EFFECTS OF SOME PROTEINS THAT INACTIVATE THE EUKARYOTIC RIBOSOME

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1. Introduction

The glycoproteins abrin and ricin ([1] review) and the proteins PAP [2], alpha sarcin (J. E. Davies, personal communication), crotin II [3], curcin II [3], enomycin [4] and phenomycin [5] have been reported to block translation by eukaryotic ribosomes. The glycoproteins abrin and ricin are very active in intact cells since they are composed of two subunits, one of which (the B chain) facilitates the entrance into the cell of the other subunit (the A chain), which catalytically inactivates the 60 S ribosomal subunits ([1] review). However ricin A chain and the individual proteins abrin A chain, PAP, alpha sarcin, crotin II, curcin II, enomycin and phenomycin are very active in blocking translation in cell-free systems but far less so in intact cells probably owing to the cellular permeability barrier [1–5]. All these toxins might have a similar or related mechanism of action since abrin A chain [6], ricin A chain [6], PAP [2], crotin II [3], alpha sarcin (J. E. Davies, personal communication) and enomycin [4,7] were observed to have a certain inhibitory effect on the EF 1-dependent binding of aminoacyl-tRNA to the ribosome. However the mode of action of the toxins has been studied independently by different groups using different cells and cell-free systems and it is not possible to conclude from the results available whether or not the toxins act in a similar manner.

We have therefore studied comparatively the effects of ricin and abrin with PAP, alpha sarcin and enomycin on the EF 1-dependent binding of aminoacyl-tRNA, formation of the EF 2-GTP–ribosome complex, peptidyl-tRNA translocation and EF 2- and ribosome-dependent GTP hydrolysis using identical cell-free systems. The results obtained are reported here.

2. Materials and methods

The preparation of rabbit reticulocyte ribosomes and elongation factors EF 1 and EF 2, [^{14}C]Phe-tRNA (specific activity 495 Ci/mol), yeast ribosomes, and yeast polysomes was as previously described [6]. The assay systems for poly(U)-directed polyphenylalanine synthesis, enzymic binding of [^{14}C]Phe-tRNA, EF 2 binding to ribosomes in the presence of [^{3}H]GTP (spec. act. 2.26 Ci/mM) and peptidyl-tRNA translocation by yeast polysomes have also been described [8]. Otherwise, specific conditions concerning each experiment are described in the legends of figures and tables.

Ricin, abrin, ricin A chain and abrin A chain were kindly given to us by Dr S. Olsnes (Norsk Hydro's Institut for Kreftforskning, Oslo). PAP was given to us by Dr B. Hardesty (University of Texas, Austin), alpha toxin by Dr J. E. Davies (University of Wisconsin, Madison) and enomycin by Dr N. Tanaka (Institute for Applied Microbiology, Tokyo).

3. Results

3.1. *The effects of the inhibitors on polyphenylalanine synthesis and the enzymic binding of [^{14}C]Phe-tRNA to ribosomes*

The four proteins tested under identical conditions inhibit polyphenylalanine synthesis (not shown). The extent of the inhibition by these toxins varies con-

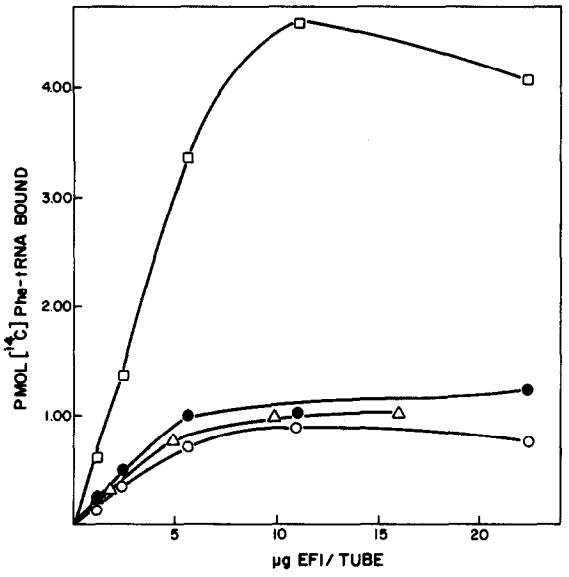

Fig.1. Effects of toxin treatment of rabbit reticulocyte ribosomes on the enzymic binding of [^{14}C]Phe-tRNA: dependence on EF 1 concentration. Reaction mixtures, 100 µl containing 50 mM Tris–HCl, pH 7.4, 60 mM KCl, 6 mM $MgCl_2$, 39.5 pmol ribosomes and the required toxin were incubated for 10 min at 37°C; GTP (0.1 mM), poly(U) (5 µg), the indicated amounts of EF 1 and 4.7 nCi [^{14}C]-Phe-tRNA were then added. The mixtures were incubated for 15 min at 37°C and the reactions were stopped and the mixtures filtered as previously described [7]. Control without inhibitor (□—□) and ribosomes treated with 2 µg PAP (●—●), 2 µg alpha sarcin (○—○) and 25 µg enomycin (△—△).

siderably with the system used since it depends on the length of time the ribosomes are preincubated with the toxin and on the concentrations of EF 1, EF 2, ribosomes and [^{14}C]Phe-tRNA in the assays [1,2,9].

