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BINDING OF T-2 TOXIN TO EUKARYOTIC CELL RIBOSOMES

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Abstract—The binding of radiolabeled T-2 to eukaryotic ribosomes was studied. The toxin bound to ribosomes in a time-, temperature- and concentration-dependent manner. The binding was saturable (0.3 nM), reversible at 37° (half-time ~ 2.5 hr) and specific. The stoichiometry was one toxin molecule bound per ribosome. Binding of T-2 appeared to stablize the toxin recognition site to thermal degradation. A synthetically derived epimer of T-2 bound to the same ribosomal site as authentic T-2, but apparently with lower affinity. Two other trichothecene toxins tested blocked the binding of T-2 to ribosomes in a manner reflecting their protein synthesis inhibitory potencies. Anisomycin blocked the binding of T-2 to both isolated ribosomes and cells, whereas emetine blocked binding only to cells. Our data, together with that in the accompanying paper (Middlebrook JL and Leatherman DL, *Biochem Pharmacol* **38**: 3093–3102, 1989), suggest that T-2 interaction with CHO cells is best viewed as a free, bidirectional movement of toxin across the plasma membrane and specific high-affinity binding to ribosomes.

The trichothecene toxins are potent inhibitors of eukaryotic protein synthesis. Studies carried out in several laboratories are consistent with a view that these toxins bind to a common site on the 60S ribosomal subunit [1]. Curiously enough, occupancy of this site by some trichothecenes, such as T-2, leads to a block of protein synthesis at initiation [2], whereas other trichothecenes, such as trichodermin, appear to block elongation or termination [3]. There is no published evidence that the trichothecenes act catalytically, as do certain protein toxins such as diphtheria toxin or ricin. Moreover, in at least one instance, the stoichiometry for trichothecene–ribosome binding was shown to be 1:1 [1, 4–6]. Therefore, it is probable that these toxins act by steric hindrance of a ribosomal interaction with one or more of the components involved in protein synthesis.

As the binding of trichothecenes to target cell ribosomes appears to be critical for the expression of toxicity, it would be valuable to define the biophysical parameters of this interaction. Some information is available on the binding of trichodermin to eukaryotic ribosomes [4–6]; the dissociation constant is about 1 μM and the stoichiometry approximately one toxin molecule per ribosome. It appears that several other trichothecenes will compete for trichodermin–ribosome binding, although incompletely in some instances [1]. To obtain further information into trichothecene–ribosome binding and to compare it with data on toxin–whole cell interactions, we investigated several aspects of T-2–ribosome binding. We found that significant differences exist between the ribosomal interactions of initiation and elongation/termination-acting trichothecenes.

MATERIALS AND METHODS

Cells and cell culture. Seed stock for the Chinese hamster ovary (CHO) line was obtained from the American Type Culture Collection (ATCC No. CCL-61). Cells were maintained in 75-cm^2 T-flasks (Costar No. 3075) with Earle's Minimal Essential Medium (EMEM), 10% fetal bovine serum and 50 μg/ml gentamycin. To obtain large numbers of cells for ribosomal preparations, CHO cells were seeded into 4-L spinner flasks and cultured with α-MEM supplemented with 10% fetal calf serum, 50 μg/ml gentamycin and 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes), pH 7.4. Yeast cells (*Saccharomyces cerevisiae*) were obtained as a block from a local bakery.

Purification of ribosomes. Ribosomes from CHO cells were purified as described by Gupta and Siminovitch [7]. Yeast ribosomes were prepared by washing 25 g of cells in buffer A (120 mM KCl, 10 mM $MgCl_2$, 6 mM mercaptoethanol, 10 mM Tris, pH 7.2) and resuspending them in a volume of 250 ml. The cells were homogenized by three 90-sec bursts with a Beadbeater (Biospec Products, Bartlesville, OK) separated by 90-sec cooling periods. After centrifugation at 26,000 *g*, the clear supernatant fraction was used to purify ribosomes exactly as was done with the CHO cell ribosomes.

Toxins. T-2 was purchased from Calbiochem (La Jolla, CH); the other trichothecene toxins were obtained from the Sigma Chemical (St Louis, MO). T-2 was tritium labeled by New England Nuclear (Boston, MA) (9.0 Ci/mmol) or Amersham/Searle (Arlington Heights, IL) (14 Ci/mmol) according to a previously published procedure [8]. All toxins were prepared in methanol and diluted so that the maximal alcohol concentration exposure to ribosomes was 0.1%.

