Site of Action of Ricin on the Ribosome

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ABSTRACT: The extent of the inhibitory effect of ricin in polyphenylalanine synthesis by eukaryotic ribosomes is strongly dependent upon the concentration of ribosomes and the elongation factors EF 1 and EF 2. Maximal inhibition by ricin, in this assay, is observed when either ribosomes or the two elongation factors are added under limiting conditions, whereas ricin-treated ribosomes support protein synthesis at saturating concentrations of elongation factors and ribosomes. Similarly, the enzymatic binding of Phe-tRNA to ribosomes is drastically blocked in ricin-treated ribosomes when low EF 1 concentrations are added to the reaction mixture, but there is no inhibition when EF 1 is at saturating concentrations. Furthermore, formation of the complex EF 2–guanosine triphosphate–ribosome, using free ribosomes pretreated with ricin, is strongly inhibited at limiting concentrations of EF 2, but is not affected at saturating concentrations of this factor. However, ricin does not inhibit the EF 2-dependent translocation of peptidyl-tRNA by polysomes, although the toxin is very active in preventing amino acid incorporation by polysomes. Our results suggest that the damaging effect of ricin on the ribosome causes a decreased affinity for both elongation factors EF 1 and EF 2. Thus, the toxin inhibits the enzymatic binding of aminoacyl-tRNA to ribosomes. The lack of inhibition of translocation by ricin suggests that the toxin cannot interact with ribosomes with substrate bound to the acceptor site. Essentially similar results are observed with ricin, abrin, ricin A chain, abrin A chain, and ricinus agglutinin A chain. A possible effect of the toxins on initiation and/or termination is further discussed.

Ricin and abrin are lectins closely related in their structure and mode of action. Both glycoproteins are composed of two glycopeptide chains. The B chain binds to the membrane of mammalian sensitive cells by recognizing galactose-containing receptors (Olsnes and Pihl, in press; review). The A chain enters the cell in a subsequent step and specifically blocks protein synthesis (Olsnes and Pihl, 1972a; Montanaro et al., 1973) by enzymatically damaging the larger ribosome subunit (Sperti et al., 1973). The k_m of this inactivation process is 2×10^{-7} and 1.4×10^{-7} M for the A chains of ricin and abrin, respectively (Olsnes et al., 1975). Under certain experimental conditions the inactivity of ricin-treated ribosomes can be overcome by adding higher concentrations of EF 2 (Olsnes et al., 1975).

Ricin and abrin prevent polysome breakdown in cell-free protein synthesizing systems; these results suggested an inhibitory effect of the toxins on the elongation of the polypeptide chain (Olsnes and Pihl, 1972a, 1972b). A strong inhibitory effect of ricin on the enzymatic binding of aminoacyl-tRNA has been described (Carrasco et al., 1975). Since ricin does not affect nonenzymatic binding, it was concluded that the toxin prevents the interaction of EF 1 with the ribosome (Carrasco et al., 1975). Indeed, an inhibitory effect of ricin on EF 1-dependent GTP[1] hydrolysis by ricin has also been observed (Benson et al., 1975; Sperti et al., 1975).

Moreover, GTP-dependent binding of EF 2 to ribosomes is also inhibited by ricin (Carrasco et al., 1975; Montanaro et al., 1975) and this inhibition is also observed when GTP is replaced by either GDP or GDPPCP (Carrasco et al., 1975). Therefore, ricin inhibits the EF 2- and ribosome-dependent uncoupled GTP hydrolysis (Montanaro et al., 1973; Sperti et al., 1975). Ricin has no effect on substrate translocation from the acceptor to the donor site of the ribosome (Montanaro et al., 1973; Carrasco et al., 1975; Sperti et al., 1975) but the toxin has been repeatedly quoted as an inhibitor of translocation on the basis of its effect on the EF 2-dependent GTP hydrolysis (Sperti et al., 1975; Montanaro et al., 1975).

We have now studied the activities of ricin-treated ribosomes using either limiting or saturating concentrations of ribosomes, EF 1 and EF 2. The results presented in this study support the previous finding that ricin affects a site of the 60S ribosome subunit involved in the interaction of EF 1 and, therefore, blocks the enzymatic binding of aminoacyl-tRNA. The same or an overlapping site appears to be involved in the interaction of EF 2 and, therefore, the interaction of this factor is also blocked by ricin; however, it appears that ricin cannot interact and damage ribosomes or polysomes with substrate bound to the acceptor site and, consequently, it does not affect translocation.

Materials and Methods

The preparation of rabbit reticulocyte ribosomes and elongation factors EF 1 and EF 2, [^{14}C]Phe-tRNA (463 mCi/mmol) and yeast polysomes was as previously described (Carrasco et al., 1975). The assay systems for poly(U)-directed polyphenylalanine synthesis, enzymatic binding of [^{14}C]Phe-tRNA, EF 2 binding to ribosomes in the presence of [^{3}H]GTP (15 Ci/mmol), and peptidyl-tRNA translocation in yeast polysomes have also been described (Carrasco et al., 1976). Otherwise, specific conditions concerning each experiment are indicated in the legends of figures and tables.

