TREATMENT OF ABRIN AND RICIN WITH β-MERCAPTOETHANOL. OPPOSITE EFFECTS ON THEIR TOXICITY IN MICE AND THEIR ABILITY TO INHIBIT PROTEIN SYNTHESIS IN A CELL-FREE SYSTEM

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1. Introduction

Recently we have presented evidence that the highly toxic plant proteins abrin and ricin inhibit peptide chain elongation in a cell-free system from rabbit reticulocytes [1, 2]. Our results, together with those of other authors [3–6], indicate that the two proteins exert their toxic effects *in vivo* by inhibiting protein synthesis.

In the present paper we wish to report that when abrin and ricin are treated with β-mercaptoethanol, their ability to inhibit protein synthesis in a cell-free system is strongly increased, while concomitantly their toxic effects *in vivo* are substantially reduced. Abrin and ricin both consist of two polypeptide chains held together by disulphide bonds [2], and the results suggest that the observed effects are due to separation of the two polypeptide chains which may have different biological functions.

2. Materials and methods

Abrin and ricin were extracted respectively from *Semen jequiriti* (the seeds of *Abrus precatorius*) and castor beans (the seeds of *Ricinus communis*) and purified to homogeneity by chromatography on a DE-52 column and on a CM-52 column [1–3].

Treatment of the toxins with β-mercaptoethanol was carried out by incubating solutions of toxin (0.5 mg per ml) in 5 mM Tris-HCl, pH 7.7, at 4° overnight with 1% β-mercaptoethanol. Polyacrylamide electrophoresis revealed that the reduction lead to dissociation of abrin and ricin into their two constituent chains.

Dilutions of the toxins were made in solutions containing 15 μg of bovine serum albumin per ml. Control experiments showed that the traces of albumin and β-mercaptoethanol present together with the toxins had no inhibitory effect on protein synthesis.

The protein synthesizing system consisted of a lysate from rabbit reticulocytes, prepared as described earlier [1, 2, 7].

Partially purified peptidyl-transferase was prepared by fractionated ammonium sulphate precipitation as described by Arlinghaus et al. [8]. Diphtheria toxin was kindly supplied by Dr. Unn Kjennerud, National Institute of Public Health, Oslo, Norway.

3. Results and discussion

In fig. 1 is shown that when 0.1 μg of abrin (fig. 1A) or ricin (fig. 1B) was added to the cell-free system from rabbit reticulocytes, protein synthesis was strongly inhibited within 10 min and in the presence of 1 μg of the toxic proteins the incorporation of radioactivity was inhibited within 5 min. The results are in agreement with our previous findings [1, 2]. The striking fact apparent from fig. 1 is, however, that when the toxic proteins had been pretreated with β-mercaptoethanol the reduced toxins inhibited protein synthesis much more strongly than the untreated toxins. Thus, 0.1 μg of reduced

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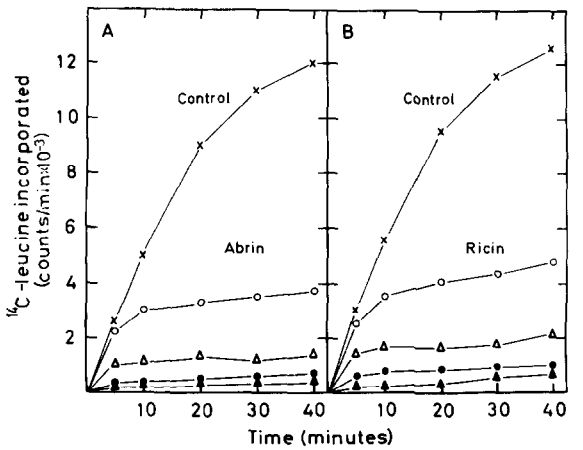

Fig. 1. Effect of β-mercaptoethanol on the ability of abrin (A) and ricin (B) to inhibit protein synthesis in a lysate from rabbit reticulocytes. Abrin and ricin were treated with β-mercaptoethanol as described under Materials and methods. One of the tubes was used as control (X–X–X),the others contained: 0.1 μg of intact toxin (○–○–○),1.0 μg of intact toxin (△–△–△), 0.1 μg of reduced toxin (●–●–●), 1.0 μg of reduced toxin (▲–▲–▲).

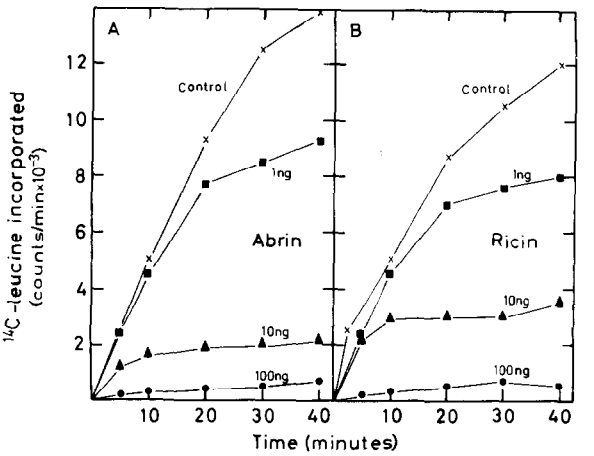

Fig. 2. Effect of different amounts of reduced abrin (A) and ricin (B) on protein synthesis in a lysate from rabbit reticulocytes. One of the tubes was used as control (X–X–X), others contained: 1 ng of reduced toxin (■–■–■), 10 ng of reduced toxin (▲–▲–▲), 100 ng of reduced toxin (●–●–●).

abrin and ricin exhibited stronger effects than 1 μg of the untreated toxins, indicating that reduction of disulphide bonds had increased more than 10-fold the inhibitory effect of abrin and ricin on protein synthesis.