Binding assay. Two techniques were employed to measure T-2-ribosome binding. For binding isotherms and Scatchard analyses, a multi-chambered dialysis apparatus was used. Identical chambers of

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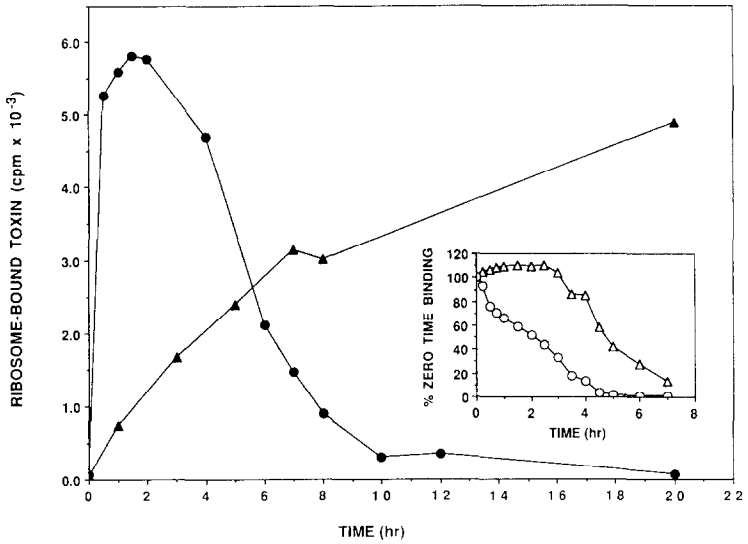

Fig. 1. Kinetics of T-2 toxin association with ribosomes. Yeast ribosomes were suspended in buffer D at 10 O.D.$_{260}$/ml and incubated with 0.1 μCi/ml [^{3}H]T-2 toxin (8.9 Ci/mmol) for the specified times at either 4° (▲——▲) or 37° (●——●). Ribosome-bound toxin was then determined by filtration, as described in Materials and Methods. Values are the averages from duplicate determinations. Inset: Loss of T-2 toxin association with ribosomes at 37°. Yeast ribosomes were suspended in buffer D at 10 O.D.$_{260}$/ml and incubated at 4° for 18 hr with (△——△) or without (○——○) 1 μCi/ml [^{3}H]T-2 toxin (14 Ci/mmol). After preincubation, 100-μl aliquots of toxin-bound ribosomes were transferred to 37° for the indicated times and then washed immediately by filtration assay, as described in Materials and Methods. Similar aliquots of unbound ribosomes were transferred to 37° for the specified times, then incubated with 1.0 μCi/ml [^{3}H]T-2 toxin for an additional 30 min, and washed by filtration. Values are the averages of duplicate determinations at each point.

either 0.1 or 0.5 ml were separated by a dialysis membrane. Typically, with the larger unit, 0.5 ml of a 10 O.D.$_{260}$/ml ribosomal preparation in buffer D (20 mM Hepes, pH 7.5; 120 mM KCl; 1.5 mM magnesium acetate; 6 mM 2-mercaptoethanol) was introduced into one side and 0.5 ml of buffer D containing toxin(s) was placed in the other. After incubation under the specified conditions, aliquots were removed, their absorbances were measured, and each was counted in a liquid scintillation spectrophotometer.

For kinetic and certain other experiments, a filter assay was employed. Usually, 10–20 μl of toxin(s) was added to 100 μl of a 10 O.D.$_{260}$/ml ribosomal preparation, and incubation was carried out under the conditions stipulated. Binding was terminated by addition of the entire sample to a Whatman GF/F glass fiber filter, followed by four 4-ml washes. Each filter was then counted using Aquasol in a liquid scintillation spectrophotometer.

Binding of T-2 to cells was assayed as detailed in the accompanying paper [9].

RESULTS

Kinetics of T-2-ribosome association and dissociation. The kinetics of T-2-ribosome binding are depicted in Fig. 1. At 37°, the association of T-2 with ribosomes was biphasic. In the initial phase, binding increased rapidly to what appeared to be a plateau equilibrium state by 30 min. However, if the incubation was continued further, a second phase was observed in which the binding gradually declined to essentially baseline values. In contrast T-2-ribosome binding at 4° was much slower, taking 24–28 hr to reach completion. Continuing the incubation for up to an additional 24 hr at 4° did not result in the appearance of a descending limb of the binding curve.

