ACTION OF THE MYCOTOXIN OF Fusarium sporotrichiella v. sporotrichioides ON LYSOSOMAL MEMBRANES

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The effect of the mycotoxin of Fusarium sporotrichiella v. sporotrichioides (sporofusarin) was studied in vitro on the total and nonsedimenting activity of eight lysosomal enzymes: acid ribonuclease, aryl sulfatases A and B, β-glucuronidase, α- and β-galactosidases, β-glucosidase, β-acetylglucosaminidase, and α-mannosidase. Incubation of a suspension of rat liver lysosomes with an aqueous solution of sporofusarin led to inhibition of the total activity of the membrane-bound lysosomal enzyme β-glucosidase. In a dose of only 1.6×10^{-5} M sporofusarin caused a significant increase in the nonsedimenting activity of nearly all the enzymes; in a concentration of 1.6×10^{-3} M most of the enzymes of the lysosomal matrix (β-glucuronidase, β-galactosidase, aryl sulfatases A and B) were liberated almost completely into the supernatant, and nearly all the β-glucosidase also was liberated. It is postulated that damage to the subcellular membranes is an important component of the toxic action of sporofusarin.

KEY WORDS: mycotoxin (sporofusarin); lysosomes and their enzymes.

Among the so-called antialimentary substances found in food products an important place is occupied by a group of toxic metabolites produced by microscopic fungi: the mycotoxins [1, 16, 17]. The mechanism of the biological action of some mycotoxins either has not been studied or is not yet completely established. This group of mycotoxins includes the highly toxic metabolite of Fusarium sporotrichiella v. sporotrichioides.

This species of microscopic fungus reproduces in the usual way with the formation of a toxin during the period of the thaw in grain covered with snow in the winter [1, 2]. The main features of the pathogenesis of poisoning are degenerative and necrotic changes in the cells of the bone marrow, leading to its aplasia and accompanied by the development of leukopenia, anemia, and thrombocytopenia; on this basis the disease thus produced is called "alimentary toxic aleukia" [4, 7, 11, 12].

The purified highly toxic metabolite (sporofusariogenin glucoside, or sporofusarin) belongs to the group of sterols of the cyclopentanphenanthrene series and it contains a lactone ring with two double bonds [5-7]. (See scheme on the following page.)

The mechanism of its action has received little study.

Analysis of the results of toxicological and morphological investigations led the writers to postulate interaction with biological membranes as a possible mechanism of the biochemical action of sporofusarin.

In the investigation described below the effect of sporofusarin was studied in vitro on the membranes of subcellular structures, especially lysosomes.

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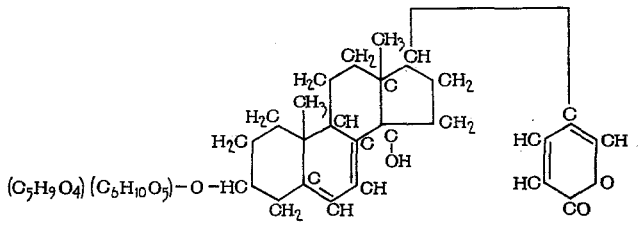
TABLE 1. Effect of Sporofusarin in vitro on Total and Nonsedimenting Activity on Rat Liver Lysosomal Enzymes (M ± m)

	Concentration of sporofusarin in sample (in M)	Protein	Acid ribonuclease E.C. 2.7.7.16	Aryl sulfatases A and B E.C. 3.1.6.1	β-glucuronidase E.C. 3.2.1.31	β-Galactosidase E.C. 3.2.1.23	β-Glucosidase E.C. 3.2.1.21	β-Acetylglycosaminidase E.C. 3.2.1.30	α-Galactosidase E.C. 3.2.1.22	α-Mannosidase E.C. 3.2.1.24
Total activity (in moles/min/g tissue)	Control	5,2±0,51	0,113±0,003	0,430±0,01	0,27±0,02	0,053±0,001	0,093±0,006	0,65±0,005	0,094±0,001	0,141±0,003
	$1{,}6\cdot10^{-5}$	5,4±0,5*	0,087±0,010**	0,384±0,02*	0,26±0,01*	0,050±0,001*	0,090±0,010*	0,56±0,010	0,073±0,007*	0,117±0,001
	$1{,}6\cdot10^{-4}$	5,1±0,6*	0,072±0,001	0,393±0,01*	0,25±0,01*	0,048±0,010*	0,080±0,003**	0,55±0,006	0,070±0,001**	0,105±0,008
	$1{,}6\cdot10^{-3}$	5,3±0,5*	0,068±0,002	0,414±0,03*	0,26±0,01*	0,050±0,001*	0,044±0,006	0,55±0,007	0,060±0,001**	0,108±0,001
Nonsedimenting activity (in % of total activity)	Control	30,2±3,8[2]	0	6,3±0,5	14,8±1,5	15,1±1,3	10,7±0,5	6,5±0,2	8,3±0,6	17,0±1,4
	$1{,}6\cdot10^{-5}$	35,2±5,6*	0	13,3±0,5	19,2±2,7*	24,0±2,0	12,2±0,9**	10,7±0,4	9,6±0,7*	22,4±1,7**
	$1{,}6\cdot10^{-4}$	41,2±5,9*	37,5±1,4	82,8±5,4	59,2±4,0	62,5±6,2	25,0±1,3	63,6±0,2	44,3±1,4	39,1±2,9
	$1{,}6\cdot10^{-3}$	62,3±9,4**	79,4±4,4	87,9±0,3	100,0±3,8	100,0±2,0	84,1±6,8	83,6±2,9	100,0±1,7	83,3±1,9

Note. Mean data of 4-6 experiments are given. *) $P > 0.05$; **) $P < 0.05$. In all other cases $P < 0.001$.
[1]Protein concentration in suspension of lysosomes (in mg/ml)
[2]Protein concentration in supernatant (in % of total protein of suspension).



