RICIN – A POTENT INHIBITOR OF PROTEIN SYNTHESIS

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1. Introduction

Ricin, a protein present in castor beans (the seeds of *Ricinus communis L.*) is extremely toxic to animals and man [1]. The effect of ricin on living animals has been extensively studied [2–5]. The reduction in serum protein in treated animals has been taken as evidence that ricin might exert its toxic action by inhibiting protein synthesis [4], and recently it has been found that in Ehrlich ascites tumour cell cultures protein synthesis is inhibited more stongly than the RNA and DNA synthesis [6]. In the present study it is shown that in a cell-free system very small amounts of ricin completely inhibit protein synthesis. The results indicate that ricin interferes with the completion of already initiated peptide chains.

2. Materials and methods

Ricin was extracted at pH 3.8 from defatted castor beans and purified as described by Ishiguro et al [7]. The procedure involves precipitation of the protein with NaCl and $(NH_4)_2SO_4$ and chromatography on a DE-52 column and subsequently on a CM-52 column. Although the protein was not crystallized, it was obtained in a highly purified state, as shown by the fact that it was completely free from proteases and hemagglutinating activity [8, 9].

The protein-synthesizing system consisted of a lysate from rabbit reticulocytes, prepared as described by Lingrel [10]. The incubation mixture (0.5 ml) contained 0.2 ml lysate, 10 mM Tris-HCl, pH 7.4, 100 mM ammonium acetate, 2 mM magnesium acetate, 1 mM ATP, 0.2 mM GTP, 15 mM creatine phosphate, 50 μg/ml creatine phosphokinase, and amino acids in concentrations varying from 0.01 mM – 0.1 mM, except the labelled amino acids which were added in amounts described in legend to figures.

The incubations were carried out at 28°. At the times indicated 25 μl samples were removed and poured into 1 ml of 0.1 N KOH and incubated at room temp for 30 min. Trichloroacetic acid was then added to a final concentration of 10% (w/v). The precipitated protein was collected on Gelman glass fiber filters type A, and the radioactivity was counted in a Beckman LS-130 scintillation system as described elsewhere [11].

Sucrose gradient centrifugation was carried out by layering the material onto 0.5–1.0 M sucrose gradients made up in 0.15 M KCl containing 1 mM $MgCl_2$ and 10 mM triethanolamine, pH 7.5 and centrifuged in Rotor SW-50.1 at 130,000 *g* for 60 min. Fractions were collected by puncturing the bottom of the tubes, and after addition of 0.8 ml of H_2O, the absorbance at 260 nm was determined. Since some of the fractions contained a considerable amount of hemoglobin which may quench the samples during the scintillation counting, the heme was converted into a colorless compound by the addition of 1% H_2O_2 to the samples prior to filtration. Because of the high amount of protein present in some of the fractions, each fraction from the sucrose gradients was treated with 0.5 ml of Soluene for 60 min at 60° prior to the addition of the scintillation solution.

3. Results and discussion

The results in fig. 1 demonstrate that under the pre-

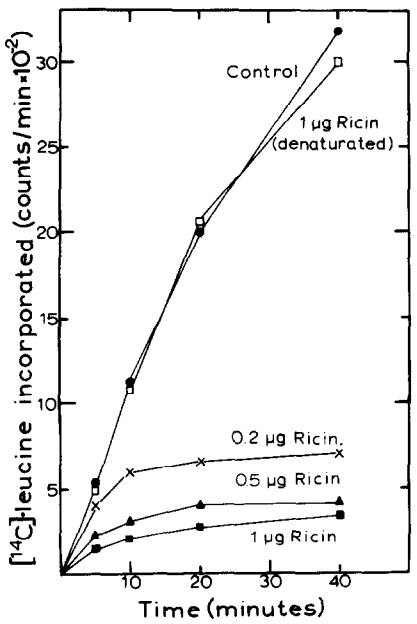

Fig. 1. Effect of ricin on protein synthesis in a lysate from rabbit reticulocytes. One of the tubes was used as control (●-●-●), whereas the other ones contained: 0.2 μg ricin (X-X-X), 0.5 μg ricin (▲-▲-▲), 1 μg (■-■-■), and 1 μg ricin preincubated at 80° for 10 min (□-□-□). The tubes contained 1.25 μCi of ^{14}C-leucine (uniformly labelled, specific activity 331 mCi/mmole) and were incubated at 28°. At the times indicated 25 μl samples were removed and the acid-precipitable radioactivity was determined.

sent conditions the incorporation of labelled amino acids into protein in the cell-free system from rabbit reticulocytes proceeded almost linearly for 40 min. After addition of 0.2 μg of ricin the incorporation occurred at an almost normal rate for the first 5 min, but then declined and virtually stopped after about 10 min. When 1 μg of ricin was added the incorporation was rapidly inhibited. On the other hand ricin denatured by incubation at 80° for 10 min had no ability to inhibit protein synthesis. Previous workers have shown that ricin treated in this way has no toxic effect on living animals [1].

In fig. 2 is shown that also the translation of the artificial messenger poly-U is inhibited by ricin. In this experiment, the endogenous protein synthesis was reduced by preincubating the reticulocyte lysate at 28° for 40 min in order to demonstrate more clearly the stimulating effect of poly-U in the absence of ricin.

