Polyribosome Disaggregation in Rat Liver following Administration of the Phytotoxic Proteins, Abrin and Ricin

Jung-Yaw Lin, Chia-Chu Pao, Shyr-Te Ju, and Ta-Cheng Tung

Institute of Biochemistry, College of Medicine, National Taiwan University, Taipei, Taiwan, Republic of China

SUMMARY

As the possible mechanisms of inhibition of protein biosynthesis, it was demonstrated that the toxic proteins abrin and ricin cause the degradation of polyribosomes of rat liver or Ehrlich ascites tumor cells. Abrin and ricin have no direct effect on the structural and functional integrity of polyribosomes but act indirectly by increasing the RNase activity in the postmicrosomal supernatant fractions of rat liver or Ehrlich ascites tumor cells. These increases of RNase activity coincided in time with reduction in the amino acid incorporation and degradation of polyribosomes in vivo and the ultrastructural changes in the rough endoplasmic reticulum of hepatic parenchymal cells.

INTRODUCTION

The remarkable inhibitory effects of abrin and ricin on the growth of Ehrlich ascites tumor cells were demonstrated by the i.p. injection of abrin or ricin (9, 12, 15, 21).

As the possible mechanism of antitumor activity, it was shown at molecular level that abrin or ricin has a strong inhibitory effect on protein biosynthesis, a moderate inhibitory effect on DNA biosynthesis, but no effect on the RNA biosynthesis (10, 13). The 2 following possibilities have been considered for the inhibition of protein and DNA biosynthesis of Ehrlich ascites tumor cells: (a) cellular energetics and (b) permeability. The fact that the addition of glucose did not reverse the inhibitory action of abrin on the protein and DNA biosynthesis (10) decreases the likelihood that energy metabolism is a primary site of abrin action in Ehrlich ascites tumor cells. The possibility that abrin might act on the site(s) of the cellular membrane was almost ruled out by the observation that the uptake of amino acids by the tumor cells was not affected in the presence of abrin or ricin (13).

The present investigation demonstrates that abrin and ricin are irreversible inhibitors of protein biosynthesis in rat liver and Ehrlich ascites tumor cells and that the inhibition occurs by means of disaggregation of polyribosomes. The disaggregations of polyribosomes are due to the increase of RNase activity in the postmicrosomal fractions of rat liver and Ehrlich ascites tumor cells, in vivo.

MATERIALS AND METHODS

L-Leucine-4,5-3H with a specific activity of 5 Ci/mmole was purchased from New England Nuclear, Boston, Mass. Adult male Sprague-Dawley rats, weighing 200 to 250 g, were used in this experiment. Male mice of Swiss white strain weighing 20 ± 2 g were used to maintain the Ehrlich ascites tumor cells (10). Yeast RNA’s were obtained from Sigma Chemical Co., St. Louis, Mo. The yeast RNA’s were further treated as follow. A 7% solution of each sample was prepared, adjusted to pH 7.0, and dialyzed against distilled water for 48 hr at 0° with several changes of distilled water. Bovine pancreatic RNase was obtained from Worthington Biochemical Corp., Freehold, N. J.

Preparation of Abrin and Ricin. Abrin and ricin were isolated and crystallized as described before (11, 14, 16).

Preparation of Polyribosomes from Rat Liver. Twelve-hr fasting animals, which were given water during starvation, were killed by decapitation, and the livers were quickly removed and cut into small pieces with a pair of scissors. After being rinsed with TKM2-0.25 M sucrose buffer, the pieces of liver were homogenized with a Teflon homogenizer that was precooled to 0°. The homogenate was centrifuged at 15,000 X g for 10 min in a Sorval RC-2B ultracentrifuge to remove the nuclei, mitochondria, and cell debris. To the postmicrosomal supernatant fraction, Triton X-100 in TKM buffer was added. Cell lysis was immediate and complete. The intact nuclei and mitochondria were separated by centrifugation at 105,000 X g for 180 min. The postmitochondrial supernatant was then centrifuged (14, 16).

Preparation of Polyribosomes from Ehrlich Ascites Tumor Cells. Polyribosomes of Ehrlich ascites tumor cells were prepared according to the method of Hogan and Korner (5). The mice bearing 10-day-old Ehrlich ascites tumors were given i.p. injections of 10 µg of either abrin or ricin per mouse and, at indicated times, were killed by decapitation and immediately washed twice with cold TKM-sucrose buffer. For lysis by detergent, the cells were lysed with cold TKM-sucrose buffer to a concentration of 108 cells/ml, and one-ninth volume of 5% Triton X-100 in TKM buffer was added. Cell lysis was immediate and complete. The intact nuclei and mitochondria were removed by centrifugation at 105,000 X g for 180 min.

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2 The abbreviation used is: TKM, 0.05 M Tris, 0.025 M KCl, and 0.005 M MgCl2, pH 7.5.
Density Gradient Analysis of Polyribosomes. This analysis was performed according to the procedure of Blobel and Potter (3). The equivalent of 20 A260 units of rRNA was layered over a linear sucrose gradient, (10 to 35%, w/v). The gradient was centrifuged at 63,000 X g (average) in the SW 25.1 rotor of a Spinco Model L ultracentrifuge for 2.5 hr at 4°. Absorbance at 260 nm was determined with a Beckman Model DU spectrophotometer during the collection of gradients, and I-ml fractions were collected.