Ricin, abrin, ricin A chain, abrin A chain, and ricinus agglutinin A chain were prepared as described (Olsnes and Pihl, 1972b, 1973a) and kindly given to us by Dr. S. Olsnes (Norsk Hydro's Institutt for Kreftforskning, Oslo).

Results

Ribosome Inactivation by Ricin for Polyphenylalanine Synthesis; Prevention of the Inhibitory Effect of Ricin by Increasing Concentrations of Elongation Factors EF 1 and

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[1] Abbreviations used are: GDP, GTP, guanosine di- and triphosphates; NAD, nicotinamide adenine dinucleotide.

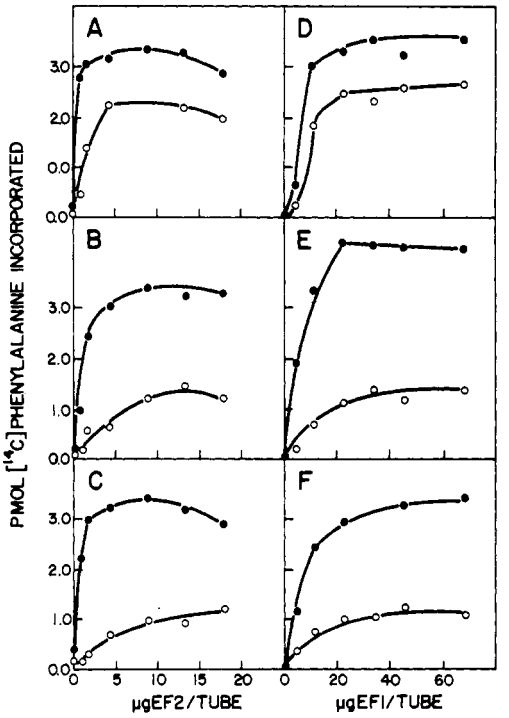

FIGURE 1: Effect of ricin A chain treatment of rabbit reticulocyte ribosomes on poly(U)-directed polyphenylalanine synthesis; dependence on the concentrations of elongation factors and ribosome. 100-μl reaction mixtures, containing 50 mM Tris-HCl, pH 7.4, 60 mM KCl, 11 mM $MgCl_2$, 9 mM 2-mercaptoethanol, ribosomes (as indicated below), and 0.12 μg of ricin A chain (where indicated), were preincubated for 3 min at 37 °C; GTP (0.5 mM), poly(U) (5 μg), EF 1 and EF 2 (as indicated), and 5.9 nCi of [^{14}C]Phe-tRNA (sp act. 463 Ci/mol) were then added and the mixtures were incubated for 15 min at 37 °C. The reaction was stopped and filtered as described (Carrasco et al., 1975). (A) 3.8 pmol of ribosomes and 35 μg of EF 1; (B) 3.8 pmol of ribosomes and 9.2 μg of EF 1; (C) 1.2 pmol of ribosomes and 35 μg of EF 1; (D) 3.8 pmol of ribosomes and 13.5 μg of EF 2; (E) 3.8 pmol of ribosomes and 2.7 μg of EF 2; (F) 1.2 pmol of ribosomes and 13.5 μg of EF 2. (●—●) Control; (○—○) ricin A chain treated ribosomes.

EF 2. Ribosomes are inactivated by ricin for polyphenylalanine synthesis in standard buffers without the addition of any cofactors (Olsnes and Pihl, 1972b; Montanaro et al., 1973; Carrasco et al., 1975). However, the subsequent addition of an excess of EF 2 in the presence of certain concentrations of EF 1 can overcome, at least partially, the damaging effect of ricin (Olsnes et al., 1975). We have reinvestigated this problem by using different amounts of EF 1 and EF 2, while maintaining the ribosome concentration invariable (Figure 1). Under these conditions, increasing concentrations of EF 2 can overcome to a great extent the inhibitory effect of the ricin treatment at saturating levels of EF 1 (Figure 1A), but not at limiting concentrations of this factor (Figure 1B). Similarly, high concentrations of EF 1 considerably reduce the inhibitory effect of the ricin treatment at saturating concentrations of EF 2 (Figure 1D), but have a reduced effect at limiting concentrations of EF 2 (Figure 1E). The high dependence of the inhibitory effect of the ricin treatment on the concentrations of EF 1 and EF 2 explains the differences in the inhibitory power of the toxin in the different polyphenylalanine synthesizing systems used by a number of workers. Furthermore, the ribosome concentration is also an important parameter to revert the effect caused by ricin; thus, ricin-treated ribosomes are moderately active at rather high concentrations (Figure 1A,D) but nt at lower concentrations, even when EF 1 and EF 2 are saturating (Figure 1C,F).

