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Biochemical mechanism of action of mycotoxins

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Abstract - Symptoms of mycotoxicosis are a result of interactions of mycotoxins with functional molecules and subcellular organelles in the animal cell. The biological effects vary mainly according to the diversity in chemical structures of the mycotoxins, but also because of biological, nutritional and environmental factors. For a few mycotoxins, especially aflatoxin B_1, so many effects on biochemical systems have been described that only a fraction could be discussed here. Many of these effects are probably secondary to one and the same primary mechanism of action, others occur at such high concentrations that they will never happen in nature. It is striking that so many mycotoxins act on the DNA-RNA level. In most cases this gives rise to consequences on many levels and with pathological pictures caused by many co-operating factors. Less frequently, the effects are as specific as the inhibition of phosphoenolpyruvate carboxykinase by ochratoxin A. Thus no generalized mechanism can apply to all mycotoxins - not even for one and the same mycotoxin in different circumstances. The mechanism of action for a mycotoxin in mammalian cells may not be applicable to plants and microorganisms if metabolic activation is involved. Despite the many data accumulated so far, some of which have been accounted for in this review, the specific lesions responsible for the acute toxicity of many mycotoxins have not yet been identified. Much work therefore remains to be done in order to fully understand their mechanism of action.

INTRODUCTION

The diversity of animal and human diseases attributable to mycotoxins (mycotoxicoses) is due to the wide variety of chemical structures of the causative mycotoxins. The various biological effects of mycotoxins are attributed largely to the alteration of basic metabolic processes. Acutely affected processes are carbohydrate metabolism, mitochondrial functions, lipid and steroid metabolism and the biosynthesis of proteins and nucleic acids.

By understanding the mechanism of action of the mycotoxins on these processes, it may ultimately be possible to develop methods for the control and prevention of mycotoxin problems.

I have limited this discussion to the most extensively studied mycotoxins. Supplementary reviews have been published by Chu (ref. 1), Hsieh (ref. 2), Hayes (ref. 3), Stark (ref. 4),

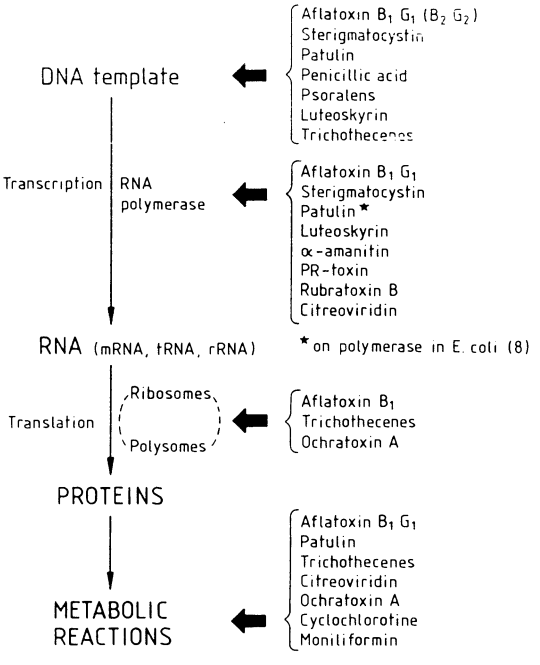


Fig. 1. Mycotoxins affecting major sites in RNA and protein synthesis

Busby and Wogan (ref. 5), Bamburg (ref. 6) and Moulé (ref. 7).

By means of a figure (Fig. 1) the major sites of mycotoxin action on RNA and protein synthesis can be indicated. Thus the primary mechanism of action of a mycotoxin may be to modify the DNA template, to impair the transcription process, or inhibit the translation process in protein synthesis. In certain cases the mycotoxin reacts directly with the enzyme protein or coenzyme. All these primary events may lead to secondary effects in terms of modified enzyme activities and, hence, changes in metabolic activity and regulation.

Effects on the DNA level

At least 14 mycotoxins are known in the laboratory to be carcinogens, including several aflatoxins, sterigmatocystin, cyclochlorotine, griseofulvin, and patulin. With the exception of cyclochlorotine they are all active on the DNA level (ref. 4).

Two types of interaction have been shown to occur between aflatoxins and nucleic acids. One is a non-covalent, weak and reversal binding (ref. 9), the other is an irreversible covalent binding requiring mammalian metabolizing systems (Fig. 2). Most of the carcinogenic and genetic activities of aflatoxins and related compounds such as sterigmatocystin have been observed with metabolically activated mycotoxins. Crucial for the covalent binding is the C_2-C_3 unsaturated bond, which means that aflatoxins B_1 and G_1 are more active than B_2 and G_2.

