INHIBITORY EFFECT OF NATURALLY OCCURRING AND CHEMICALLY MODIFIED AMATOXINS ON RNA POLYMERASE OF RAT LIVER NUCLEI

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1. Introduction

The toxic cyclopeptides of the toadstool Amanita phalloides belong to two groups: amatoxins and phallotoxins [1]. α-Amanitin, the main component of amatoxins, was found to inhibit mammalian RNA polymerase (II) by binding to the enzyme and not to template DNA [2].

In the present experiments we studied the action on RNA polymerase (II) of other naturally occurring as well as chemically altered amatoxins (fig. 1). The effect on RNA polymerase of some phallotoxins was also tested.

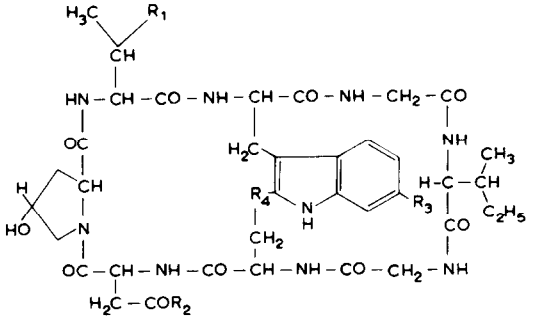

Fig. 1.

No.	Peptide	R_1	R_2	R_3	R_4
1	α-Amanitin	$CHOH\text{-}CH_2OH$	NH_2	OH	O = S–
2	β-Amanitin	same	OH	OH	same
3	*O*-Methyl-α-amanitin	same	NH_2	OCH_3	same
4	Amanin	same	OH	H	same
5	O-Methyl-aldoamanitin	CHO	NH_2	OCH_3	same
6	*O*-Methyl-demethyl-γ-amanitin	CH_2OH	NH_2	OCH_3	same
7	Dethio-α-amanitin	$CHOH\text{-}CH_2OH$	NH_2	OH	H– H
8	*O*-Methyldethio-α amanitin	same	NH_2	OCH_3	same
9	Deoxy-α-amanitin	same	NH_2	OH	S –

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Table 1
In vivo toxicity and in vitro action on RNA polymerase of amatoxins (fig. 1) (and in 2 phallotoxins).

Substance no. (see fig. 1)	Concentration (nM)	Inhibition of RNA polymerase (%)	i.p. LD_{50} in white mouse (mg/kg)
1	5	50	0.3
2	5	43	0.4
	10	69	
	20	88	
3	5	44	0.2
	10	56	
4	1	12	0.5
	5	31	
	10	57	
	50	81	
5	100	0	>50
	500	14	
	1000	38	
6	5	33	3
	10	42	
7	1000	0*	∞
8	10	0	∞
	1000	0	
9	(6)	50	∿1.0
Phalloidin	50000	0	2.0
Norphalloin	50000	0	1.2

* Tested by Prof. C.E. Sekeris in his system [8].

2. Materials and methods

RNA polymerase was solubilized from rat liver according to Jacob et al. [3] and its activity measured as described by Novello et al. [4]. *O*-Methyl-α-amanitin (*3*) was prepared by methylation of the 6-OH group of α-amanitin with diazomethane [5]. *O*-Methyl-aldoamanitin (*5*) was obtained by oxidation of *3* with periodate [6]. *O*-Methyl-demethyl-γ-amanitin (*6*) was obtained by reduction of *5* with $NaBH_4$ [6]. Dethio-α-amanitin (*7*) and *O*-methyl-dethio-α-amanitin (*8*) were prepared by treatment of *1* and *3*, respectively, with Raney-Nickel [7]. The methyl derivative *8* was also prepared from dethio-α-amantin (*7*) by methylation with diazomethane without further purification of the product.

3. Results

The results (table 1) show that there is a parallelism between in vivo toxicity of the compounds tested and their in vitro action on RNA polymerase. Amatoxins *1–4*, which are almost equally toxic, all inhibit RNA polymerase by about 50% at concentrations of about 5 nM; amanin (*4*) is a little less toxic. Derivative *6* is an exception since it causes an inhibition of the same order of magnitude but is 10 times less toxic. This discrepancy will be understood only when the causal connection between inhibition of nuclear RNA polymerase and the final symptoms of liver intoxication has been fully explained.

It is always possible that the inhibitory and toxic actions of amatoxins could be due not to the compounds themselves, but to trace contamination by other amatoxins during their preparation. Thus, the inhibition produced by the non-toxic aldehyde *5* at concentrations ca. 500 times those of compounds *1–4* may be

due to traces of compound *3* from which it was derived and whose presence in such low amounts could not be detected in the toxic assay. Dethio-α-amanitin (*7*) and *O*-methyl-dethio-α-amanitin (*8*) did not inhibit RNA polymerase even at concentrations of 1 μM. Previously, a sample of *7* had been shown to inhibit RNA polymerase by 50% at a concentration of 200 nM and was distinctly toxic (LD_{50} = 30 mg/kg body wt. of white mouse); the compound was found to be contaminated by traces of deoxy-α-amanitin (*9*), an intermediate product of desulfurization with Raney Nickel of α-amanitin, in which only the oxygen atom had been removed from the sulfoxide bridge [9]. Since the LD_{50} for compound *9* was about 1 mg/kg, the LD_{50} observed for *7* (30 mg/kg) indicates a 3% contamination of compound *7* by compound *9*. This value enables us to calculate a 50% inhibitory action of compound *9* at a concentration of 6 nM.

Similarly, inhibition of RNA polymerase by different samples of phallotoxins was also shown to be due to contaminating amatoxins, since synthetic norphalloin [10] and a sample of several-fold purified phalloidin showed no inhibition at concentrations as high as 50 μM.

References

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