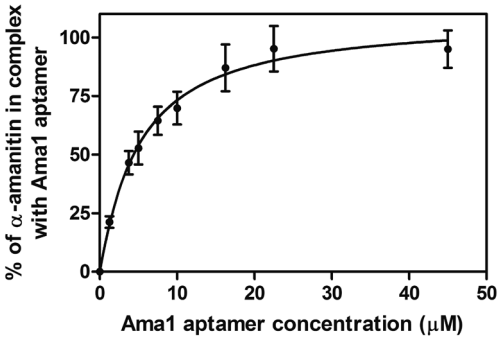

Figure 7. Determination of the Ama1 aptamer/α-amanitin complex dissociation constant using the pull-down assay.
5′-biotinylated Ama1 aptamer was immobilized on HCSA resin (concentration 0–45 μM). Subsequently it was incubated with 14 nM α-amanitin. Next, the amount of unbound amanitin was determined using the Buhlmann Laboratories AC Amanitin ELISA Kit. Using the equilibrium concentration of Ama1 aptamer/α-amanitin as a function of free Ama1 aptamer (total Ama1 aptamer), the dissociation constant (Kd) of the analyzed complex was calculated, with the help of GraphPad Prism. Results are the mean of three measurements. Error bars represent the standard deviation.

Taking into account that the antidote for α-amanitin poisoning is not available, the aim of this project was the development of a DNA aptamer that could be used to capture α-amanitin from blood, for example as a component of an extracorporeal device. In contrast to our expectations, the dissociation constant for the α-amanitin/Ama1 aptamer complex was in the μM range, similarly as reported for example for the aptamer/ATP complex (Huizenga & Szostak, 1995). The α-amanitin/Ama1 aptamer complex K_d value determined by pull-down assay should be verified in future by other more precise methods e.g. isothermal titration calorimetry or Biacore analysis. Moreover, the K_d value suggests that the strength of binding between the tested molecules is not sufficient for medical applications of the present version of the developed aptamer. Taking into account that the number of ssDNA library molecules used for aptamer selection did not provide saturation of all possible combinations of the aptamer random region, we cannot exclude that a ssDNA molecule characterized by stronger affinity to α-amanitin could be identified if other pools of ssDNA library molecules would be used.

Aptamers are a common subject of studies in different fields for example analytics and medicine (James, 2000; Keefe *et al.*, 2010). The most spectacular and successful example of therapeutic application of an aptamer-based product is Macugen, which is used for treatment of wet age-related macular degeneration (Ng *et al.*, 2006; Vavvas & D'Amico, 2006). There are also many other examples of aptamers currently under clinical investigation (Wang *et al.*, 2011; Zhu *et al.*, 2015). Moreover, the issue of toxin inactivation by aptamers is also investigated and the studies relating to this topic were listed in a recent review article (Hong & Sooter, 2015). Among the presented examples are an RNA aptamer neutralizing anthrax toxin produced by *Bacillus anthrax* (Lee *et al.*, 2015), a DNA aptamer selected against *Staphylococcus aureus* α-toxin (Vivekananda *et al.*, 2014), aptamer S8-7 recognizing whole cell of *Salmonella* Typhimurium (Dwivedi *et al.*, 2013) and a DNA aptamer against Brevetoxin-2 from *Karenia brevis* (Tian *et al.*, 2016).

CONCLUSIONS

In this study, we demonstrated that the Ama1 aptamer can capture α-amanitin from aqueous solution. The analysis of aptamer stability in blood, as well as improvement of the affinity between the Ama1 aptamer and α-amanitin are necessary before trials of Ama1 aptamer application for α-amanitin elimination from blood.

Competing financial interests

The authors declare no competing financial interests.

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