CORRELATION OF INHIBITION OF RIBOSOMAL GTPase AND PROTEIN SYNTHESIS BY ABRIN AND RICIN

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SUMMARY

The rate of inhibition of protein synthesis and ribosome-dependent GTPase activity by abrin and ricin was determined. Ribosomes were treated with toxin A-chains for increasing periods of time and they assayed for their ability to synthesize polyphenylalanine and hydrolyze GTP. The rate of inhibition of GTPase activity of the 60S subunit derived 8S ribonucleoprotein complex was also determined. Although the degree of maximal inhibition of protein synthesis and GTPase activity was different, the rates of inhibition were almost identical. The results support a conclusion that abrin and ricin inhibit protein synthesis by inactivating ribosome-dependent GTPase.

INTRODUCTION

The toxic effect of the plant proteins abrin and ricin is due to their ability to inhibit protein synthesis by irreversibly inactivating the 60S ribosomal subunit (1,2). It has been shown that the elongation factor 1 (EF-1) and elongation factor 2 (EF-2) stimulated ribosome dependent GTPase activity is reduced to about half the control value after treatment with the toxin A-chains (1,2). It was further shown (1) that an 8S ribonucleoprotein complex which can be released from the 60S ribosomal subunit and which has GTPase activity is inhibited similarly after treatment with toxin A-chains. If inhibition of ribosomal GTPase is causally related to inhibition of protein synthesis it is important to demonstrate that the ability of ribosomes to hydrolyze GTP decreases in parallel with their ability to synthesize

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protein. This study reports the close correlation between the rate of poly-phenylalanine synthesis inhibition and the rate of inhibition of GTPase from intact ribosomes and isolated 8S particles.

MATERIALS AND METHODS

Rabbit reticulocyte ribosomes were prepared and washed free of elongation factors according to Carrasco et. al. (3). The ribosomes were suspended in 50 mM Tris-HCl (pH 7.4), 25 mM KCl, 5 mM, $MgCl_2$ and 1 mM dithiothreitol and stored in liquid nitrogen until used. A reticulocyte S-100 was fractionated by ammonium-sulfate precipitation and the 35-70% fraction was collected and subjected to gel filtration on Sephadex G-200 to separate elongation factor 1 from elongation factor 2. Elongation factor 2 was further purified by DEAE-cellulose, hydroxyapatite and phosphocellulose chromatography (3). An 8S complex consisting of 5 S RNA and a single ribosomal protein from EDTA-treated 60 S subunits as earlier described (1).

Abrin and ricin and their isolated A-chains were prepared as earlier described (4,5). Anti-toxins were prepared by immunizing rabbits with formaldehyde-treated toxins, and the A-chain antibodies were isolated by passing the sera through columns containing covalently bound abrin and ricin A-chains as earlier described (6).

Poly (U)-directed [^{14}C]-phenylalanine incorporation was measured in a total volume of 100 µl buffer A containing: 5µg poly(U), 0.5 mM GTP, 0.8 mg rat liver (pH 5) supernatant (7), 2 µl anti-A-chains antibodies, 3.9 pmoles ribosomes, and 78 pmoles [U-^{14}C] phenylalanyl-tRNA (spec. act. 127 m Ci/mmole, New England Nuclear, Boston, Mass.). The mixture was incubated at 37° for 15 minutes, and the reaction was stopped by the addition of 3 ml 5% trichloro-acetic acid. The samples were heated at 90° for 10 minutes, and the precipitate collected on Gelman glass fiber filters (type A). Radioactivity was measured by liquid scintillation counting.

Unless otherwise indicated GTPase activity of ribosomes was measured in an incubation mixture consisting of the indicated amounts of ribosomes or 8S complex in 100 µl of buffer B containing 2 µl anti-abrin or anti-ricin A-chains, 120 µM [γ-^{32}P] GTP (spect. act. 1.27 Ci/mmole, Amersham). Radio-active GTP was added last and the reaction mixture incubated at 37° for the indicated time. After incubation the samples were chilled and 100 µl of 1N $HClO_4$ was added, followed by 1.0 ml of a Norite suspension (250 mg per ml 1N HCl). The samples were mixed and centrifuged for 10 minutes at 6000 x g. A 100 µl aliquot of supernatant was withdrawn and released ^{32}P-radioactivity by scintillation counting. The appropriate blank in the absence of ribosomes was substracted from all values. Buffer A: 50 mM Tris-HCl (pH 7.4), 60 mM KCl, 11 mM $MgCl_2$, 9 mM 2-mercaptoethanol. Buffer B: 50 mM Tris-HCl (pH 7.8), 1 mM KCl, 1 mM $MgCl_2$, 6 mM 2-mercaptoethanol.