EF 1-Dependent Binding of Phe-tRNA to Ricin-Treated Ribsomes. The inhibitory effect by ricin on the enzymatic binding of aminoacyl-tRNA appears to be due to a lack of EF 1 interaction with ricin-treated ribosomes, since nonenzymatic binding of aminoacyl-tRNA to ribosomes is not inhibited by the toxin (Carrasco et al., 1975). However, the irreversible damage caused by ricin on the ribosome does not appear to impair but only to decrease the affinity of EF 1 for the ribosome, since (a) ricin-pretreated ribosomes enzymatically bind aminoacyl-tRNA at saturating concentrations of EF 1 (Figure 2A), even when this saturating concentration of factor is added

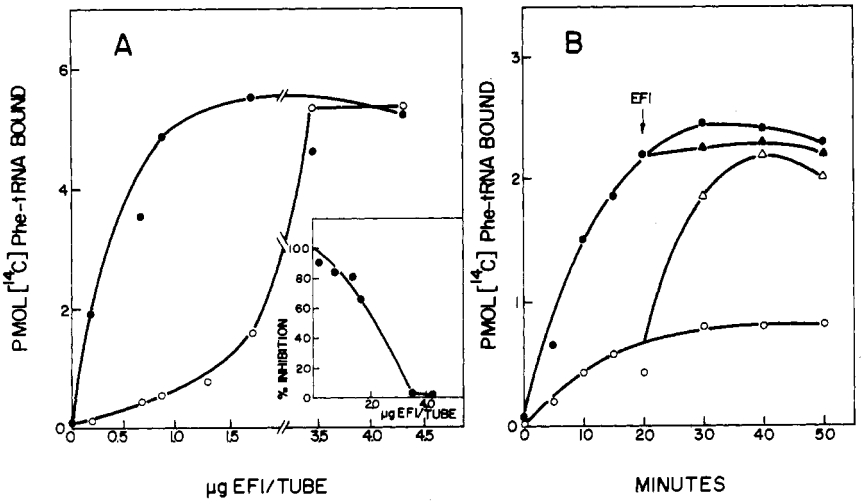

FIGURE 2: Effect of ricin treatment of rabbit reticulocyte ribosomes on [^{14}C]Phe-tRNA binding; dependence on EF 1 concentration and kinetics of the reaction. (A) 100-μl reaction mixtures, containing 50 mM Tris-HCl, pH 7.4, 60 mM KCl, 6 mM $MgCl_2$, 10 mM 2-mercaptoethanol, 30 pmol of ribosomes, and 10 μg of ricin, were preincubated for 1 h at 37 °C; GTP (0.1 mM), poly(U) (5 μg), the indicated amounts of EF 1, and 6.4 nCi of [^{14}C]phenylalanyl-tRNA (sp act. 463 Ci/mo) were then added; the mixtures were incubated for 20 min at 37 °C and the reactions were stopped and filtered as previously described (Carrasco et al., 1975). (●—●) Control; (○—○) ricin-treated ribosomes. (B) 1-ml reaction mixtures, containing 50 mM Tris-HCl, pH 7.4, 60 mM KCl, 6 mM $MgCl_2$, 10 mM 2-mercaptoethanol, 300 pmol of ribosomes, and 1.2 μg of ricin A chain were preincubated for 1 h at 27 °C; 0.1 mM GTP, 50 μg of poly(U), 4.6 μg of EF 1, and 64 nCi of [^{14}C]Phe-tRNA were then added, and the mixtures were incubated at 37 °C. 75-μl samples were taken at the indicated times into 2 ml of 20 mM Tris-HCl, pH 7.4, 50 mM KCl, 20 mM $MgCl_2$, and immediately filtered through nitrocellulose filters. (●—●) Control; (○—○) ricin A chain-treated ribosomes; (▲—▲) control + 23 μg of EF 1/ml added after 20 min of incubation; (△—△) ricin A treated ribosomes + 23 μg of EF 1/ml added after 20 min of incubation.

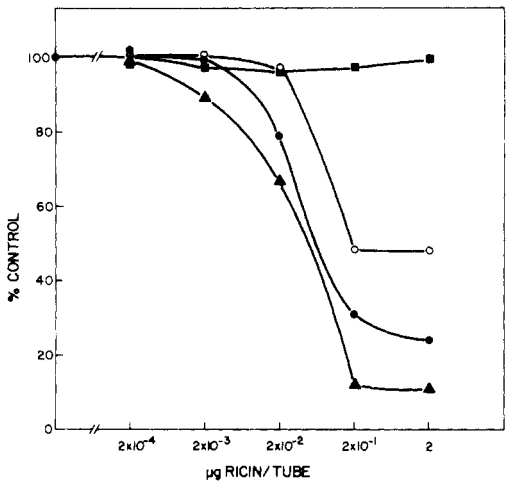

FIGURE 3: Effect of ricin treatment of rabbit reticulocyte ribosomes on [^{14}C]Phe-tRNA binding. Dependence on EF 1 concentration. The reaction was carried out as described in Figure 2A. (■—■) 57 μg of EF 1/ml; (○—○) 23 μg of EF 1/ml; (●—●) 9.2 μg of EF 1/ml; (▲—▲) 4.6 μg of EF 1/ml.