The results in fig. 2 show that as little as 1 ng of the reduced proteins gave a clearly demonstrable inhibition of the protein synthesis. Since the total volume of the cell-free system was 0.5 ml and the proteins have a molecular weight of about 65 000 daltons, this corresponds to a concentration of about 3×10^{-11} M. It can be calculated that this concentration corresponds to one molecule of toxic protein per approx. 3000 ribosomes present in the system. This very high biological action per molecule supports the view that abrin and ricin may exert their effect on protein synthesis by some catalytic mechanism [1, 9].

The effect of reduction of abrin and ricin on their toxic effects in mice is shown in table 1. It is apparent that the β-mercaptoethanol treatment reduced the toxicity of abrin, as measured by the LD_{50} dose, by a factor of more than 50 and that of ricin by a factor of about 10. It is thus clear that reduction of the toxic proteins resulted in opposite effects on their toxicity *in vivo* and their capacity to inhibit protein synthesis in a cell-free system.

The above results are very similar to those obtained by other authors with diphtheria toxin, a protein (molecular weight 62 000) which after trypsin treatment consists of two peptide chains held together with disulphide bonds (for review see [9]). The inhibitory effect of trypsin-treated diphtheria toxin on protein synthesis in a cell-free system is strongly increased after treatment with thiols, whereas the toxic effects *in vivo* are strongly reduced [10–13]. We therefore examined whether abrin and ricin inhibit peptide chain elongation by a similar mechanism as diphtheria toxin. The latter toxin is known to act by catalyzing the incorporation of adenosine diphosphoribose from NAD into peptidyl transferase II, thus inactivating the enzyme [14]. The reaction may conveniently be followed by incubating labelled NAD with peptidyl transferase II and measuring the amount of radioactivity that becomes bound to acid-precipitable material [15]. The results in fig. 3 show that whereas small amounts of diphtheria toxin promoted rapid incorporation of radioactivity into acid-precipitable material, no incorporation above background was obtained with abrin and ricin.

Table 1
Toxicity in mice of intact and reduced toxins.

Toxin	LD_{50} dose in μg	
	Untreated toxin	Reduced toxin
Abrin	0.2	>10.0
Ricin	0.5	5.0

The LD_{50} dose was estimated by injecting increasing amounts of toxin intraperitoneally into mice weighting 20-30 g. Only deaths occurring within 3 days were recorded.

Similar results were obtained with the reduced toxins. It is therefore clear that abrin and ricin inhibit protein synthesis by a mechanism different from that of diphtheria toxin.

Recently it has been found that in trypsin-treated diphtheria toxin one of the peptide chains liberated after reduction inhibits protein synthesis whereas the other chain seems to be required for attachment of the toxin to the cell surface [10–13, 16]. On the basis of the structural similarity between trypsin-treated diphtheria toxin and abrin and ricin and their similar responses to reduction with thiols, it is tempting to suggest that in the case of the plant toxins a similar situation may apply. Thus, the loss in toxic action *in vivo* upon reduction of abrin and ricin suggests that the intact molecules are required for this activity. On the other hand, the striking concomitant increase in the ability to inhibit protein synthesis in a cell-free system suggests that the inhibitory activity may be associated with one (or both) of the free polypeptide chains. Preliminary experiments with the separated polypeptides of abrin, isolated after chromatography, indicate that only one of its polypeptide chains possesses the ability to inhibit protein synthesis.

Acknowledgements

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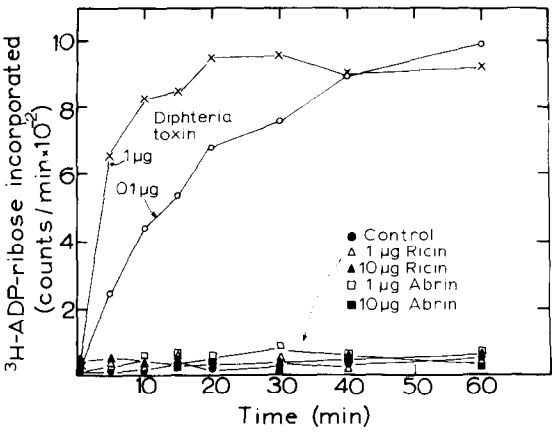

Fig. 3. The ability of different toxins to promote incorporation of adenosine diphosphoribose into peptidyltransferase II. 7 mg of partially purified transferase was mixed with 17.5 μCi of NAD-[^{3}H]adenosine (G) (specific activity 1.13 Ci/mmole, New England Nuclear, Boston) in 7 ml of 0.15 M KCl, 1 mM $MgCl_2$, 10 mM triethanolamine (pH 7.5) and 5 mM mercaptoethanol. The mixture was divided into 7 equal parts, one of which was used as control, whereas toxins were added to the others as indicated. The incubation was carried out at 28° and 100 μl-samples were removed and poured into cold 10% trichloroacetic acid at the times indicated.

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