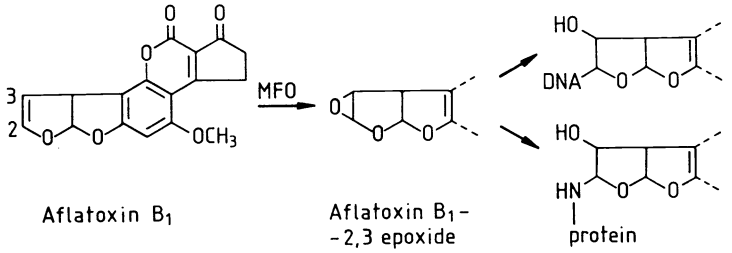


Fig. 2. Metabolic activation of aflatoxin B_1 (and G_1) by mixed function oxidase (MFO). The MFO in the endoplasmic reticulum of the liver catalyses the formation of the 2,3 epoxide of aflatoxin B_1 (ref. 10, 11). The epoxide formed can give rise to adducts with nucleic acids and proteins, which may render them biologically inactive (ref. 12, 13).

A strong correlation can be found between carcinogenicity, mutagenicity and the extent of covalent DNA binding among aflatoxins and their metabolites and precursors (ref. 4). Guanine in the DNA is the principal target for the attack of activated aflatoxins. The formation of mutations is made possible by the covalent binding to DNA (Fig. 2) which may lead to cancer (ref. 13).

A direct interaction between aflatoxin and nuclear DNA as a primary mechanism of action has been questioned by Kunimoto et al. (ref. 14) and by Niranjan et al. (ref. 15). Results published by Kunimoto et al. demonstrated that the aflatoxin B_1 induced inhibition of DNA synthesis (in HeLa and L 51784 cells) was due primarily to reduction of thymidine transport into the cell and not to decreased DNA template activity. However, as normal cells do not need external thymidine - they synthesize their own thymidine from uridine - the significance of this transport for the mechanism by which aflatoxins inhibit nucleic acids in normal cells may be questioned.

Recent work by Niranjan et al. showed that aflatoxin B_1 administered to experimental animals is covalently linked to liver mitochondria more efficiently than to nuclear DNA. The concentration of carcinogenic adducts in mitochondrial DNA remains unchanged even after 24 hours, possibly because of lack of excision repair. In consequence, mitochondrial transcription and translation remain inhibited for up to 24 hours. The authors suggest that this long-term inhibition of mitochondrial biosynthetic processes and possible mitochondrial mutational events may contribute to the carcinogenic process. In view of the large number of mitochondria per cell, this hypothesis is obviously open to certain objections.

There are several structural similarities between aflatoxin B_1 and sterigmatocystin, e.g. the dimensions and absolute configuration of the bisdihydrofuran moiety are very similar (ref. 16). This suggests a metabolic activation on the same site, the C_2-C_3 double bound, as in aflatoxin B_1. Experimental data support this (ref. 17). The mycotoxins may therefore operate by a common biochemical mechanism. Thus inhibition of DNA and RNA synthesis should be one of the primary modes of sterigmatocystin action.

A direct interaction of patulin and penicillic acid with cellular DNA has been consistently observed in appropriate bacterial and mammalian cell systems. Single- and double-strand breaks were induced by both toxins in HeLa and mammary carcinoma cell DNA (ref. 18, 19). An approximately fourfold larger total dose of penicillic acid was used to achieve carcinogenic effects in rats and mice, similar to those obtained with patulin (ref. 20, 21).

Even for another group of mycotoxins, the psoralens (furocoumarins), it is now apparent that they can react with DNA (photoreaction) which is very likely the primary mechanism by which these compounds function. There are no prerequirements for prior metabolic activation of the psoralens. The mechanism by which psoralens form adducts with DNA proceeds in three steps - one which can proceed in the dark, the other two by absorption of quanta of radiant energy (ref. 22).

Luteoskyrin is weakly carcinogenic in the liver of mice (ref. 23). It forms two types of complex with DNA in vitro - one rapidly with single-stranded nucleic acids, and the other slowly with double-stranded nucleic acids, including native DNA (ref. 24, 25).

The action of T-2 toxin on DNA was studied by Lafarge-Frayssinet et al. (ref. 26). No effect was observed on hepatic DNA, but single-strand breaks were induced in the DNA of lymphoid cells, both in vitro and in vivo. This is consistent with previous results (ref. 27-30). The lack of effect on liver DNA may be attributable to the presence of glutathione transferases in the hepatic cytosol capable of catalysing the conjugation of the trichothecene, since epoxides, like T-2 toxin, react with SH groups. It has been argued whether the effect observed with T-2 toxin is primarily on the DNA or whether it is an indirect effect. Pokrovsky et al. (ref. 30) have suggested that proliferation of lysosomes with the ensuing increase of hydrolytic enzyme activities, e.g. DNAases, in the lymphoid cells was responsible for the sensitivity of these cells to the toxin.