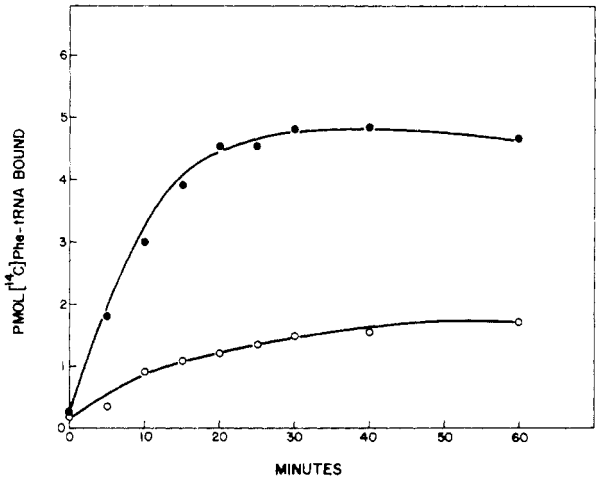

FIGURE 4: Kinetics of the enzymatic binding of [^{14}C]Phe-tRNA by normal and ricin-treated ribosomes. 1-ml reaction mixtures, containing 50 mM Tris-HCl, pH 7.4, 60 mM KCl, 6 mM $MgCl_2$, 10 mM 2-mercaptoethanol, 300 pmol of ribosomes, and 1.2 μg of ricin A chain (when required), were preincubated for 15 min at 37 °C; 64 nCi of [^{14}C]Phe-tRNA, GTP (0.1 mM), poly(U) (50 μg), and 4.6 μg of EF 1 were then added and the mixtures were incubated at 37 °C. At the indicated times, 100-μl samples were taken into 2 ml of 20 mM Tris-HCl, pH 7.4, 50 mM KCl, 20 mM $MgCl_2$, and immediately filtered through nitrocellulose filters (Carrasco et al., 1975). (●—●) Control; (○—○) ricin A chain treated ribosomes.

after the binding reaction reaches the plateau level (Figure 2B), and (b) the concentrations of EF 1 required for overcoming the damaging effect of ricin are strongly dependent on the concentration of the toxin (Figure 3). The effects observed in these curves are not just due to different kinetics of the aminoacyl-tRNA binding by ricin-treated ribosomes, since a similar extent of inhibition was observed after different times of incubation (Figure 4). Furthermore, when saturating concentrations of EF 1 are added to overcome the damaging effect of ricin, the binding of [^{14}C]Phe-tRNA is similar in the control and ricin-treated ribosomes at the different times of incubation (results not shown).

The above results might explain the failure of some workers to detect an inhibitory effect of ricin in aminoacyl-tRNA binding to ribosomes because they were probably working

TABLE I: [^{14}C]Phe-tRNA Binding to Ricin-Treated Ribosomes; Effect of EF 1.[a]

Ribosome Preincubations	Enzymatic Binding of Phe-tRNA in the Presence of: Low (cpm × 10^{-1})	EF 1 (% control)	High (cpm × 10^{-1})	EF 1 (% control)
Control	196		350	
Ricin	64	33	279	80
Low EF 1 (control)	157		313	
Ricin; low EF 1	63	40	252	80
High EF 1 (control)	162		261	
Ricin; high EF 1	71	44	287	109

[a] One milliliter reaction mixtures, containing 50 mM Tris-HCl, pH 7.4, 60 mM KCl, 6 mM $MgCl_2$, 10 mM 2-mercaptoethanol, 300 pmol of rabbit reticulocyte ribosomes, and 10 μg of ricin (when indicated), were incubated for 15 min at 37 °C. After this preincubation, either 4.6 (low EF 1) or 58 of μg of EF 1 (high EF 1) was added and incubated for 20 min at 37 °C when indicated. The mixtures were taken to 9 ml with 20 mM Tris-HCl, pH 7.4, 60 mM KCl, 6 mM $MgCl_2$, and 10 mM 2-mercaptoethanol and centrifugated at 45 000 rpm for 3 h. The pellet was resuspended in 50 μl of the same buffer and 260-nm absorption was measured. The enzymatic binding of [^{14}C]Phe-tRNA was carried out essentially as described in Figure 2A using 2.0 units (260 nm) of preincubated ribosomes and either 0.9 (low EF 1), or 5.75 μg of EF 1 (high EF 1). Reaction mixtures were incubated, for 20 min, at 37 °C and filtered on nitrocellulose filters.

under saturating conditions of EF 1 (Montanaro et al., 1973).

