α-AMANITIN: A POSSIBLE SUICIDE SUBSTRATE-LIKE TOXIN INVOLVING THE SULPHOXIDE MOIETY OF THE BRIDGED CYCLOPEPTIDE

D. Michelot* and R. Labia
Laboratoire de Chimie et de Biochimie
des Interactions Fongiques et Microbiennes
URA 401 CNRS. Muséum National d'Histoire Naturelle
63, rue de Buffon, 75231-Paris Cedex 05, France

CONTENTS

SUMMARY

The highly hepatotoxic natural compound, α-amanitin, is noteworthy for its uncommon properties: high and specific affinity for the enzyme RNA polymerase type II, and, furthermore, high potency, severity and irreversibility of the poisoning. Considering the structural uniqueness of the tryptathionine bridge bearing a sulphoxide function, a few mechanistic and biochemical observations suggest that an enzyme-induced process may underlie the characteristics of the poisoning evolution which leads to the cytolysis of the hepatocytes.

I. INTRODUCTION

The green death cap, *Amanita phalloides* (Vaillant, Fries, *pro parte*, 1821, Quelet 1872) Secr., some of the various species of the genus *Amanita* (*A. virosa*, *A. verna*...), and, occasionally, a few species of the genus *Lepiota* and *Galera*, account each year in France and in the whole of Europe for nearly hundred cases of fatal mushroom intoxication /1-3/. It is important to point out the particularities of this intoxication: latency, severity and irreversibility of the poisoning. Besides the discovery and development of an

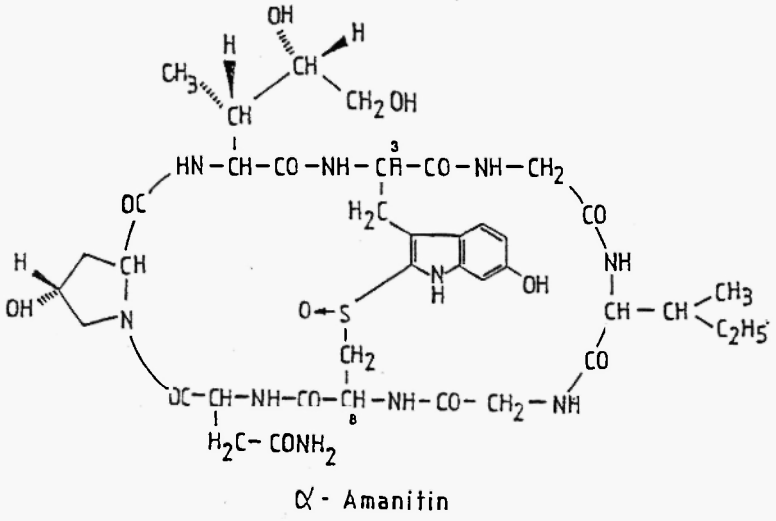


Fig. 1: Structural formula of α-amanitin.

efficient chemotherapy, we direct our attention, in this short article, to an understanding of the interactive entities (toxins and receptors) and at their mode of action.

Several chemical, biochemical and molecular biological studies of Wieland /4/ have marked the approach to the understanding of the phenomenon. They have isolated, identified, and sometimes synthesized the active compounds, i.e. phalloidins, amatoxins, virotoxins, and recently phallolysins. Among these different classes of compounds, we particularly turned our attention to the amatoxins, and, more precisely, to α-amanitin 1, since it is the major toxic component of the *Amanita* mushrooms, and because of the intrinsic features related to its toxicity.

II. STRUCTURE AND BIOLOGICAL PROPERTIES OF THE TOXIN

While phallotoxins generally exert their toxicity during the first six hours following ingestion, amatoxins exhibit their irreversible lethal toxicity after an average of twelve hours and never before the following six hours. It has been shown that they selectively bind to hepatic cells, then inducing the fatal hepatic cytolysis.

Amatoxins are cyclic octapeptides in which the indole ring of the tryptophan N° 3 is substituted at position-2 with a sulphur atom, which is part of the cysteine residue N° 8 already present in the peptide; this crosslinkage unit, called tryptathionine, is presumably achieved by a previous biochemical oxidation. In amatoxins, the oxidation state of the sulphur is typically a sulphoxide function, but, the synthetic sulphide corresponding to α-amanitin has been demonstrated to be as toxic as the natural compound /5/; this is likely, since, *in situ*, *S*-oxidation may represent the metabolic activation route to reactive/toxic metabolites /6/.

Among the various natural and semi-synthetic amatoxins, a few chemical modifications are present on the side chains of the aminoacids: proline, aspartic acid and dihydroxyisoleucine; since these modifications might involve a change of conformation or hydrophilic behaviour, one can presume that the shape of the molecule also plays an important role in its toxicity. These modifications could, of course, be responsible for an appropriate

hindrance or affinity towards the active site, or, presumably, for an ion complexing effect. In the present article, we will consider the chemical and mechanistic aspects of the inactivation of the receptor.