Other mycotoxins which cause single-strand breaks in DNA are patulin, penicillic acid and PR toxin (ref. 18, 31).

EFFECT ON RNA POLYMERASE

One prominent effect of aflatoxin B_1 administration to an animal is the decrease in RNA content and in RNA polymerase in the nuclei of the liver (ref. 9, 33-36). Aflatoxin B_1 was about three times as active as G_1, whereas B_2 had no effect at all (ref. 37), which led to the conclusion that the 2,3 unsaturated double bond in the dihydrofurfuran moiety was important for activity.

Other mycotoxins with a similar mechanism of action are α-amanitin (ref. 38-41), luteoskyrin (ref. 24, 42, 43), PR toxin (ref. 44, 45), rubratoxin B (ref. 46), sterigmatocystin (ref. 47), citreoviridin (ref. 48) and patulin (ref. 8). Schachtschabel et al. (ref. 49), studying various cell strains, found that luteoskyrin primarily inhibits DNA synthesis and only to a lesser extent protein and RNA synthesis by inhibition of RNA polymerase activity.

EFFECTS ON TRANSLATION LEVEL

Inhibitory effects on both the transcriptional and the translational level may result in inhibition of protein synthesis and a consequent change in metabolic activity and regulation. As for trichothecenes, e.g. T-2 toxin, their action on protein synthesis is complicated, since direct effects on both transcription and translation have been reported.

The trichothecenes inhibit protein synthesis as well as DNA synthesis in eukaryotic cells. As early as in 1968, Ueno and Fukushima (ref. 50) inhibited thymidine incorporation into DNA by up to 60% preincubating Ehrlich ascites tumour cells with nivalenol.

Simultaneously, protein synthesis was markedly suppressed (ref. 50). Ueno (ref. 51) and McLaughlin et al. (ref. 52) have suggested that although DNA synthesis is inhibited to the same degree as protein synthesis, the primary mechanism of trichothecene activity is probably disruption of protein synthesis.

A classification of the trichothecenes into different protein synthesis inhibitor groups has been reviewed by Doyle and Bradner (ref. 53). The toxins act either on the initiation or the elongation-termination steps (Fig. 3).

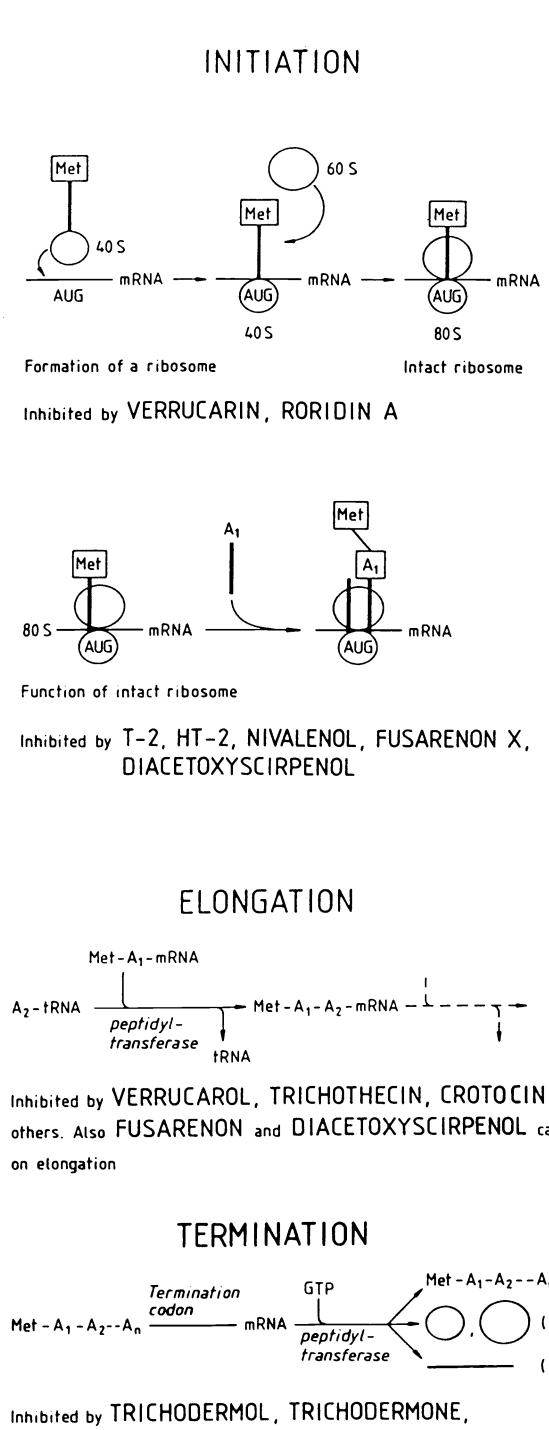


Fig. 3. The effect of trichothecenes on translation. Genetic messages coded by RNA are translated by the ribosomes into protein structure in three steps.

