# Understanding the mechanism of enzymatic mycotoxin degradation

Proteins are essential building blocks of the human body and of all living organisms. They fulfil a wide variety of functions with using merely 20 different amino acids. Antibodies defend the body from foreign molecules or pathogens; small proteins such as insulin are the means of communication between the cell; pumps transport molecules from and into cells; molecules such as collagen provide structure; proteins also act as storage for ions such as ferritin; and finally enzymes catalyse the transformation of small molecules during the catabolism of food molecules and the anabolism of complex molecules.

**by Dr Zsofia Bata,**
**Scientific Director, Dr Bata Ltd.**
**www.drbata.com**

The four levels of structural organisation are the key to fulfilling such plethora of functions. The primary structure of a protein is its amino acid sequence. Amino acids or residues are bound by strong covalent bonds according to the information encoded by the DNA. Hydrogen bonds between amino acids form the two particularly stable secondary structural elements: alpha helices and beta sheets. These structures are then folded into a compact globular shape, creating the pockets and binding sites for substrate molecules. Two or more polypeptide chains may come together to form the functional molecules, like in case of virus capsids that are formed of hundreds of individual protein molecules.

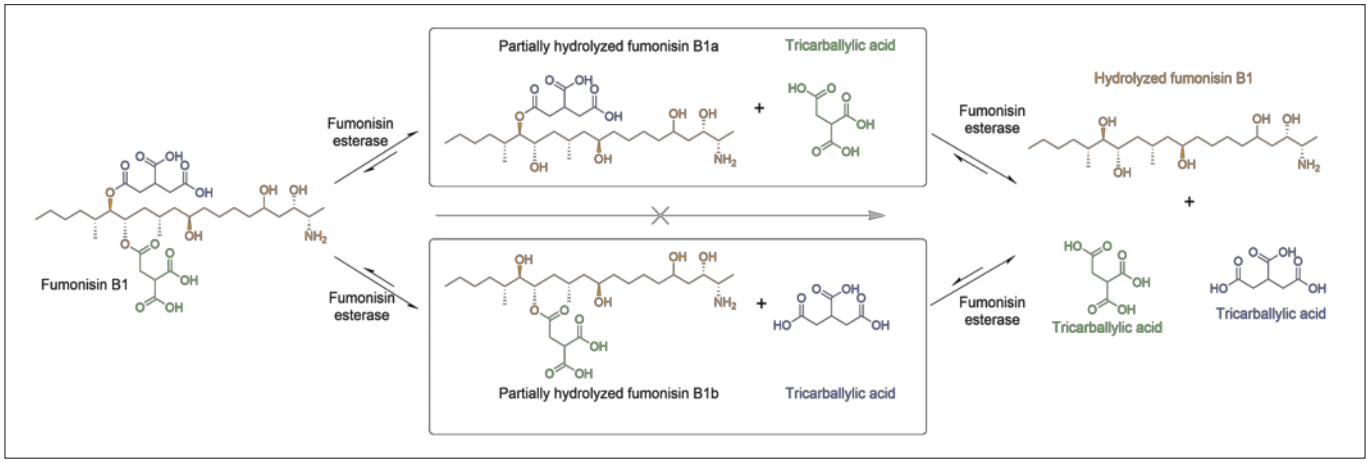
Enzymes or biocatalysts are special proteins that catalyse the transformation of a variety of compounds. All biological molecules are built up by enzymes during the anabolic processes. In parallel, solutions exist in nature for the biodegradation of these compounds.

Enzymes are the key players in digestion, they break down the biopolymer molecules, such as proteins or polysaccharides to oligomers, then continue the processing to produce monomers, that in turn can be absorbed by the cells. For example, proteases such as trypsin or chymotrypsin catalyse the digestion of polypeptides (proteins) to amino-acid monomers. Vertebrates endogenously produce trypsin and chymotrypsin in their digestive tract, thus can easily digest protein.

## Intestinal bacteria produce additional endogenous enzymes

However, some biopolymers are indigestible for certain phyla as the enzymes capable for catalysing such processes are absent from their genetic material, from their DNA. For example, the genetic information for the production of phytase is absent from vertebrates, thus these organisms cannot

**Fig. 1. Fumonisin esterase catalyse the hydrolysis of the two tricarballylic acid side chains of fumonisin B1.**



degrade phytic acid, an organic form of phosphorus commonly found in grains and oil seeds.

To overcome this challenge, ruminants developed a symbiotic relationship with bacteria in their gut, that can produce phytases. The phytases produced by the symbiotic bacteria break down the phytic acid to phosphate monomers, which in turn can be adsorbed by the ruminant.