However, the results presented might be explained if either EF 1 or a contaminant component of the preparation was able to repair the lesion caused by ricin on the ribosome. To test this hypothesis, ricin-treated ribosomes were incubated with EF 1, sedimented by centrifugation, and then resuspended and incubated with the different components required for the enzymatic binding of aminoacyl-tRNA to take place. Table I clearly shows that the preincubation treatment with EF 1 is hardly relevant for the results obtained in further studies on the enzymatic binding of aminoacyl-tRNA. The damage of ricin on the ribosome can be overcome only when saturating concentrations of EF 1 are added in the assay for the enzymatic binding of aminoacyl-tRNA.

EF 2 Binding and Peptidyl-tRNA Translocation by Ricin-Treated Ribosomes. The site of action of ricin on the ribosome and the EF 2 binding site are mutually exclusive or overlapping, since (a) ricin inhibits the formation of the EF 2-GTP-ribosome complex (Carrasco et al., 1975; Montanaro et al., 1975) and the EF 2- and ribosome-dependent GTP hydrolysis (Montanaro et al., 1973; Sperti et al., 1975), and (b) preformation of the EF 2-GTP-ribosome complex protects the ribosome from ricin inactivation (Fernández-Puentes et al., in press). Moreover, ricin-treated ribosomes form the EF 2-GTP-ribosom complex in the presence of saturating concentrations of EF 2 (Figure 5), showing that the ribosome damage caused by ricin does not totally abolish, but only decreases the affinity of EF 2 for the ribosome. Similar results were obtained with rabbit reticulocyte (Figure 5) and yeast ribosomes (results not shown).

No inhibition of peptidyl-tRNA translocation by ribosomes has been observed in the presence of ricin (Montanaro et al., 1973; Carrasco et al., 1975), although the toxin inhibits the interaction of EF 2 with free ribosomes (Carrasco et al., 1975;

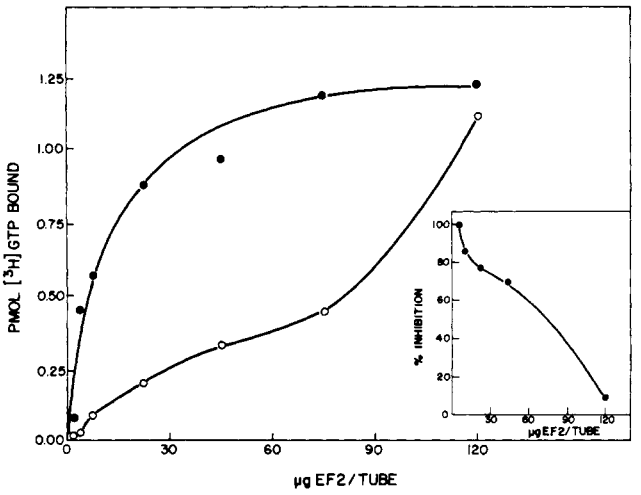

FIGURE 5: Effect of the EF 2 concentration on the inhibition of EF 2-GTP-ribosome complex formation by ricin-treated ribosomes. 100-μl reaction mixtures, containing 50 mM Tris-HCl, pH 7.4, 60 mM KCl, 8 mM $MgCl_2$, 10 mM 2-mercaptoethanol, and 6 pmol of ribosomes, were preincubated with 2 μg of ricin for 15 min at 37 °C; EF 2 and 0.5 μCi of [^{3}H]GTP (sp act. 17 Ci/mol) were then added and the mixtures incubated for 5 min at 37 °C; reactions were stopped and filtered as described (Carrasco et al., 1975). (●—●) Control; (○—○) ricin-treated ribosomes.

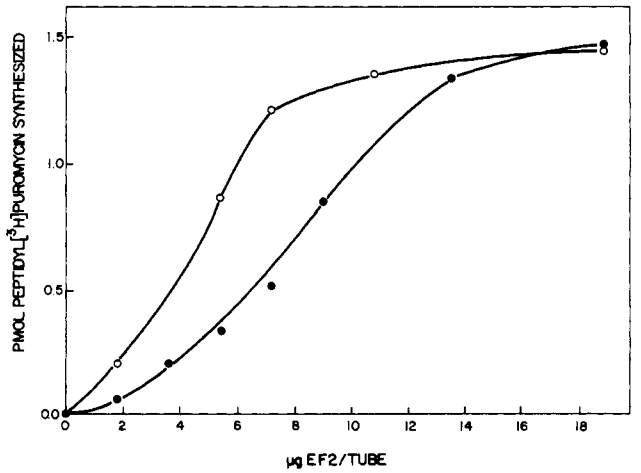

FIGURE 6: Effect of ricin A chain in the translocation of the peptidyl-tRNA by yeast polysomes. 50-μl reaction mixtures, containing 50 mM Tris-HCl, pH 7.4, 80 mM KCl, 5 mM $MgCl_2$, 10 mM 2-mercaptoethanol, 44 pmol of yeast polysomes, and 0.12 μg of ricin A chain, when required, were preincubated for 5 min at 37 °C; 0.8 mM GTP, the indicated amounts of EF 2, and 4 μM [^{3}H]puromycin (3.7 Ci/mmol) were then added and, after 10-min incubation at 37 °C, the reaction was stopped by addition of 2 ml of cold 10% trichloroacetic acid, filtered through GF/C Whatman glass fiber filters, and washed twice with 5% Cl_3CCOOH and 10 ml of ethanol. The filters were dried and radioactivity was estimated in a scintillation counter. (●—●) Control; (○—○) ricin A chain treated polysomes.