Effect on Polyribosomes in Vitro. A typical series of sedimentation patterns (Chart I) clearly illustrated the progressive degradation of rat liver polyribosomes resulting from the action of abrin. Chart IA illustrates the ribosomal pattern of normal rat liver. The i.p. injection of a dose of abrin, 100 μg/100 g body weight, causes a distinct shift of polyribosomes toward smaller aggregates (Chart 1, B and C), which are reflected in a conspicuous increase of the amount of single ribosomes at 3 and 6 hr after i.p. injection of abrin. At 9 and 12 hr, a drastic change has occurred, most of the polyribosomes have broken down into single ribosomes, and the peak resulting from the larger aggregates has almost completely disappeared (Chart 1, D and E). After i.p. injection of leucine-3H for 1 hr, nascent peptide was detected in the polyribosomal region (Chart 1A). At 6 hr after i.p. injection of abrin, the radioactivity in the polyribosomal region decreased considerably (Chart 1C), and most of the radioactivity disappeared from the polyribosomal area at 12 hr (Chart 1E). The effect of ricin on the profile of polyribosomes of rat liver after the i.p. injection of ricin was similar to that of abrin.

RESULTS

Effect of Abrin and Ricin on Protein Biosynthesis in Rat Liver Slices. The inhibition of protein biosynthesis was rapid, and about 80% inhibition was observed at 6 hr after treatment with abrin. The effect of ricin on macromolecular biosynthesis in rat liver slices was similar to that of abrin.

Effect on Polyribosomes and on Attachment of Nascent Peptide in Vitro. A typical series of sedimentation patterns (Chart 1) clearly illustrated the progressive degradation of rat liver polyribosomes resulting from the action of abrin. Chart IA illustrates the ribosomal pattern of normal rat liver. The i.p. injection of a dose of abrin, 100 μg/100 g body weight, causes a distinct shift of polyribosomes toward smaller aggregates (Chart 1, B and C), which are reflected in a conspicuous increase of the amount of single ribosomes at 3 and 6 hr after i.p. injection of abrin. At 9 and 12 hr, a drastic change has occurred, most of the polyribosomes have broken down into single ribosomes, and the peak resulting from the larger aggregates has almost completely disappeared (Chart 1, D and E). After i.p. injection of leucine-3H for 1 hr, nascent peptide was detected in the polyribosomal region (Chart 1A). At 6 hr after i.p. injection of abrin, the radioactivity in the polyribosomal region decreased considerably (Chart 1C), and most of the radioactivity disappeared from the polyribosomal area at 12 hr (Chart 1E). The effect of ricin on the profile of polyribosomes of rat liver after the i.p. injection of ricin was similar to that of abrin.

Effect on Polyribosomes in Vitro. We investigated the possibility that abrin degraded the polyribosomes directly by incubating the polyribosome preparation with abrin at 37° for 15 min. The results indicated that the addition of abrin to the polyribosome preparation in vitro had no effect on the polyribosome pattern at a concentration of 250 μg/ml, which is much higher than that for in vivo experiments.

It is possible that the inhibition of protein biosynthesis by abrin in rat liver is due to the factor(s) that exist in the fractions other than polyribosomes. Therefore, postmicrosomal supernatant fraction of abrin-treated rat liver was incubated with the polyribosome preparation from normal rat liver. The results clearly indicated that incubation of 10 mg of protein of postmicrosomal supernatant fraction of 12-hr, abrin-treated rat liver with 2.2 mg of polyribosome resulted in complete disaggregation of the polyribosomes (Chart 2), while incubation of polyribosome preparation and postmicrosomal supernatant fraction from normal rat liver did not affect the polyribosome profile.

Degradation of Polyribosomes by RNase. The nature of factor(s) causing the destruction of polyribosomes was studied by dialysis of the postmicrosomal supernatant fraction of rat liver poisoned with abrin against TKM buffer for 48 hr at 4°, with several changes of the buffer. The results indicated that the factor was nondialyzable and hence was macromolecular in nature. Judging from the properties of the factor(s) described above, the most possible candidate is RNase. The appearance of RNase activity is the postmicrosomal supernatant fraction of rat liver poisoned with abrin was studied. Table 1 shows that RNase activity in the postmicrosomal supernatant fraction increases about 15-fold after the injection of abrin.

Effect on Polyribosomes of Ehrlich Ascites Tumor Cells. The administration of a single dose of abrin i.p. caused pronounced degradation of polyribosomes (Chart 3). The increase in RNase activity in postmicrosomal supernatant was also demonstrated; it increased about 20-fold at 3 hr after the i.p. injection of abrin (Table 2).
Effects of Administration of Abrin and Ricin

Chart 2. Effect of postmicrosomal supernatant fractions of liver from a rat treated with abrin for 12 hr on the polyribosomes of normal rat liver. The reaction mixtures were incubated at 37° for 15 min and then were subjected to sucrose density gradient analysis. •, normal rat liver polyribosomes + supernatant of normal rat liver; o, normal rat liver polysomes + supernatant of rat liver treated with abrin for 12 hr.