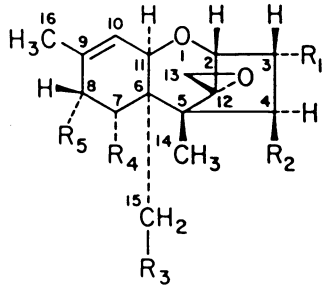
(A) Initiation: In eukaryotic cells, polypeptides begin with a methionine residue (Met), donated by a special initiating methionyl - tRNA which can only attach to an AUG-initiation codon in the mRNA. The trichothecenes in (A) inhibit either the formation of the 80S complex or the function of the intact ribosome (ref. 54). Breakdown of polyribosomes is another mode of action of these initiation inhibitors (ref. 52, 55-58).

(B) Elongation: An aminoacyl-tRNA complex, specified by the next coding triplet in the mRNA, is bound to the 80S complex and a peptide bond is formed, catalysed by peptidyl transferase. This happens repeatedly until the polypeptide chain is completed. The probable mode of action of trichothecenes on elongation is interaction with the peptidyl transferase centre on the 60S subunit an suppression of peptide bond formation (ref. 59- 61).

(C) Termination: The termination of the polypeptide is signalled by a "nonsense triplet" and the polypeptide is released in free form by hydrolytic cleavage from its terminal tRNA. Trichothecenes interfere with termination in the same way as with elongation, by interaction with the peptidyl transferase centre on the 60S ribosomal subunit. They also prevent release of the polypeptide, as they can suppress hydrolysis of the peptidyl-tRNA at termination (ref. 60, 61).

The most toxic trichothecenes, e.g. verrucarin, mono- and diacetoxyscirpenol, T-2 toxin and nivalenol, act as inhibitors of the initiation step partly by binding to the 60S ribosome unit and partly by inhibiting the peptidyl transferase activity (ref. 54), whereas the others, e.g. deoxynivalenol, trichothecin, and verrucarol, act on the elongation-termination step. Wei et al. (ref. 59) have shown that trichodermin acts exclusively on the peptidyl transferase required for termination. The expression of either elongation or initiation inhibition type behaviour depends upon the concentration of certain trichothecenes (ref. 62). Thus Steele et al. (ref. 63) showed that diacetoxyscirpenol inhibited initiation at a dose producing up to 70% inhibition of protein synthesis, but both initiation and elongation at higher doses.

The toxicity of a particular trichothecene depends, apart from the presence of the 12,13-epoxy-ring and the C9-10 double bond, on the number and positions of the hydroxy groups and on the type of the esterifying acids. Thus little or no substituent at the C_4 makes the trichothecene a termination inhibitor. An ester group at only the C_4 position makes the compound an elongation inhibitor. With another ester group on the C_{15} the compound may inhibit initiation besides elongation. If C_3 or C_4 are acetylated besides esterification of C_{15}, the trichothecene acts as an initiation inhibitor.



Aflatoxin B_1 is another mycotoxin that suppresses protein synthesis (ref. 64), whereas B_2 and G_2 have not been found to inhibit in vivo synthesis. Polysome disaggregation may be the mechanism by which aflatoxin B_1 disrupts protein synthesis. The simultaneously formed monosomes lack RNA and peptidyl-tRNA and contain only low levels of tRNA (ref. 65).

Recently, ochratoxin A has also been included in the group of mycotoxins acting on the translation level. The mycotoxin acts by degrading the mRNA coding for the phosphoenolpyruvate carboxykinase in the kidney (ref. 66). Furthermore it has been established why ochratoxin A (but not ochratoxin α) is toxic. It was found that ochratoxin A may act as an analogue of phenylalanine as regards aminoacylation of tRNA in bacteria (ref. 67, 68). This reaction is catalysed by phenylalanyl-tRNA synthetase and is fundamental for all living organisms. Ochratoxin A competitively inhibits the formation of phenylalanyl-tRNA. The affinity of the phenylalanyl-tRNA synthetase for ochratoxin A was, however, about one-threehundredth of that of phenylalanine and the question then came up as to how ochratoxin A toxicity arises in intact animal cells. Part of the answer is, according to Creppy et al. (ref. 69, 70) that the mammalien synthetase has a high affinity for ochratoxin A and a low affinity for phenylalanine. Another contributory factor may be that ochratoxin A and its metabolites, some of which are still toxic, accumulate inside cells (ref. 70. For a review, see Roschenthaler et al. (ref. 71).