Montanaro et al., 1975). This apparently anomalous result might be due either to the use of a saturating concentration of EF 2 in the translocation reaction or because ricin is not able to interact with a ribosome carrying the peptidyl-tRNA in the A ribosomal site. Therefore, we have studied the effect of ricin on peptide bond formation with [^{3}H]puromycin in a natural system using yeast polysomes to which different concentrations of EF 2 were added in order to translocate the peptidyl-tRNA initially bound to the ribosomal A-site (Figure 6). We found no inhibition but rather some stimulation of the overall reaction. Therefore, ricin treatment of the ribosomes does not inhibit either peptide bond formation (Carrasco et al., 1975) or translocation in agreement with previous observations (Montanaro et al., 1973; Carrasco et al., 1975).

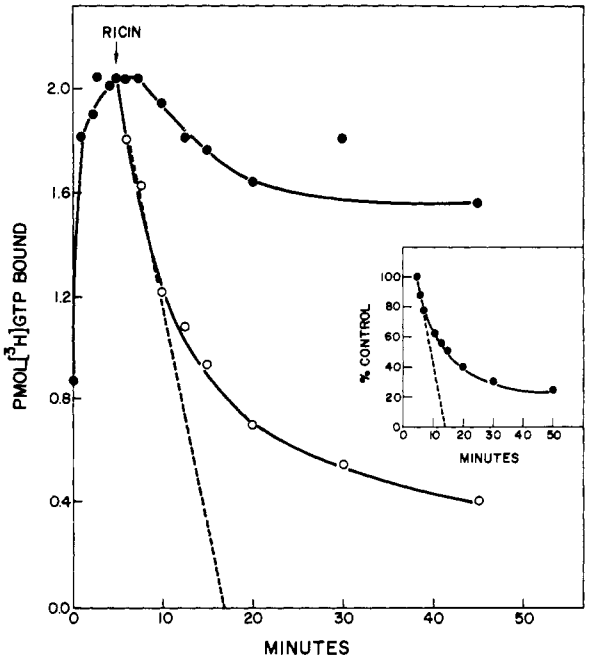

FIGURE 7: Kinetics of the breakdown of the EF 2-GTP-ribosome complex. 500 μl of reaction mixtures, containing 50 mM Tris-HCl, pH 7.4, 60 mM KCl, 8 mM $MgCl_2$, 10 mM 2-mercaptoethanol, 30 pmol of ribosomes, 375 μg of EF 2, and 2.5 μCi of [^{3}H]GTP, were incubated at 37 °C; 20-μl samples were taken at the indicated times into 2 ml of 20 mM Tris-HCl, pH 7.4, 50 mM KCl, 20 mM $MgCl_2$, and filtered as described under Materials and Methods. After 5 min of incubation, a 180-μl aliquot was taken mixed with 10 μg of ricin, incubated as above, and samples were taken at intervals. (●—●) Control; (○—○) in the presence of ricin.

The preformed EF 2-GTP-ribosome complex is reduced by 40% by the subsequent addition of ricin with 5 min of treatment (Figure 7). Since EF 2 binding has a protective effect on the damaging action of ricin on the ribosome (Fernández-Puentes et al., 1976), it appears that the toxin does not directly cause a dissociation of the EF 2-GTP-ribosome complex but merely interacts with free ribosomes, which are continuously formed in the system due to breakdown of the complex. If this is so, the initial rate of complex breakdown in the presence of ricin (Figure 7) would allow us to calculate that the half-life of the complex is of 6-7 min. Ricin has a reduced effect on the destruction of the complex formed in the presence of either fusidic acid (Table II, Expt 1) or GDPCP (Table II, Expt 2), whereas the EF 2-GTP-ribosome complex, which is less stable, is more affected by the toxin (Table II, Expt 1).

Comparative Effects of Lectins and Their A Chains. In order to study ricin and abrin and their A chains, we have compared their effects, under similar conditions, on the enzymatic binding of [^{14}C]Phe-tRNA, formation of the EF 2-GTP-ribosome complex, and translocation (Table III). The results obtained show that, contrary to diphtheria toxin which specifically inhibits translocation (Vázquez, 1974; review), ricin, abrin, ricin A chain, abrin A chain, and ricinus agglutinin A chain inhibit the enzymatic binding of aminoacyl-tRNA and EF 2-GTP-ribosome complex but do not affect translocation, indicating a similar mechanism of action for all of them.