Table 1
Effect of abrin on the RNase activity in the postmicrosomal supernatant fraction of rat liver

<table>
<thead>
<tr>
<th>Duration of abrin treatment</th>
<th>RNase activity (0.03 μg)</th>
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<tbody>
<tr>
<td>0 hr</td>
<td>63.5</td>
</tr>
<tr>
<td>3 hr</td>
<td>55.0</td>
</tr>
<tr>
<td>6 hr</td>
<td>82.5</td>
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<tr>
<td>9 hr</td>
<td>410</td>
</tr>
<tr>
<td>12 hr</td>
<td>785</td>
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DISCUSSION

The remarkable antitumor activity of phytotoxic proteins has been demonstrated previously (9, 12, 15, 21), and, as the possible mechanisms of antitumor activity, it was shown at a molecular level that the toxic proteins inhibit the protein and DNA biosynthesis in the Ehrlich ascites tumor cells (10, 13). Although the toxic proteins could act in both processes, the observed partial inhibition of DNA biosynthesis in the tumor cells probably results from the primary effect on protein biosynthesis, since protein biosynthesis is required for the biosynthesis of DNA in the animal cells (7).

The inhibitory effect was not found to be due to interference with glucose metabolism or cellular respiration (8, 13). By use of the nonmetabolizable amino acid, aminoisobutyric acid, it was also demonstrated that abrin and...
and all of those that inhibited the growth of tumor cells show agents has been used for treatment of P1798 lymphosarcomas, published in the treatment of lymphosarcomas. A variety of biosynthesis in vivo. The increase in RNase activity supports in time with the disaggregation of polyribosomes and protein of toxic protein-poisoned rat liver was determined quantita
demonstrated by polyribosomal pattern analysis. The
found that these toxic proteins produced marked disaggrega-
cause complete disaggregation of polyribosomes within 1 hr
after injection. The delayed effect of abrin and ricin may be
due to the molecular size of the toxic proteins and the i.p. route of administration.

**REFERENCES**


The degradation of polyribosomes, similar to that induced by abrin or ricin, can be caused in rat liver cells by some hepatotoxins such as ethionine (2), lipomycin (23), puromycin (22), 4-dimethylaminoazobenzene (6), and lasiocarpine (4). The effect of abrin and ricin is not immediate and is different from the effects of puromycin and pyrrolizidine alkaloid, which cause complete disaggregation of polyribosomes within 1 hr after injection. The delayed effect of abrin and ricin may be due to the molecular size of the toxic proteins and the i.p. route of administration.

**Table 2**

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>0.03 µg</td>
<td>63.5</td>
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<tr>
<td>0 hr</td>
<td>30</td>
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<tr>
<td>0.5 hr</td>
<td>45</td>
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<td>1 hr</td>
<td>80</td>
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<td>2 hr</td>
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ricin did not affect the rate and level of accumulation of the amino acids in Ehrlich ascites tumor cells (13).

In this report, we present evidence that abrin and ricin inhibit protein biosynthesis by degradation of the polyribo-
somes. By i.p. injection of a single dose of abrin or ricin, it was found that these toxic proteins produced marked disaggrega-
tion of polyribosomes of rat liver. This was clearly demonstrated by polyribosomal pattern analysis. The appearance of RNase activity in the postmicrosomal fraction of toxic protein-poisoned rat liver was determined quantita-
tively, and the increase of enzyme activity seemed to coincide in time with the disaggregation of polyribosomes and protein biosynthesis in vivo. The increase in RNase activity supports the previous findings (8). Similar results also have been published in the treatment of lymphosarcomas. A variety of agents has been used for treatment of P1798 lymphosarcomas, and all of those that inhibited the growth of tumor cells show an increase in RNase activity (1). Furthermore, injection of Escherichia coli asparaginase to host mice bearing asparaginase-sensitive 6C3HED or P1798 lymphosarcomas causes an increase of RNase activity in tumor, but no such increase occurred in the asparaginase-resistant strain (18, 19).

**Chart 3.** Effect of abrin on the ribosomal patterns of postmitochondrial supernatant fractions of Ehrlich ascites tumor cells. Mice bearing 10-day-old tumors were given injections of 10 µg of abrin. At the indicated time, the tumor cells were collected, and polyribosomes were prepared as described in "Materials and Methods." •, ribosomal pattern of normal tumor cells treated with 0.9% NaCl solution; ○, ribosomal pattern of tumor cells treated with abrin.

**Figure 1.** Effect of abrin on the RNase activity in the postmicrosomal supernatant fractions of Ehrlich ascites tumor cells. Figures in the table represent absorbance × 10² obtained with 0.1 ml of the postmicrosomal supernatant of a 1:10 homogenate of Ehrlich ascites tumor cells. Readings were made on 1:15 to 1:150 dilutions of acid-soluble filtrates. The data are the average of 3 experiments, differing from the mean by less than 15%.

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