INHIBITION BY MYCOTOXINS OF KEY ENZYMES IN METABOLIC PROCESSES

Major biochemical effects of mycotoxins involve the modification of normal metabolic and other vital processes. The mode of their action appears to be based primarily on their ability to interact with macromolecules, subcellular organelles and organs. Many of these mycotoxin-induced effects may be derived from and secondary to their disruption of nucleic acid or protein synthesis.

Carbohydrate metabolism

The effect of mycotoxins on carbohydrate metabolism is generally discerned as reduced hepatic glycogen and increased blood glucose levels. Mycotoxins that can cause these effects are aflatoxins, ochratoxin A, rubratoxin B, cyclochlorotine and citreoviridin.

Aflatoxin B_1, cyclochlorotine and citreoviridin all decrease the liver glycogen level, by inhibiting biosynthetic enzymes such as glycogen synthetase and by increasing the activity of enzymes metabolizing glycogen precursors, e.g. the NADP-reducing enzyme glucose 6-phosphate dehydrogenase (ref. 72, 73).

In their study, Shankaran et al. (ref. 72) injected 2.7 mg aflatoxin B_1 in one-day-old chicks and noted, besides the above-mentioned effects, also decreased

activities of glycogen phosphorylase, phosphoglucomutase and glucose 6-phosphatase. It is difficult, however, to forecast how these effects may influence the glycogen level, as the first two enzymes mentioned catalyse glycogen breakdown and the third enzyme, glucose 6-phosphatase, catalyses glucose 6-phosphate elimination. These contradictory results were probably secondary effects, as neither aflatoxin B_1 nor its metabolites inhibited the enzymes involved in glycogen metabolism in vitro (ref. 72).

Ochratoxin A is another mycotoxin which decreases liver glycogen (ref. 74, 75). Especially in adrenalectomized rats, liver glycogen was almost eintirely depleted after ochratoxin A treatment, but this effect could be eliminated by pretreating with hydrocortisone, suggesting an interaction of ochratoxin A with hormone balance mechanisms (ref. 74).

Pitout (ref. 76) described an inhibition of the phosphorylase system by ochratoxin A. However, as no single enzyme in this system was inhibited by the toxin, Pitout suggested that the protein kinase B activation by cAMP might be impaired. Heller and Röschenthaler (ref. 77) found that ochratoxin A competitively inhibited cAMP binding to bovine receptor protein, but only at very high concentrations.

In 1979 Meisner and Selanik (ref. 78) found that ochratoxin A, at comparatively low concentrations (2 mg/kg per day for 2 days), selectively decreased gluconeogenesis in kidney slices by depressing phosphoenolpyruvate carboxykinase activity by 50%. This enzyme is a link between the citric acid cycle and gluconeogenesis and makes possible transformation of citric acid cycle intermediates and their precursors via oxaloacetate - phosphoenolpyruvate into glycogen (Fig. 4). Recently, Meisner et al. (ref. 80) showed that this effect of ochratoxin A was caused by a decrease in the concentration of the mRNA coding for the phosphoenolpyruvate carboxykinase in the kidney rather than by an inhibition of the enzyme itself. There was no effect on transcription.