Ricin A chain, PAP, alpha sarcin and enomycin inhibit the EF 1-dependent binding of [^{14}C]Phe-tRNA to the ribosomes (fig.1). Inhibition by enomycin is lower than previously observed [4] probably owing to partial inactivation of our preparation during storage. The inhibitory effect of the toxins appears to be due to a decrease in the affinity of the toxin-treated ribosomes for EF 1 since the non-enzymic binding of [^{14}C]Phe-tRNA to ribosomes is not affected by the toxins (results not shown). Similar results were previously observed with ricin [6].

3.2. *Effects of the toxins on the formation of the EF 2–[^{3}H]GTP–ribosome complex and EF 2- and ribosome-dependent GTP hydrolysis*

Enomycin appears not to inhibit but rather to enhance the formation of the complex (fig.2A) whereas PAP, alpha sarcin and abrin A chain inhibit the formation of the EF 2–[^{3}H]GTP–ribosome complex (fig.2B).

Enomycin and to a lesser extent alpha sarcin inhibit the EF 2- and ribosome-dependent GTP hydrolysis uncoupled from translocation (table 1). On the other hand neither PAP nor abrin A chain inhibit the reaction but rather enhance it at limiting concentrations of EF 2. Similarly to abrin A chain, ricin has no effect on the reaction (not shown). The effects of alpha sarcin, PAP and abrin A chain were further tested on the ribosomal GTPase activity in the absence of EF 2; a certain stimulation in this activity was observed in the presence of PAP and abrin A chain whereas alpha sarcin has no effect (not shown).

3.3. *The effects of the toxins on translocation*

In order to test the activity of the toxins in translocation we have studied their effects on peptide bond formation with [^{3}H]puromycin in a natural system using yeast polysomes to which different concentrations of EF 2 had been added to translocate the peptidyl-tRNA initially bound to the ribosomal A-site (table 2). Neither of the toxins has any significant inhibitory effect on the reaction and we therefore conclude that they do not inhibit either peptide bond formation or peptidyl-tRNA translocation.

4. Discussion

The toxins that we have studied in this contribution have in common their inhibitory effect on EF 1-dependent binding of aminoacyl-tRNA and a lack of inhibition on translocation. However enomycin, unlike the other toxins, does not inhibit the formation of the EF 2–GTP–ribosome complex. Furthermore enomycin and alpha sarcin inhibit the EF 2- and ribosome-dependent GTP hydrolysis whereas the other toxins tested (ricin, abrin A chain and PAP) do not. Obviously, inhibitory effect of ricin, abrin and PAP on the formation of the EF 2–GTP–ribosome