RESULTS AND DISCUSSION

When ribosomes were incubated in the presence of EF-2 [γ-^{32}P] GTP, ^{32}P was liberated with time at an almost linear rate (Fig. 1). A 10 fold decrease in ribosome concentration resulted in a reduction of the ^{32}P liberated to 1/10 indicating that the rate of ^{32}P liberated was linearly

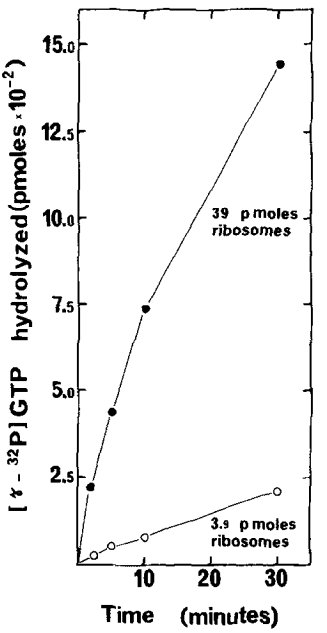

Figure 1. EF-2 dependent ribosome GTPase activity. The indicated amount of ribosomes and 22 pmoles of elongation factor 2 were assayed for GTPase activity as described in Materials and Methods. Values obtained in parallel tubes without ribosomes and EF-2 were substracted from the total amount of ^{32}P released. The specific activity of the γ-^{32}P GTP was 645 counts/min/pmole.

related to the amount of ribosomes.

It was earlier shown (1) that protein synthesis can be inhibited 70-90% by abrin and ricin A-chains depending upon the system used, whereas ribosomal GTPase can only be inhibited 40-50%. When ribosomes are treated with low amounts of toxin A-chains, their ability to synthesize protein decreases with time, due to the fact that the A-chains inactivate the ribosomes enzymatically (8). In order to correlate the rate of inhibition of the GTPase activity and protein synthesizing activity, 78 pmoles of ribosomes were incubated with 0.5 ng of abrin and ricin A-chains. After various periods of time, aliquots were transferred into tubes containing specific antibodies which immediately stops further ribosome inactivation (8). The ribosomes were then tested for their ability to polymerize phenylalanine and to hydrolyze GTP. The results