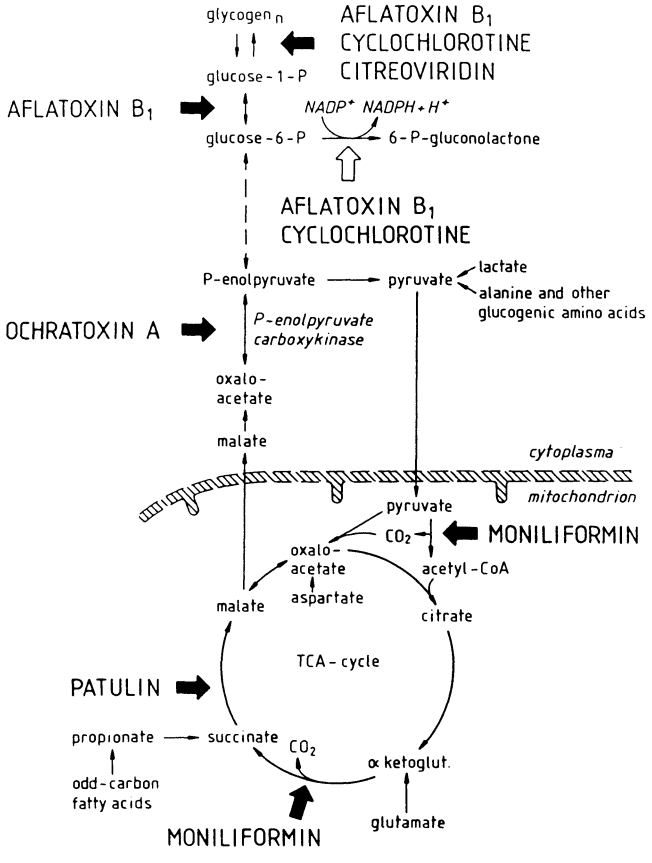


Fig. 4. Sites where mycotoxins interfere with carbohydrate metabolism. Aflatoxin B_1 and cyclochlorotine inhibit glycogen synthesis by decreasing glycogen synthetase and transglycosylase activities, enzymes which catalyse elongation and rearrangement of the glycogen molecule (ref. 72, 73). Another enzyme, citreoviridine, also inhibits glycogen synthetase (ref 48). Aflatoxin B_1 decreases the activity of phosphoglucomutase which reversibly converts glucose 6-phosphate into glucose 1-phosphate (ref. 72). Furthermore, aflatoxin B_1 and cyclochlorotine reduce hepatic glycogen by accelerating glucose 6-phosphate oxidation. Ochratoxin A inhibits synthesis of phosphoenolpyruvate carboxykinase, the enzyme which transforms oxaloacetate into phosphoenolpyruvate (ref. 78). Moniliformin inhibits oxidative decarboxylation of the two α-ketoacids pyruvate and α-ketoglutarate (ref. 79).

➡ inhibition

⇨ activation

Ochratoxin A is a nephrotoxin (ref. 81-83). The question remains open as to how this organ specificity is brought about. Galtier et al. (ref. 84) found high levels of ^{14}C-radioactivity in kidneys but only traces in liver of rats after applying labelled ochratoxin A. Creppy et al. (ref. 85), found that inhibition by ochratoxin A of protein synthesis was weak in liver compared with in kidney and spleen. One explanation for the weak effect in liver could be the high capacity of the liver to metabolize and, consequently, detoxify ochratoxin A to ochratoxin α. The fact that spleen is even more affected than kidney may be due to the high content of lymphocytes in this organ, as protein synthesis in lymphocytes is considered to be very sensitive to ochratoxin A (ref. 69).

Several trichothecenes react with proteins. Ueno and Matsumoto (ref. 86) found that fusarenon x, neosolaniol and T-2 toxin inhibited thiol-containing enzymes such as lactate dehydrogenase, probably by reacting with sulfhydryl groups.

Lipid metabolism

Lipid metabolism is affected by numerous mycotoxins, e.g. aflatoxins (ref. 87-89), ochratoxin A (ref. 90), citrinin (ref. 91), luteoskyrin (ref. 92), trichothecenes (ref. 93), penitrem A (ref. 94, 95) and rubratoxin B (ref. 96). Many of these give rise to accumulation of hepatic lipids. Aflatoxins have been shown by Chou and Marth (ref. 89) to cause this effect by increasing the cytosolic level of NADPH necessary for fatty acid synthesis in liver. But both aflatoxins and luteoskyrin also inhibit triglyceride transport, causing fatty liver (ref. 88, 92, 97). Even at comparatively low doses (0.6 ppm), aflatoxin B_1 affects not only transport of triglyceride but also of phospholipid and cholesterol. This led Tung et al. (ref. 88) to suggest that this effect on transport is a primary lesion, at least in chicken, and not secondary to the previously described effects on nucleic acid metabolism.

Effects on oxidative phosphorylation and other mitochondrial functions

Not unexpectedly, aflatoxins also fall in this category. Other mycotoxins for which mitochondrial effects have been described in the literature are ochratoxin A, luteoskyrin-emodin, patulin, rubratoxin B, sporidesmin, moniliformin and citreoviridin.

Aflatoxin B_1 inhibits electron transport in mitochondria (ref. 98, 99), both ADP-coupled and DNP-uncoupled. Since the inhibition could be reversed by the electron acceptor TMPD, the site of inhibition must be situated between cytochrome b and c (site II in Fig. 5). Pai et al. (ref. 100) showed that aflatoxin M_1 and B_1 could act as uncouplers of oxidative phosphorylation. Obidoa and Siddiqui (ref. 105) showed that aflatoxin B_1 is an inhibitor of electron transport at the cytochrome oxidase level (Fig. 5).