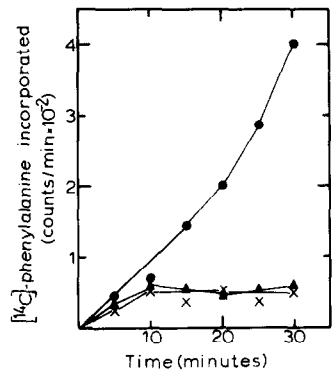

Fig. 2. Effect of ricin on polyphenylalanine synthesis. The cell-free system described in fig. 1 was preincubated at 28° for 40 min in the absence of labelled compounds. The concentration of $MgCl_2$ was then adjusted to 7 mM, 4 μCi of ^{14}C-phenylalanine (uniformly labelled, specific activity 513 mCi/mmole) was added, and the system was divided into 3 equal samples. One sample (▲-▲-▲) was used as a control, whereas another sample (●-●-●) contained 100 μg of poly-U and a third sample (X-X-X) contained 100 μg of poly-U and 0.5 μg of ricin. The samples were incubated at 28° and aliquots were removed as indicated and the acid-precipitable radioactivity was determined.

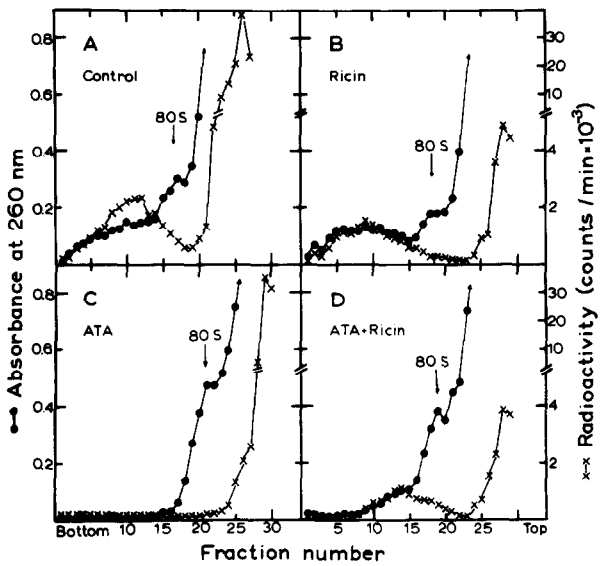

Fig. 3. Effect of ricin and ATA on the polysome pattern and nascent peptide chains in a lysate from rabbit reticulocytes. The cell-free system was prepared in 4 parallel tubes, one of which (A) was used as control, whereas to the others were added: (B) 1 μg ricin, (C) 5 × 10^{-5} M ATA, and (D) 5 x 10^{-5} M ATA + 1 μg ricin. Then 3 μCi of ^{14}C-leucine was added and the tubes were incubated at 28° for 15 min. After incubation a 300 μl aliquot from each tube was layered onto sucrose gradients and centrifuged at 130,000 *g* for 60 min. Absorbance at 260 nm and acid-precipitable radioactivity were determined in each fraction.

To study which step in the protein synthesis is inhibited by ricin, the cell-free system was incubated for 15 min in the absence and presence of ricin and subsequently analyzed by sucrose gradient centrifugation. In the control (no ricin present) the absorbance at 260 nm revealed (fig. 3A) that monoribosomes, as well as polysomes were present in the system. Part of the radioactivity (about 10%) was recovered from the polysomes region demonstrating the formation of nascent peptide chains. Most of the radioactivity (about 90%) was present on top of the gradient as evidence that considerable amounts of globin chains were completed under these conditions. It appears from fig. 3B that after incubation with ricin the polysome pattern was better preserved. Thus, the amount of polysomes relative to that of monosomes was higher than in the control. Moreover, in this case the total radioactivity was much lower than in the control, and significantly, about half of the radioactivity was found in the polysome region. The results indicate that some elongation of peptide chains had taken place in the presence of ricin, but that few globin chains were completed and released from the polysomes under these conditions.

When the incubation was carried out in the presence of aurin tricarboxylic acid (ATA), a compound known to inhibit specifically the initiation of new peptide chains [12, 13], only monoribosomes were observed (fig. 3C) and virtually all the radioactivity was found on top of the gradient, demonstrating that the peptide chains initiated prior to the addition of ATA were completed during the incubation. If ricin inhibits the elongation of already initiated peptide chains as suggested by the above experiments, it would be expected to inhibit also in the presence of ATA the completion and liberation of the peptide chains already initiated and to preserve the polysome structure. The results in fig. 3D bear out this expectation. It is clear that when both ATA and ricin were present, the polysome structure was partly preserved in contrast to the results in fig. 3C and about half of the total radioactivity was found in the polysome region.

The present data demonstrate that ricin in small concentrations block protein synthesis in the cell-free system, and they are consistent with the view that this is due to an interference with the completion of already initiated peptide chains. The effect of ricin is not an immediate one (at least in low concentration) but appears to require a few min to manifest itself (fig. 1). Since the toxin does not appear to affect the charging of tRNA (unpublished data), the results suggest that ricin gradually inactivates some component in the cell-free system essential for the elongation. The possibility should be considered that ricin, like diphtheria toxin [14] may exert its action by possessing enzymatic activity.

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