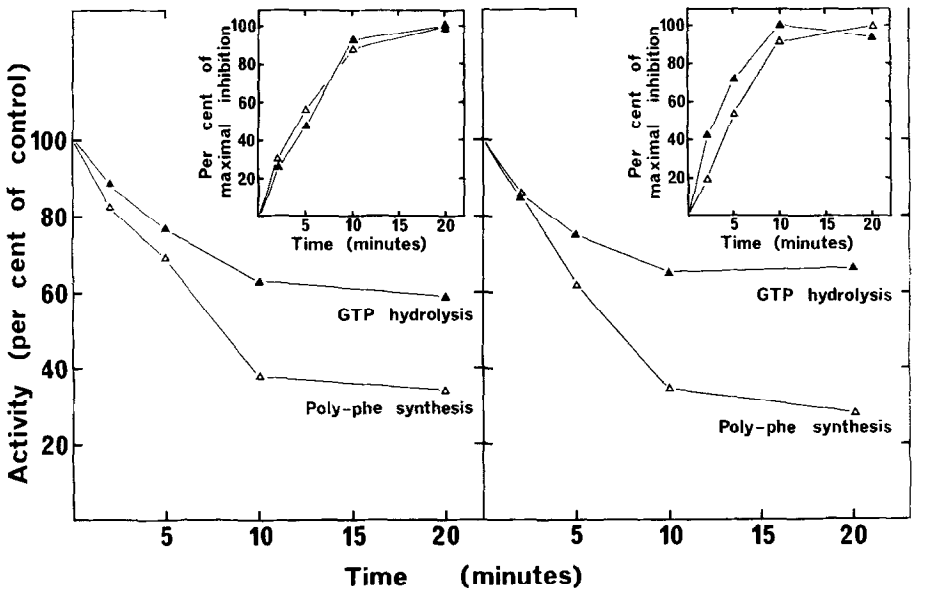

Figure 2. Inhibition of [^{14}C] phenylalanine incorporation and GTPase activity of ribosomes by A-chains of abrin (A) and ricin (B). Ribosomes (79 pmoles) were preincubated in a volume of 200 µl of buffer A for 20 minutes at 37°. 0.5 ng of A-chains were then added, and 10 µl aliquots were transferred at the indicated times into 50 µl buffer A or buffer B containing 2 µl anti-toxin. Subsequently, 3.9 pmoles of ribosomes were tested for their ability to polymerize phenylalanine and hydrolyze GTP as described in Materials and Methods. The activity measured in the control samples were 261 (A) and 231 (B) pmoles [^{14}C]-phenylalanine incorporated and 558 (A) and 511 (B) pmoles ^{32}P liberated.

(Fig. 2) show that the ability of ribosomes to synthesize polyphenylalanine decreased as a function of time of preincubation with the toxin A-chain. The GTPase activity also decreased with time, but whereas the polyphenylalanine synthesis was reduced to about 30% of the control value after 30 minutes incubation with toxin A-chains, the GTPase activity was only reduced to about 60% of the control value. However, it is clear from Fig. 2 that the length of incubation time of ribosomes with toxin required to achieve maximal inhibition of the two activities was approximately the same. To further examine the inhibition of the two activities, the data were replotted as per cent of maximal inhibition (insertion in Fig. 2). The two lines closely parallel

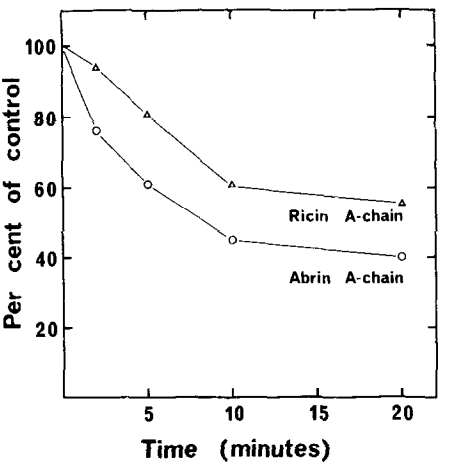

Figure 3. Inhibition of 8S complex associated GTPase by A-chains of abrin and ricin. 8S complex (0.088 OD_{260} units) was preincubated in 240 µl of 20 mM Tris-HCl (pH 7.5), 10 mM KCl, 1mM $MgCl_2$, 6 mM 2-mercaptoethanol for one minute at 37°. The 2.5 ng of A-chain were added, and 40 µl aliquots were removed at the indicated times to 30 µl buffer B containing 2 µl of anti-toxin. Subsequently, 0.015 OD_{260} of 8S complex was tested for GTPase activity as described in Materials and Methods. The activity measured in the control was 43 pmoles ^{32}P liberated in 2 hours. (O), abrin A-chain; (V), ricin A-chain.

each other demonstrating that the rate of inhibition of ribosomal GTPase activity and polyphenylanine synthesis by the toxin A-chain was the same.

As shown earlier an 8S complex consisting of 5S RNA and one ribosomal protein (1) can be released from the 60S ribosomal subunit by EDTA treatment. The 8S complex has a GTPase activity which can be inhibited by abrin and ricin A-chains (1). To examine whether this GTPase activity was inhibited by the toxin A-chains at a rate similar to the inactivation of the intact ribosomes, freshly isolated 8S complex was incubated with toxin A-chains and after different periods of time aliquots were transferred to buffer containing the specific antibodies and then assayed for GTPase activity. The results (Fig. 3) show a rate of inactivation similar to that observed with intact ribosomes.

The fact that maximal inhibition of protein synthesis expressed as

percentage of control was greater than that of ribosomal GTPase activity may be due to GTPase activity which is insensitive to the toxins. A high background value in GTP hydrolysis may be due in part to the fact that GTP hydrolysis was measured uncoupled from polypeptide synthesis. Furthermore, Mazumder (9) has demonstrated a 40S ribosomal subunit GTPase activity which is stimulated by EF-2 and a 0.5 M KCl wash of A. salina ribosomes. Since we have previously shown that elongation factors and 40S subunits are not inactivated by toxin A-chains (1), it is possible that ribosomal GTPase activity remaining after abrin and ricin treatment is partly due to 40S subunit GTPase activity. Altogether the present observations support our conclusion that the toxins inactivate ribosomes by enzymatically modifying a site on the ribosomes at or near the binding site of EF-2 and GTP (10).

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