III. α-AMANITIN AND RNA-POLYMERASE TYPE II INHIBITION

α-Amanitin has been shown to be a potent inhibitor of mammalian RNA polymerase type II (or B, DNA dependent), especially during the elongation stage /7,8/. This toxin rapidly binds to the B3 subunit, non covalently, but with a strong affinity (Kd = 10^{-9} M). In mice, after *in vivo* administration of the toxin, the resulting protein synthesis is already decreased one hour later /9/. Nevertheless, the first clinical symptoms present an average of twelve hours after ingestion.

It should be mentioned that Wieland et al. /10,11/, have identified and isolated *in vitro* a new complex proteinaceous entity which has been proved not to be part of the enzyme. This entity shows an affinity for the toxin comparable with that of the enzyme. This protein is present in centrioles and spindle apparatus during mitosis of cells, and might have a predominent role in the hepatic cytolysis.

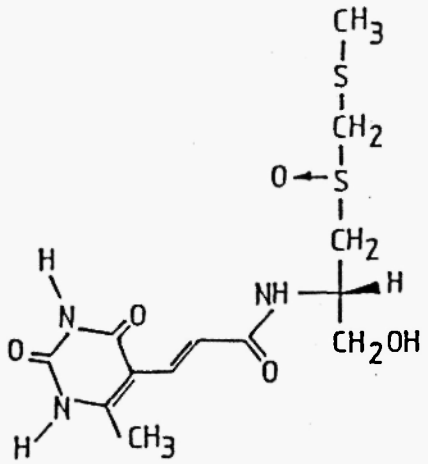
A few other results would lead us to presume that this RNA polymerase is not the only species involved in the intoxication process. Biochemical and toxicological data have shown that there is no direct relationship between the α-amanitin-enzyme affinity and the LD_{50} in mice /12/; for instance, the natural α-amanitin exhibits a four fold greater affinity for the enzyme than amanullin (a semi-synthetic derivative with a reduced dihydroxyisoleucine residue); the LD_{50} for α-amanitin is 0.1 mg/kg, while the LD_{50} of amanullin is greater than 20 mg/kg. Therefore, it seems that, *in vivo*, the correlation between affinity for the enzyme and toxicity is not a direct one.

IV. α-AMANITIN AND SUICIDE SUBSTRATE MECHANISM: IMPLICATION OF THE SULPHOXIDE GROUP

In the preceeding paragraph, we emphasized the fact that the toxins interact with RNA polymerase type II, but the toxicity

potencies correlate poorly with enzyme affinities; in addition, the *in vitro* inhibition is immediate, whereas, *in vivo*, the first clinical symptoms appear twelve hours after ingestion /13/. Among the different explanations, we could presume the importance of the following factors: α-amanitin and amanullin pharmacokinetics, a relevant initial liver protein pool, or a slow access to the target enzyme.

In another attempt to understand this process, we could also consider a substrate-induced irreversible inhibition of the receptor protein (an enzyme or another biomolecule). Several natural and synthetic toxins and drugs act as suicide substrates /14-17/; among them, we can report two cases in which the sulphoxide moiety of the compound is directly involved. Sparsomycin 2 is a sulphoxide-containing antibiotic of natural origin /18/ which is a novel "A-site" inhibitor of ribosomal protein biosynthesis /19/. It has been demonstrated that the sulphur atom needed the correct oxidation state for its peptidyl transferase inhibitory effect /20,21/. Similarly, the sulphoxide 2-amino-4-chloro-5-(*p*-nitrophenylsulphinyl) pentanoic acid 3 has been shown to induce mechanism-based inactivation of two enzymes: cystathionine γ-synthetase and methionine γ-lyase /22/.



Sparsomycin

Fig. 2: Structural formula of sparsomycin.

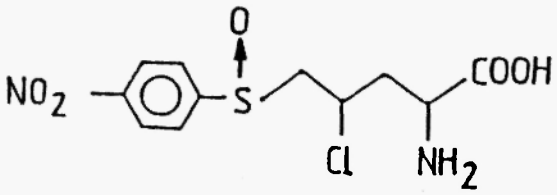


Fig. 3: Structural formula of 2-amino-4-chloro-5-(*p*-nitrophenylsulphinyl) pentanoic acid.

In both cases, the targets are pyridoxal phosphate-dependent enzymes. The proposed inactivation mechanisms necessitate the *in situ* generation of a sulphenate type entity from the sulphoxide which in turn gives the desired electrophilic species attack by an active site nucleophile.