Discussion

The lectins ricin an abrin are very interesting glycoproteins presenting some unique features in their mode of action, since

(a) they are the only known enzymes which selectively recognize the eukaryotic ribosomes as a substrate having no effect on the prokaryotic ribosome (Carrasco et al., 1976; review), (b) they are the only known inhibitors that, acting on the 60S ribosome subunit, specifically prevent the enzymatic binding of aminoacyl-tRNA without having any effect on the nonenzymatic binding (Carrasco et al., 1975), and (c) they inactivate a site on the 60S subunit involved in GTP hydrolysis (Montanaro et al., 1973; Benson et al., 1975). This ribosomal moiety is a key site on the ribosome which is involved in the alternate interaction with the elongation factors EF 1 and EF 2 (Carrasco and Vazquez, 1973; Carrasco et al., 1975).

However, there are still some important unresolved problems concerning the mode of action of ricin, since (a) intact cells and crude cell-free systems are very sensitive to ricin (Olsnes and Pihl, 1972b) but the sensitivity in purified and resolved cell-free systems is smaller and very variable depending on the systems and conditions used by the different workers (Montanaro et al., 1973; Benson et al., 1975; Sperti et al., 1975; Carrasco et al., 1975), and (b) the toxin does not inhibit translocation in model systems, although it prevents EF 2 interaction with free ribosomes (Carrasco et al., 1975).

The results presented in this paper clearly show that ricin damage to the ribosome does not totally prevent the enzymatic binding of aminoacyl-tRNA but only decreases EF 1 affinity for the ribosome. This explains the high activity of ricin in intact cells and cell-free systems, since it has been observed that EF 1 is limiting in the cell (Girgis and Nichols, 1972). On the other hand, EF 1 is saturating in most cell-free systems used, which are, therefore, less sensitive or not inhibited by ricin.

Our finding in this paper that translocation in polysomal systems is not inhibited by ricin confirms similar findings in model systems (Montanaro et al., 1973; Carrasco et al., 1975). Indeed, the enzymatic binding of aminoacyl-tRNA to the acceptor site of the ribosome has been shown to protect the ribosome from ricin action (Fernández-Puentes et al., 1976). It is likely that ricin cannot interact with ribosomes with peptidyl-tRNA bound to the acceptor site and, therefore, in the pretranslocated state. Furthermore, we find some enhancement in peptidyl-[^{3}H]puromycin formation in our polysomes in the presence of ricin (Figure 6). The stimulation by ricin occurs when EF 2 is limiting; this factor has a higher affinity for free ribosomes that are always present in polysomal preparations and, therefore, sequester some of the EF 2 available for translocation. Since EF 2 cannot interact with free ribosomes in the presence of ricin (Montanaro et al., 1973; Carrasco et al., 1975, 1976), the EF 2 is not sequestered in the presence of the toxin. Therefore, translocation takes place to a higher extent in polysomes in the presence of the toxin when EF 2 is limiting, but not when there is an excess of this factor (Figure 6).

It is widely accepted that there is a single site on the 60S ribosome subunit involved not only in elongation factor de-

TABLE II: Stability of the EF 2–Ribosome Complex.[a]

Additions	Ricin	cpm × 10^{-2}	% Control
Expt 1: binding [^{3}H]GTP:			
None	−	1012	100
None	+	461	45
+ Fusidic acid (1 mM)	−	1355	100
+ Fusidic acid (1 mM)	+	889	66
Expt 2: binding [^{3}H] GDPCP:			
Control	−	90	100
Control	+	67	75
+ Fusidic acid	−	84	100
+ Fusidic acid	+	69	81

[a] Mixtures of 100 μl, containing 50 mM Tris-HCl, pH 7.4, 60 mM KCl, 8 mM $MgCl_2$, 10 mM 2-mercaptoethanol, 60 pmol of ribosomes, 75 μg of EF 2, 1 mM fusidic acid (when required), and either 500 nCi of [^{3}H]GTP (sp act. 17 Ci/mol) or 73 nCi of [^{3}H]GDPCP (sp act. 82 Ci/mol), were incubated for 5 min at 37 °C. Two micrograms of ricin was then added where indicated and incubated for 30 min at 37 °C. The reaction was stopped and the reaction mixtures were filtered (Carrasco et al., 1975).

