Mycotoxin Detoxifying Aptamer Technology

[QUESTIONS (Q) & ANSWERS (A)]

Q. What are aptamers?

A. An aptamer is a single-stranded DNA or RNA molecule that is able to bind to a target molecule such as a mycotoxin with high affinity and specificity. An aptamer could also be a peptide. Aptamers interact with and bind to their targets through structural recognition, a process similar to that of antigen and antibody reaction. These single-stranded, synthetic oligonucleotides fold into 3-dimensional structures capable of binding non-covalently with high affinity and specificity to a toxin with a dissociation constant usually in the Pico to Nano molar range. Aptamers specifically bind to various molecular targets such as small molecules, proteins, nucleic acids, and even cells and tissues. The majority of the clinically tested aptamers act as inhibitors, by binding and inhibiting enzyme activities or protein-protein interactions. Aptamers have also been used to target the delivery of nanoparticles, therapeutics, and imaging agents. As more researchers devote themselves to rational aptamer development, next generation aptamer-based therapeutics with superior biological functions and pharmacokinetic profiles are highly anticipated. Furthermore, factors such as the market demand for cost-effective treatments, technological advancements in synthesis and formulation, and expiration of the SELEX patent, provide a strong impetus for the development of this promising class of therapeutics for both humans and animals.

Q. How do aptamers (chemical antibodies) compare with antibodies?

A. Aptamers are produced chemically or biologically in a readily scalable process, whereas antibodies are produced using mammalian cells or transgenic plants/animals. The development of procedures for the production of smaller quantities of less expensive antibodies in transgenic plants and animals could take 2-3 years at least 10 months for the production of antibodies in grams (50-100) quantities. On the other hand, chemical synthesis of aptamers in grams or kg quantities needs only 2 weeks. The chemical process is not prone to viral or bacterial contamination. Aptamers are essentially non-immunogenic. While typical antibody size is 180 kDa, the size of an aptamer is usually less than 30 kDa. Aptamers can usually be reversibly denatured, whereas degradation of antibodies is not reversible. The incorporation of phosphodiester bonds in aptamers greatly increases stability. Furthermore, conjugation chemistries for the attachment of functional groups can be readily introduced during synthesis of the aptamer. The original IP covering the SELEX (Synthetic Evolution of Ligands by Exponential Enrichment) technique has expired in 2014.

Q. Does pH and enzymes affect aptamers?

A. Aptamers without optimization are reasonably stable in a pH range of from 5.5 to 8. However, aptamers can be optimized for persistence under physiological conditions, such as a low pH or the presence of hydrolytic enzymes, during selection or during structure-activity relationship studies.
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[QUESTIONS (Q) & ANSWERS (A)]

**Q.** Does temperature affect aptamers?

**A.** Unlike antibodies, aptamers withstand wide range of temperatures. Aptamers are known to exhibit excellent stability under extreme heat (over 60°C) conditions as they can be renatured if denatured without losing the functional integrity. Aptamers can be stored indefinitely at any temperature if they are lyophilized and rehydrated upon use. Furthermore, aptamers can be optimized during selection to withstand a wide range of temperatures if necessary.

**Q.** How do you protect aptamers from nuclease degradation?

**A.** DNA and RNA based natural aptamers being readily hydrolyzed by nucleases must be stabilized against cleavage by endo- and exonucleases if they are intended to be used in a biological environment, and particularly for therapeutic use. Since these nucleases are abundant in biological fluids, several modifications of nucleotides have been introduced that protect the vulnerable 2’-position of the ribose-phosphate backbone against endonucleases (2’-fluoro, 2’-amino, 2’-O-methyl, etc.) and the terminal nucleotides against exonucleases. Lipi et al. (Lipi et al., RNA Biology, 13: 1232-1245, 2016) has reviewed the extensive literature published on the production and use of many different chemically modified nucleic acid aptamers. As indicated above, many different approaches have been used to stabilize aptamers. The use of mirror-image RNA or DNA that is not recognized by the ubiquitous plasma nucleases is a promising approach (Hoffmann et al., Curr. Protoc. Nucleic Acid Chem., 46: 1-30, 2011; Vater A. and S. Klussmann., Drug Discovery Today, 20: 147-155, 2015). In a mirror-image aptamer, also known as a Spiegelmer, all chirality centers are only located in the (deoxy)ribose sugars of the nucleotide and, as a result, are mirror-inverted. These L-(deoxy) ribose-based nucleotides do not occur in nature but are perfect mirror-images of the corresponding natural D-nucleotides (Vallazza, M. et al., Acta Crystallogr. D: Biol. Crystallogr., 60:1-7, 2004). Thus, a left-turning helix instead of the typical right-turn helix is observed (Bouchard et al., Annu. Rev. Pharmacol. Toxicol., 50:237-257, 2010). Because naturally occurring enzymes are stereo selective, Spiegelmers, unlike D configured aptamers, enjoy a native bio-stability. L-configured aptamers are ideal for use in biological systems as they have the same physical and chemical properties as D-aptamers but unlike these aptamers, are not degraded by nucleases. The original IP covering spiegelmers will expire in 2022.