In our hypothesis, we also suggest a suicide substrate inactivation (Figure 4). The starting step is the amidolysis at the modified cysteine N° 8 *N*-terminal position by an acylase. The first intermediate is the Schiff base that forms between the aldehyde group of pyridoxal and the amino group of the aminoacid (i.e. pyridoxaldimine). Subsequent mechanism depends upon a well known deprotonation-protonation process which leads to the formation of a carbanion adjacent to the Schiff base. At the end of the rearrangement, the covalent pyridoxal-α-amanitin adduct shows two reactive positions: on the one hand a highly reactive sulphenium ion (reaction pathway a), on the other hand, a Michael acceptor (reaction pathway b). Such a mechanism has already been described in the case of penicillin sulphoxide rearrangement which was elucidated by Morin et al. /23/; after opening of the sulphur-carbon bond, a sulphenic acid intermediate was confirmed by the work of Barton et al. /24/.

V. CONCLUSIONS

Both of the new reactive sites can then undergo attack by an enzyme nucleophilic group (i.e. lysine, histidine or serine) and therefore yield the intermediate inactivated enzyme (e.g. RNA polymerase type II), or any vitamin B6 dependent enzyme implicated in the hepatic detoxication mechanisms. It is interesting to note that

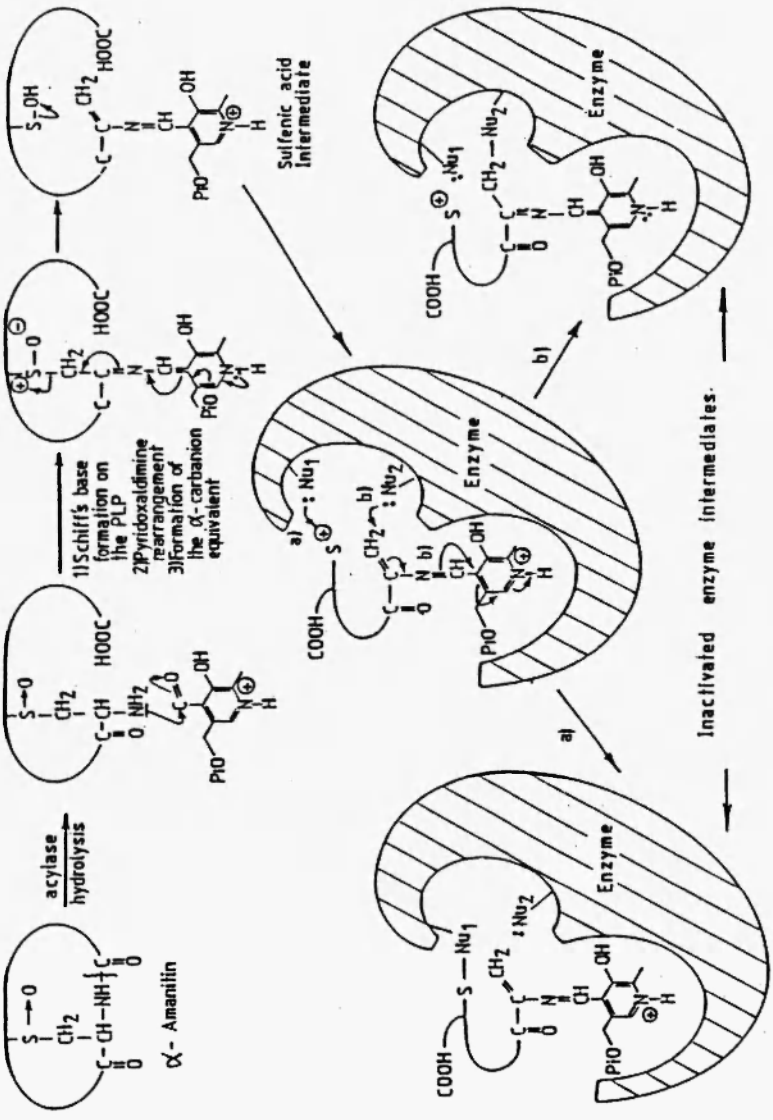


Fig. 4: Mechanism-based inactivation by α-amanitin.

in some cases, the clinical use of reducing agents as drugs, like ascorbic acid, thioctic acid or penicillin (probably via its degradation products: penicilloic acids, and penicillamine) have been successful in circumventing the intoxication, since they are able to scavenge the sulphenic acid entities and thus lower the toxicity.

Finally, experimental observations and assumptions suggest that besides its significant role, the RNA polymerase type II-α-amanitin interaction might not be the only factor inducing the toxicity and another receptor would be involved. The mode of action probably implicates a suicide substrate mechanism. Further biochemical investigations in the field of liver metabolism, and, more precisely, those concerning the fate of the sulphur atom of this class of hepatotoxin, would not only explain this process but might also yield chemotherapeutic hints or solutions to specific liver disease problems.

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