Monogastric animals have no such symbionts, thus cannot naturally digest phytic acid. Modern nutrition has however overcome this by using biotechnologically produced exogenous phytases, to enable monogastric animals to digest phytic acid.

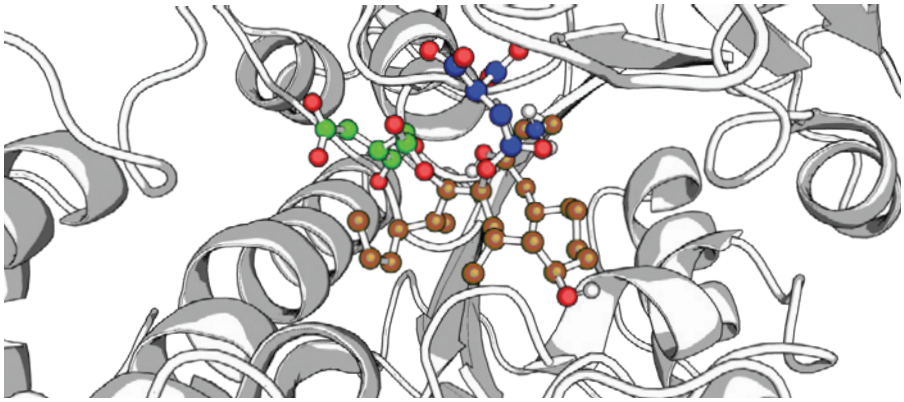


**Fig 2. Binding of fumonisin B1 by fumonisin esterase.**

## Enzymes produce and break down mycotoxins in nature

Mycotoxins, like all biological molecules are built up and can be broken down by enzymes.

Mycotoxins are toxic compounds synthesised by fungi growing on grains. Fungal enzymes synthesise thousands of secondary metabolites that may have atoxic effect on vertebrates. In practice however, only a handful of toxins are responsible for the majority of mycotoxicoses.

The acute and chronic problems caused by fumonisin are common in all animal species and all geographical regions worldwide, thus fumonisin will be used as an example to explore the key enzymes for its production and breakdown.

The enzymes FUM1 and FUM2 play key roles in the synthesis of fumonisin toxins by Fusarium moulds. Studies have shown a correlation between the expression of these proteins, and fumonisin production. The last gene, FUM19 is thought to transport the toxin to the surface of the mould, thus protecting the producing mould from its effects.

These Fusarium toxins are found all around the globe in a wide array of crops. The toxins continue to be present for long periods of time in grains and biological systems.

However, their amount does not appear to increase on a global scale. Thus, there must exist an organism that degrades these toxins. In fact, numerous organisms encode the enzymes of the fumonisin degradation pathway. The key enzyme of this is called fumonisin esterase, that catalyse the breakdown of fumonisin to the amino-pentanol backbone and two molecules of tricarballylic acid as shown in Fig. 1.

Fumonisin esterase and the fumonisin degradation pathway limit the amount of fumonisin present in the environment.

None of the mycotoxins are present in large quantities in the environment, thus the observations of the fumonisin example can be extended to all mycotoxins, in that biological degradation pathways to all mycotoxins already exist in nature.

This article will now examine in detail the example of fumonisin esterase, and how this enzyme may be employed as an exogenous enzyme for mycotoxin degradation within the digestive system of farm animals. Understanding the detailed reaction mechanism is key for the effective application of enzymes to the detoxification of fumonisins

## Understanding the detailed reaction mechanism of fumonisin esterase: structural and kinetic

The structural aspect investigates the four levels of structural organisation of fumonisin esterase, and how its substrate, fumonisin binds to this structure and which are the key residues for biotransformation, for catalysis. The kinetic aspect investigates how the reaction proceeds, and how changing environmental conditions influence the reaction.

The four levels of structural organisation of an enzyme is encoded in the DNA. Distant amino acids in the primary protein sequence come together in the three dimensional space to form the binding site of the enzyme. The active site of the enzyme is located in the bottom of the binding site, shielded away from the solvent and all environmental effects. This creates the unique and specific local environment that enables the catalysis of the chemical reaction.

Fumonisin, the substrate, forms weak, reversible secondary chemical bonds with the binding site of the fumonisin esterase (Fig. 2) and positions its ester bonds in the vicinity of the active site. As the optimal binding position is reached the enzyme cleaves the ester bonds and the hydrolysis occurs. Finally, the two products are released.