TABLE III: Comparative Effects of Different Toxins in the Enzymatic Binding of [^{14}C]Phe-tRNA, EF 2 Binding and Translocation.[a]

Additions	Enzymatic Binding of [^{14}C]Phe-tRNA		EF 2 Binding		Translocation	
	cpm × 10^{-1}	% Control	cpm × 10^{-2}	% Control	cpm × 10^{-1}	% Control
None	313	100	999	100	257	100
Ricin	76	24	10	1	259	100
Abrin	72	23	25	2	270	105
Ricin A chain	73	23	35	3	250	97
Abrin A chain	70	22	21	2	279	108
Ricinus agglutinin A chain	81	26	22	2	233	90
Diphtheria toxin (NAD^+)	296	85	925	93	0	0

[a] The enzymatic binding assay was carried out essentially as described in the legend of Figure 2A but the ribosomes were preincubated for 1 h at 37 °C with either 1 μg of ricin, 2 μg of abrin, 0.36 μg of ricin A chain, 0.28 μg of abrin A chain, or 0.020 μg of ricinus agglutinin A chain. Diphtheria toxin (0.05 mM) was preincubated for 5 min at 37 °C with 0.05 mM NAD. 0.92 μg of EF 1 was added per tube in all the cases in the binding reaction. For the binding of EF 2, 100-μl reaction mixtures, containing 50 mM Tris-HCl, pH 7.4, 60 mM KCl, 8 mM $MgCl_2$, 10 mM 2-mercaptoethanol, and 60 pmol of ribosomes were preincubated for 1 h at 37 °C with either 1 μg of ricin, 2 μg of abrin, 0.36 μg of ricin A chain, 0.28 μg of abrin A chain, or 0.20 μg of ricinus agglutinin A chain; 75 μg of EF 2 and 500 nCi of [^{3}H]GTP (sp act. 17 Ci/mol) were then added; the incubation was continued for 5 min at 37 °C and the reaction mixtures were filtered (Carrasco et al., 1975). For the translocation assay, 100-μl reaction mixtures, containing 50 mM Tris-HCl, pH 7.4, 80 mM KCl, 4 mM $MgCl_2$, 10 mM 2-mercaptoethanol, 44 pmol of polysomes from rabbit reticulocytes, and either 6 μg of ricin, 2 μg of abrin, 0.36 μg of ricin A chain, 0.28 μg of abrin A chain, or 0.20 μg of ricinus agglutinin A chain, were preincubated for 3 min at 37 °C; 0.1 mM GTP, 18 μg of EF 2, and 800 nCi of [^{3}H]puromycin (sp act. 3.7 Ci/mM) were finally added and reaction mixtures were incubated for 10 min at 37 °C. The reaction was stopped with cold 10% trichloroacetic acid, filtered on GC/C Whatmann fiber filters, and washed with 5% trichloroacetic acid and 10 ml ethanol, to remove the [^{3}H]-puromycin that had not reacted; filters were dried and counted with toluene butyl-PBD 5%.

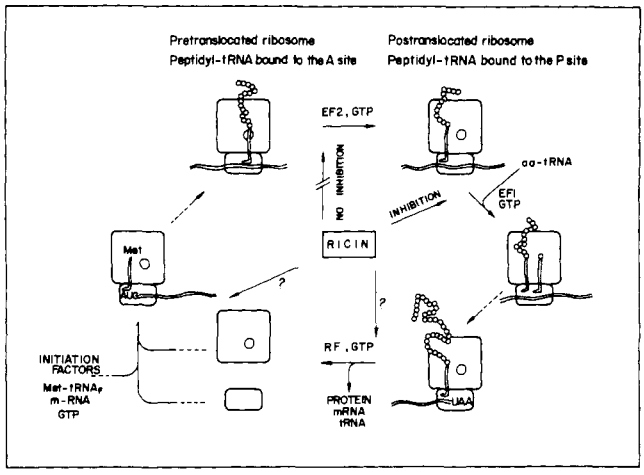

FIGURE 8: Schematic representation of the ribosome cycle showing the possible sites of inhibition by ricin.

pendent GTP hydrolysis, but also in initiation factor 2- and release factor-GTP hydrolysis, in initiation and termination, respectively (Modolell and Vázquez, 1975; Tate et al., 1973; reviews). Therefore, ricin might also inhibit initiation and termination in mammalian systems by preventing the interaction of initiation factor 2 and release factor with this common interaction site of the 60S ribosome subunit. This is schematically presented in Figure 8. The toxin interacts with ribosomes carrying the peptidyl-tRNA in the P-site blocking the interaction of the complex aminoacyl-tRNA-EF 1-GTP with the ribosome, thus inhibiting the aminoacyl-tRNA binding step. On the other hand, although the toxin blocks the binding of EF 2 to free ribosomes, it does not interfere with translocation in any of the model systems used to study this step. It is possible that ricin also prevents initiation and/or termination by blocking the binding of the corresponding factors required for GTP hydrolysis in these steps. This possibility has not been tested directly. However, it is most likely that ricin blocks initiation, since it causes polysome breakdown in intact cells (Lin et al., 1972; Grollman et al., 1974). This effect of ricin in polysome breakdown is not very strong (Grollman et al., 1974; Onozaki et al., 1975); this might be due to the further blockade of elongation by the toxin, as indicated above.

Evidence presented in this and previous studies suggests that ricin and abrin have a similar mode of action on the ribosome, although there are some differences in their chemical structures (Olsnes and Pihl, in press; review).

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