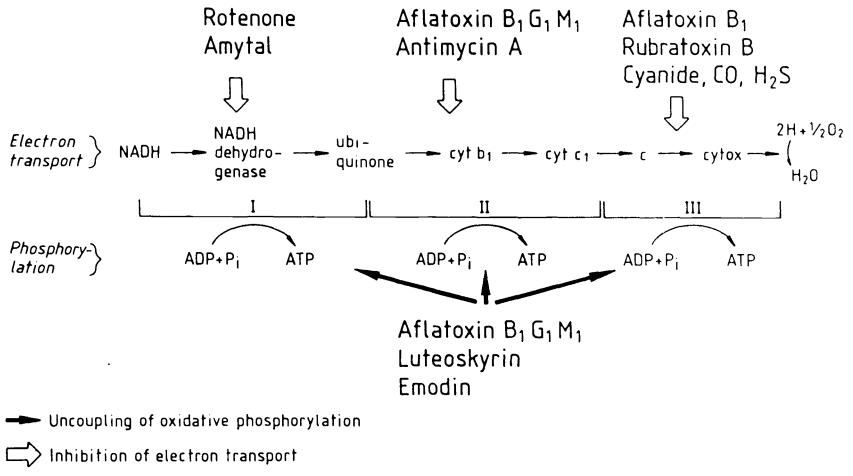


Fig. 5. Interaction of mycotoxins with oxidative phosphorylation. Aflatoxin B_1, G_1, M_1 (ref. 100) and luteoskyrinemodin (ref. 101) uncouple phosphorylation from electron transport. The aflatoxins also inhibit electron transport at site II (ref. 102, 103). Rubratoxin B inhibits electron transport at site III (ref. 104) and aflatoxin at the cytochrome oxidase (cyt.ox.) level (ref. 105).

Another inhibitor of mitochondrial respriation is luteoskyrin, possibly by affecting the transport system of the mitochondria (ref. 106). Kawai et al. (ref. 101), on the other hand, found that luteoskyrin as well as its monomer emodin, uncoupled oxidative phosphorylation.

Ochratoxin A (and ochratoxin α) was shown by Moore and Truelove (ref. 107) to inhibit state III respiration, but the necessary concentrations needed were high (8 µg/ml for a 50 % inhibition). Ochratoxin A competitively inhibited the mitochondrial uptake of the dicarbonic acids succinate and malate, of ADP and of inorganic phosphate (ref. 108). The interpretation of the authors of these results was that ochratoxin A is taken up by the mitochondria via an energy-dependent mechanism (ref. 109), thus acting as a competitive inhibitor of mitochondrial transport carriers, as there was no effect on respiration when submitochondrial particles were used.

Hayes and Hannan (ref. 110) demonstrated inhibition of mitochondrial respiration by another mycotoxin, rubratoxin B, which inhibits electron transport in the region of cytochrome c or c_1 and cytochrome oxidase (ref. 104). Also with this toxin, effects on the mitochondrial transport mechanism have been observed (ref. 111).

Several mycotoxins act as inhibitors of various ATPases. Citreoviridin is a potent inhibitor of mitochondrial respiration (ref. 112) and has a direct effect on ATPase activity. Aflatoxin B_1, patulin and rubratoxin B all inhibit the oligomycin-sensitive Mg^{2+}-ATPase in the inner mitochondrial membrane, that is, ATP synthetase (ref. 111, 113, 114).

The same toxins, as well as penicillic acid, also inhibit the Na^+/K^+ ATPase (Na^+, K^+ activated ATPase) (ref. 115), which catalyses the active transport of Na^+ and K^+ across the cell membrane.

A fusarium metabolite, moniliformin (ref. 79) decreases mitochondrial respiration by selectively inhibiting the oxidative decarboxylation of pyruvate and α-ketoglutarate. These effects may constitute the main molecular mechanism by which moniliformin acts (Fig. 4).

Another fusarium metabolite for which effects on mitochondrial respiration have been reported is T-2 toxin (ref. 116, 117). However, the dose needed to exert partial inhibition of mitochondrial respiration in subcellular systems was very high compared with that needed to cause irreversible damage to eukaryotic cells in culture. The significance of the observed effects on mitochondrial respiration is therefore doubtful.

The same objection is even more applicable to the experiments published by Pace (ref. 118). In order to obtain a 40% inhibition of mitochondrial respiration, 2.2 mM T-2 concentration (= 1000 ppm) was needed.

HORMONAL EFFECTS

Zearalenone, as well as its metabolites zearalenol α and β, can be considered a true estrogen, as it promotes estrus in adult mice (ref. 119). Most of the phenomena induced by zearalenone in animals are consistent with the effects produced by diethylstilbestrol or steroidal estrogens.

Steroid hormone activity has until recently been thought to be mediated by noncovalent binding to specific cytoplasmic protein receptors in the target cell. This complex was believed to be transported to the nucleus, being followed by interaction with the appropriate chromatin acceptor sites to induce selective RNA transcription (ref. 120-122). This hypothesis has been the basis for the following interpretation of zearalenone action. The binding of zearalenone to estrogen receptors is specific as regards structure and allows the 6'-ketone and 6'-hydroxyl derivative to compete with 17 β-estradiol at the receptor sites (ref. 123). Like 17 β-estradiol, zearalenone can effect an immediate translocation of cytosol-receptor complexes into the uterine nuclei (ref. 123). According to Boyd and Wittliff (ref. 124) zearalenone competitively inhibits association of 17 β-estradiol with its specific receptor sites. The translocation to the nuclei (ref. 123) is followed by a 3- to 4-fold increase in binding sites and a stimulation of protein synthesis.