EXPERIMENTAL METHOD

Male Wistar rats weighing 250-300 g were used. The animals were deprived of food for 12 h before sacrifice. Liver homogenates were prepared in 0.25 M sucrose containing 1 mm EDTA (pH 7.4) [10]. The lysosomal fraction (the fraction of particles sedimented at speeds of between 33,000 and 250,000 g·min) was isolated from the liver homogenate by differential centrifugation by the method of De Duve et al. [14], washed twice in 0.25 M sucrose with the same conditions of centrifugation, and the resulting sediment was resuspended in a ratio of 1 : 5. The final suspension of lysosomes contained particles from 0.2 g wet weight of liver tissue in 1 ml.

To 5 ml of the lysosomal suspension 0.1 ml of an aqueous solution of sporofusarin was added (final concentration 8×10^{-4}, 8×10^{-3}, and 8×10^{-2} M), and the samples were thoroughly mixed and then incubated for 30 min at 37°C. At the end of incubation 1 ml of the suspension was taken in order to determine the total activity of the enzymes, while the rest was again centrifuged for 25 min at 10,000 g. The nonsedimenting enzyme activity was determined in the resulting supernatant.

The protein content and activity of the following eight specific lysosomal enzymes were determined in the suspension of lysosomes and the supernatant: acid ribonuclease, aryl sulfatases A and B, β-glucuronidase, β-galactosidase, β-glucosidase, β-acetylglucosaminidase, α-galactosidase, and α-mannosidase. The activity of these enzymes was determined by spectrophotometric micromethods developed by Pokrovskii et al. [8-10]. The activity of the enzymes was expressed in micromoles of substrate converted by 1 gram wet weight of liver tissue in 1 minute.

The nonsedimenting enzyme activity was expressed as a percentage of the total activity.

The protein content was determined by the method of Lowry et al. [15], and the protein was previously dissolved in 0.2 N NaOH solution.

EXPERIMENTAL RESULTS AND DISCUSSION

The results of the investigation of the effect of sporofusarin on the total and nonsedimenting lysosomal enzyme activity in vitro are given in Table 1. Depending on the degree of change in the total activity, all the enzymes studied can be divided into three groups. The activity of the group 1 enzymes (acid ribonuclease and β-

glucosidase) was sharply inhibited by sporofusarin; the activity of the group 2 enzymes (β-acetylglucosaminidase, α-galactosidase, and α-mannosidase) was reduced by a lesser degree; the enzymes of group 3 were those whose activity was virtually unchanged by the toxin (β-glucuronidase, β-galactosidase, and aryl sulfatases A and B).

The marked solubilizing action of sporofusarin was revealed by the study of the nonsedimenting enzyme activity in vitro. In a dose of 1.6×10^{-5} M, which is close to the calculated concentrations of this toxin detectable in the liver of small laboratory animals poisoned with it (LD_{50}), sporofusarin led to a significant increase in the nonsedimenting activity of nearly all the lysosomal enzymes studied. In a concentration of 1.6×10^{-4} M the nonsedimenting activity of aryl sulfatases A and B was about 13 times higher than in the control and it accounted for 82.8% of the total enzyme activity in the original lysosomal suspension; the nonsedimenting activity of β-acetylglucosaminidase was 10 times higher than the control (63.6% of the total activity) and that of β-glucuronidase and α- and β-galactosidases was 4-5 times higher (59.2, 44.3, and 62.5% of the total activity, respectively). With a sporofusarin concentration of 1.6×10^{-3} M, corresponding to the dose of the toxin causing death of the animals during the first 10-12 h, complete liberation of most enzymes of the lysosomal matrix (β-glucuronidase, β-galactosidase, aryl sulfatases A and B) and the almost complete liberation of the membrane-bound enzyme β-glucosidase into the supernatant were observed.

The results emphasize the importance of a hitherto unstudied component in the toxic action of the mycotoxins, namely their damaging action on the membranes of the subcellular structures and, in particular, on lysosomal membranes. This fact must be taken into account by toxicologists, for damage to the lysosomal membranes must lead to the liberation of highly active hydrolases and must disturb the coordinated activity of the cell enzymes. Some of the clinical and morphological manifestations of sporofusarin poisoning can evidently be partly explained by involvement of the lysosomes in the pathological process. In particular, the extensive degenerative and necrotic changes in the tissues and the increased phagocytic activity of the leukocytes observed in poisoning by Fusarium sporotrichiella v. sporotrichioides [1-3, 13] have also been found as a result of the action of certain "labilizers" of the lysosomal membranes [18].

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