**Q.** Do you have any animal feeding study data to show that Aflatoxin B1 (AFB1)-specific aptamer neutralizes the toxic effects of AFB1 in a chosen animal model?

**A.** AFB1 is a highly toxic mycotoxin which is prevalent in food and feeds worldwide. Mycotoxin Solutions Inc.’s ‘proof of concept’ research with AFB1 using Zebrafish as a model animal demonstrated the complete prevention of lethality in Zebrafish by an AFB1-specific aptamer. In
contrast, the lethality in Zebrafish when treated with only AFB1 was 82%. There was no lethality in Zebrafish treated with AFB1-specific aptamer alone. These data strongly support the proposal that aptamers can be designed to eliminate mycotoxin pathogenicity in animals. Aptamers have been utilized in preclinical studies using animal models to target the delivery of nanoparticles, therapeutics, and imaging agents. Also, MSI has a study protocol ready for a feeding study using ducklings which are more sensitive to AFB1 compared other animal models. MSI is currently looking for a contract research lab that is familiar with toxicity studies using ducklings and AFB1.

Q. What is the cost of producing aptamers?
A. The cost of 1 g aptamer in 2011 was $20 (Ni, X., et al., Cur. Med. Chem. 2011; 18: 4206-4214). Currently the cost should have come down to $10 or $5 as many companies in North America, Europe, and Asia Pacific Countries are producing aptamers in small or large scales for the following applications: (i) Research, (ii) Diagnostics, (iii) Synthetic Biology, (iv) Therapeutics, and (v) Data Storage. Also, aptamers would be lot cheaper if non-analytical grade aptamers with about 50% purity are purchased from oligo companies in China or India. MSI used less than 50% pure AFB1-specific aptamers in their Zebrafish studies. It was able to completely neutralize the AFB1 toxicity in Zebrafish compared to AFB1 alone causing 82% lethality. Because of the growing demand for aptamers for various applications, big oligo companies are now producing aptamers in kilogram and tonne quantities. Like any other products, because of the competition and largescale production, the bulk aptamer price will come down significantly. MSI predicts that aptamers will be used on a large scale as the agent that most effectively and economically controls and mitigates mycotoxicoses in livestock.

Q. What about the regulatory path for aptamer-based feed additives in Canada/USA/Europe?
A. Since the discovery of aptamers in the early 1990’s great efforts have been made to make them clinically relevant for cancer, HIV, cardiovascular diseases, infectious diseases, and macular degeneration. In the last two decades, many aptamers have been clinically developed as inhibitors for targets such as vascular endothelial growth factor (VEGF) and thrombin. The first aptamer based therapeutic was FDA approved in 2004 for the treatment of age-related macular degeneration and several other aptamers are currently in Phase 1, Phase 2 and Phase 3 clinical trials. With advances in targeted-therapy, imaging, and nanotechnology, aptamers are readily considered as potential targeting ligands as they can be readily modified chemically to form conjugates. Preclinical studies using aptamer-siRNA chimeras and aptamer targeted nanoparticle therapeutics have been very successful in mouse models of cancer and HIV. Although aptamers have been approved for internal use in humans, they will also be approved as being safe and effective when used as a feed additive for domestic animals. MSI’s initial studies with Zebrafish have demonstrated that aptamers are safe and highly effective at preventing toxicity of a potent mycotoxin AFB1. Further studies in consultation with the Canadian Food Inspection
Agency (CFIA) and the American Food and Drug Administration (FDA) are needed for demonstrating the safety and efficacy of aptamers as feed additives. MSI anticipates that aptamers will be approved for use as an animal feed additive after completing the safety and efficacy studies.

Q. Is MSI technology patent protected?

A. Yes. The use of aptamers as agents to bind mycotoxins in animal feed to prevent mycotoxin toxicity is an unique and revolutionary approach. Based on the thorough literature survey by Mycotox Solutions Inc (MSI) and its Patent Attorney, there is not a single scientific publication or a patent on the use of aptamers as binders to mitigate mycotoxin contamination in animal feeds. Currently, MSI has a published PCT patent application (No. PCT/CA2016/051083) entitled “Aptamers for Mycotoxin Detoxification”. This patent will enable MSI to exclusively control the use of aptamers as a detoxifier of mycotoxin contaminated feed.