Hepatic estrogen receptors have also been shown to react with zearalenone derivatives (ref. 125).

In a publication from 1980 (ref. 126), Tashiro and Ueno describe experimental results where zearalenone was claimed to compete with 17 β-estradiol in the binding to the uterine cytosol and nuclei receptors, both in vivo and in vitro. After administration of zearalenone to immature rats and isolation of uterine nuclei, an increase in RNA polymerase activities was obtained.

Today, we must probably reconsider the "two step" model of steroid hormone-receptor interaction, proposed in 1968 by Gorski et al. (ref. 127) and Jensen et al. (ref. 128). A number of reports have appeared which are difficult to reconcile with their model. One crucial result presented by Welshons et al. (ref. 129) is that empty estrogen receptors do reside within the cell nucleus of steroid-sensitive cells. This led them to propose that "in intact cells, there is no nuclear translocation of receptor as part of the steroid response, but rather an increase in receptor affinity for nuclear elements". Their finding that estrogen receptors reside exclusively in target cell nuclei of estrogen-sensitive tissue is supported by King & Greene (ref. 130), who used immunocytochemical staining with the aid of a monoclonal antibody generated against the estrogen receptor protein and found specific staining to be confined to the nucleus of all stained cells, including uterus and liver cells. One explanation for earlier conclusions that nuclei contain no unfilled receptor sites may be that they were solubilized during the course of nuclear isolation. A technique to overcome this problem has been described by Welshons et al. (ref. 129).

Many natural and synthetic derivates of zearalenone have been tested in rat and mouse uterotropic assays and their potencies have been expressed relative to zearalenone and diethylstilbestrol (ref. 131, 132). According to these lists it appears that the estrogenic potency of zearalenone increases by appropriate alterations of the structure. Furthermore the isomeric configuration of zearalenone and its derivatives plays a primary role in determining the uterotropic activity of the compound (for a review, see Busby and Wogan (ref. 5). In this sense Hurd (ref. 132) suggested that compounds with estrogenic activity but without the steroid structure act like estrogen by means of remarkable similarities as regards molecule length and the nature of the organic functional groups at each end.

Zearalenone is rapidly transformed into α- and β-zearalenol by 3α-hydroxysteroid dehydrogenases in the liver (ref. 133, 134). These findings are supported by Thouvenot and Morfin (ref. 135) who showed that zearalenone competitively inhibits the 3α- and 3β-hydroxysteroid dehydrogenase transformation of 5α-dihydrotestosterone to 5α-androstanediols in human prostate gland. The formation of the two metabolites, α- and β-zearalenol, has been confirmed by Tashiro and Ueno (ref. 126), Tashiro et al. (ref. 136), Mirocha et al. (ref. 137) and James et al. (ref. 138). However, Tashiro & Ueno chose to give the name ZEN-reductase to the enzymes catalyzing the zearalenone reduction, although it seems rather farfetched to intimate the existence of a zearalenone-specific enzyme in vertebrate cells. As α-zearalenol is a three to fourfold more active estrogen compound than zearalenone (ref. 139) zearalenol formation involves activation.

The reduction of zearalenone by means of 3α-hydroxysteroid dehydrogenases may involve another mechanism of action of the toxin. The 3α-hydroxysteroid dehydrogenases (several multiple forms exist in both cytosol and microsomal fractions (ref. 140, 141)) are normally steroid-metabolizing enzymes (C_{19} to C_{26} steroids). Zearalenone is rapidly reduced to α-zearalenol in pig and conjugated with glucuronic acid in vivo (ref. 142). Soon after ingestion, conjugated zearalenone and α-zearalenol - but no free substances - can be detected in plasma. Consequently zearalenone, continuously administered in the feed and rapidly and constantly reduced to zearalenol, could constitute a serious obstacle to the metabolism of steroids catalysed by 3α- (and 3β-) hydroxysteroid dehydrogenases.

Williams and Rabin (ref. 143) and Blyth et al. (ref. 144) found that corticosterone, testosterone and estradiol, steroid hormones which may influence cellular protein synthesis, could facilitate the binding of polysomes to smooth endoplasmatic reticulum membranes. Williams and Rabin (ref. 143) also found that incubation in vitro with high concentrations of aflatoxin B_1 (40 µg/ml) caused a detachment of ribosomes from the membranes. Corticosterone - but not hydrocorticosterone - reduced the effect of aflatoxin B_1, possibly by competing with the toxin for the polysome-binding membrane sites. Blyth et al. (ref. 144, 145) and Sunshine et al. (ref. 146) also showed that the binding sites for testosterone to smoth endoplasmatic reticulum from female rat liver, and of estradiol to corresponding reticulum from male rat liver, were totally eliminated by aflatoxin B_1.

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