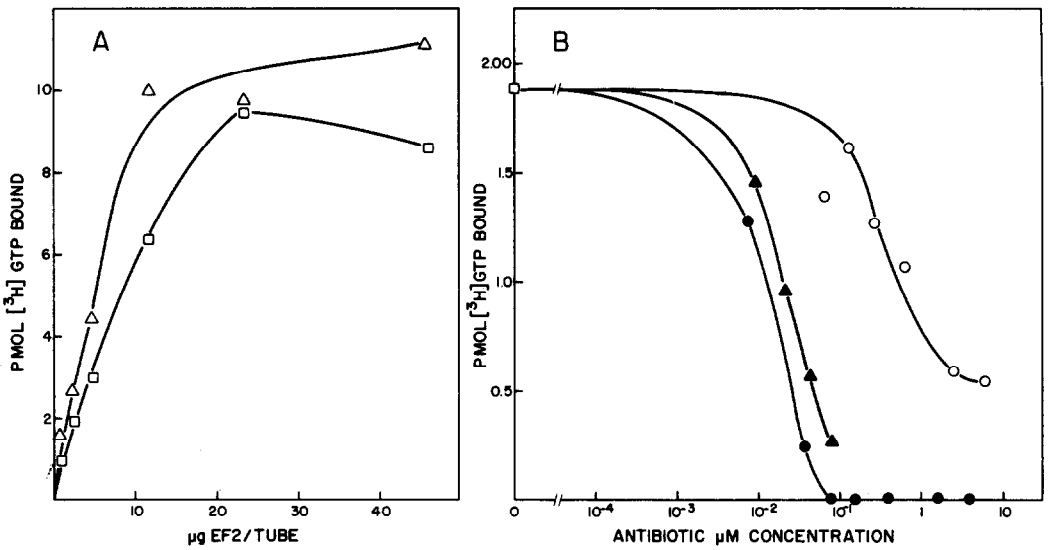

Fig.2. Effects of toxin treatment of yeast ribosomes on EF 2–GTP–ribosome complex formation. Reaction mixtures, 100 µl, containing 50 mM Tris–HCl, pH 7.4, 60 mM KCl, 8 mM $MgCl_2$, 10 mM 2-mercaptoethanol, and the amounts of ribosomes indicated below were preincubated with the required toxin for 10 min at 37°C. EF 2 (as indicated) and 35 µCi [^{3}H]GTP were then added, the mixtures incubated for 5 min at 37°C and the reactions stopped and the mixtures filtered as previously described [7]. Fig.2A: 39 pmol ribosomes were preincubated without inhibitor (□—□) and with 25 µg enomycin (△—△). Fig.2B: 26 pmol ribosomes were preincubated without inhibitor (□) and with the indicated concentrations of PAP (●—●), abrin A chain (▲—▲) and alpha sarcin (○—○). EF 2, 3 µg was added in all cases to the incubation mixtures.

Table 1
Effects of the toxins on the EF 2- and ribosome-dependent GTPase uncoupled from translocation

Experiments	GTP hydrolysis			
	+3 µg EF 2		+12 µg EF 2	
	(pmol)	(% Control)	(pmol)	(% Control)
A + Abrin A chain (0.36 µg)	200	125	640	82
B + PAP (2 µg)	150	190	500	86
C + Alpha sarcin (2 µg)	133	27	500	40
D + Enomycin (25 µg)	135	29	530	45

Reaction mixtures, 50 µl, containing 50 mM Tris–HCl, pH 7.4, 60 mM KCl, 8 mM $MgCl_2$, 10 mM 2-mercaptoethanol, 12.6 pmol yeast polysomes, were preincubated with the required toxin for 5 min at 37°C. The indicated amounts of rabbit reticulocyte EF 2 and 3.6 nCi [^{32}P]GTP (spec. act. 2.9 Ci/mol) were then added and after 5 min incubation at 37°C the reaction was stopped by addition of 150 µl perchloric acid, 2.5 mM K_2HPO_4, 4% activated charcoal [11]. After mixing and pelleting, 100 µl aliquots of the supernatant were taken and radioactivity estimated in vials containing 2 ml Bray's BPBD–Cab–*O*–sil scintillation mixture. The different toxins were tested in individual experiments with different batches of EF 2.

Table 2
The effects of the toxins in the translocation of peptidyl-tRNA by yeast polysomes

Experiments	Peptidyl-[^{3}H]puromycin formed (pmol)			
	+4 μg EF 2		+16 μg EF 2	
	(pmol)	(% Control)	(pmol)	(% Control)
A + Abrin A chain (0.36 μg)	3.4	126	10.0	81
B + PAP (2 μg)	4.6	162	9.5	109
C + Alpha sarcin (2 μg)	3.0	100	9.2	84
D + Enomycin (25 μg)	2.1	87	7.5	100

Reaction mixtures, 50 μl, containing 50 mM Tris–HCl, pH 7.4, 80 mM KCl, 4 mM $MgCl_2$, 10 mM 2-mercaptoethanol, 88 pmol yeast polysomes and the required toxin were preincubated for 5 min at 37°C. The indicated amounts of EF 2, 0.8 mM GTP and 4 μM [^{3}H]puromycin (1.2 Ci/mmol) were then added and, after 10 min of incubation at 37°C, the reactions were stopped and the mixtures filtered as described [9].

complex does not necessarily imply an inhibitory effect of the toxins on EF 2- and ribosome-dependent GTP hydrolysis. In fact our findings with these toxins confirm prior reports with PAP and ricin [1–3,9]. Alpha sarcin is a strong inhibitor of both formation of the EF 2–GTP–ribosome complex and GTP hydrolysis whereas enomycin inhibits the EF 2-dependent GTPase but does not affect or rather enhances the formation of the ternary complex, which might be more stable in the presence of the toxin.

We can summarize the overall effects of the toxins studied above by stating that all of them prevent EF 1-interaction with the ribosome and therefore inhibit the enzymic binding of aminoacyl-tRNA. All the toxins appear to have some effect on either EF 2-interaction with the ribosome or EF 2-dependent GTPase in model systems but do not inhibit translocation since the peptidyl-tRNA or EF 2 bound to the ribosomal A-site appear to prevent the interaction of the toxins with the ribosome as previously shown with abrin and ricin [9]. All these toxins act catalytically on the larger subunit of the ribosome. Their sites of action are closely connected and appear to be involved in EF 1- and EF 2-interaction. This postulate explains the results obtained with enomycin, since this toxin does not inhibit the formation of the initiation complex but prevents formation of the initial dipeptide [10]. Furthermore our results are in agreement with previous reports concerning the mode of action of PAP [2], abrin and ricin [1,3,9].

Acknowledgements

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