The reaction kinetics investigate the function of the enzyme on a measurable scale. In case of the fumonisin esterase, the key question is if the enzyme cleaves both ester bonds of the tricarballylic acid molecules simultaneously or sequentially (Fig. 1), and in the latter case if it most occurs on one site first followed by the second or both ester bonds are attacked with equal probability.

These questions are crucially important for the effective and complete detoxification of the mycotoxin. High performance liquid chromatography shows that the fumonisin biodegradation reaction proceeds via two intermediates, thus the direct path is unlikely, hence it is represented with a crossed out arrow on Fig. 1. Furthermore, HPLC results show, that

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**Table 1. Fumonisin B1 (FB1) and hydrolysed fumonisin B1 (HFB1) content (μg/g) of the pig faeces at the start (day 1) and at the end (day 28) of the trial.**

| Analyte | Negative control (NC) | | Positive control (PC) | | Trial group (T) | | p treatment | | p, NC-PC | | p, NC-T | | p, PC-T | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1d | 28d | 1d | 28d | 1d | 28d | 1d | 28d | 1d | 28d | 1d | 28d | 1d | 28d |
| HFB1 | 0.01 | 0.01 | 0.21 | 0.20 | 0.13 | 4.81 | 0.076 | 0.000 | 0.001 | 0.032 | 0.045 | 0.000 | 0.126 | 0.000 |
| FB1 | 0.03 | 0.06 | 1.01 | 14.16 | 0.54 | 0.84 | 0.288 | 0.000 | 0.003 | 0.000 | 0.118 | 0.106 | 0.148 | 0.000 |

*Continued from page 21*
the enzyme does not seem to have a preference for either of the tricarballylic acid side chains, as the intermediates are present in almost equal amounts in the reaction mixture.

Reaction kinetic investigations may also cover measuring the effects of temperature, pH or ion concentration on reaction rate.

## Exogenous fumonisin esterase counters the toxic effects of fumonisin B1 in piglet feeding trial

Fumonisin B1 (FB1) consumption results in oxidative stress, immune toxicity, reproductive toxicity, apoptosis and autophagy.

FB1 perturbs the sphingolipid metabolism due to its structural resemblance to sphingosine, thus the effects of FB1 directly correlates with the sphinganine/sphingosine ratio in blood serum.

In a 28 day in vivo trial, 36 female weaned piglets were randomly divided into three groups:

- A negative control group receiving normal diet without detectable mycotoxin content.
- A positive control group receiving feed contaminated with 4.37mg/kg fumonisin.
- A trial group receiving 4.37mg/kg fumonisin contaminated feed and Free Yeast F in 50g/t dosage.

Free Yeast F is a feed additive produced by Dr Bata Ltd. It contains exogenous fumonisin esterase, that degrades FB1 in the digestive tract of monogastric animals. In all other aspects the groups received equal feed and treatment.

Faeces samples were collected from each animal individually at the start and at the end of the trial. The FB1 and hydrolysed FB1 (HFB1) content of the faeces were measured by HPLC-MS/MS.

Results presented in Table 1 show, that at day one of the experiment, small quantities of FB1 and HFB1 appeared in the faeces of all groups, however the treatment had no statistically significant effect (p treatment 0.076 and 0.288). At the end of the trial, faeces of the NC group contained very low quantities of FB1 or HFB1.

As expected, faeces of the PC group at day 28 contained a high quantity of FB1; statistically significantly higher than the NC group or the T group. At the same time, a small amount of HFB1 was present in the faeces of the PC group, which was statistically significantly higher than the NC group, but lower than the T group.

The feed additive, Free Yeast F, fully degraded the FB1 from the feed, as the FB1 content of the faeces of the T group and the NC group showed no statistically significant difference (p=0.106).

Confirming the efficacy of the enzyme, the concentration of the reaction product HFB1 was statistically significantly higher in the T group than in the NC and PC groups. Accounting for the molar differences between FB1 and HFB1, approximately 60% of the toxin was found in the faeces of the T group compared to the PC group.

This suggests that the HFB1 may have been further degraded in the digestive tract.

## Conclusion

Enzymes are fascinating products of nature, and biotechnology has taught us how to use them in a targeted manner to produce safe food. Yet detailed understanding of their function is essential for the development and dosage of truly efficient products.

Free Yeast F contains exogenous fumonisin esterase that cleaves both ester bonds of fumonisin and results in 99% fully hydrolysed fumonisin within two hours in an in vitro model. The feed additive degrades fumonisin in vivo as well, with the use of the feed additive the concentration of fumonisin is reduced by up to 94% in the faeces of the animal. ■

References are available from the author on request