One likely explanation for the latter stage decline in T-2-ribosome binding at 37° is thermal degradation of the ribosome–T-2 binding capacity. To test that possibility, we incubated ribosomes at 37° and, at various times, removed aliquots and bound them with [^{3}H]T-2 for 30 min at 37°. As shown by the data in the inset of Fig. 1, incubation at 37° resulted in an immediate loss of binding capacity, a trend which continued to baseline values. The kinetics of this loss were roughly linear and did not describe a straight line when plotted semilogarithmically. For comparative purposes, we examined the 37° rate of degradation of ribosomes prebound at 4° with T-2. We consistently observed a time lag of approximately 4 hr before the apparent degradation process began (Fig. 1, inset). In addition, there was a small, but reproducible, increase in the binding immediately after the temperature was raised to 37°. Despite the delay in their inception, the thermal degradation rates of T-2-bound or -unbound ribosomes were, within experimental error, the same as judged by the slopes for the two lines (Fig. 1, inset). Other experiments (not shown) demonstrated that the T-2 binding capacity of ribosomes held at 4° was very stable, requiring 3 weeks to decay 50%.

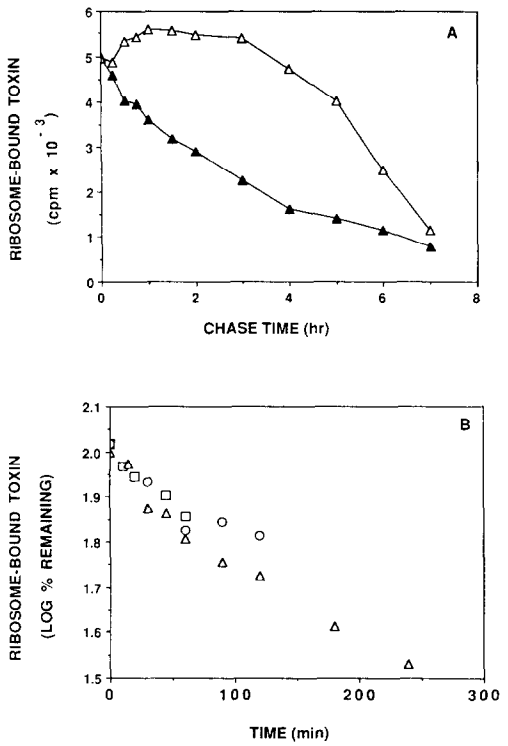

Fig. 2. Kinetics of T-2 toxin dissociation from ribosomes at 37°. (A) Yeast ribosomes were suspended in buffer D at 10 O.D.$_{260}$/ml and prebound with 0.1 μCi/ml [^{3}H]T-2 toxin (9.0 Ci/mmol) for 30 min at 37°. Incubation was then continued with (▲——▲) or without (△——△) a 100-fold molar excess of non-labeled T-2 toxin. At the specified times, ribosome-bound T-2 toxin was determined by filtration, as described in Materials and Methods. Values are the averages of duplicate determinations at each point. (B) Semi-logarithmic plots for the data obtained in three experiments performed exactly as in panel A.

The reversibility of T-2-ribosome binding was demonstrated by a "chase" experiment. After prebinding the ribosomes for 30 min with radiolabeled T-2 at 37°, a 100-fold molar excess of unlabeled T-2 was added and incubation continued. As shown by the data in Fig. 2A, the addition of a chase induced a prompt and progressive reduction in radiolabeled T-2–ribosome binding. Unlike the patterns seen in the inset of Fig. 1, the kinetics for chase-induced loss of binding were semi-logarithmic, as shown by a plot of this and two other experiments (Fig. 2B). The apparent first-order rate constant for dissociation was 0.12/hr (half-time 2.5 hr). Treatment of the data was not extended past 4 hr, since it appeared that spontaneous loss of binding became significant (Fig. 2A). Although dissociation of ribosome-bound T-2 could be readily demonstrated at 37°, the binding was apparently much tighter at 4°. When chase experiments were carried out at that temperature, no measurable dissociation was observed for up to 24 hr.

Measurement of binding constants and stoichiometry for T-2–ribosome binding. Binding isotherms for T-2–ribosome association at 4° are shown in Fig. 3. Because it was technically difficult to grow the large quantities of CHO cells required for ribosomal preparation, we performed most binding experiments with yeast cell-derived ribosomes. In almost every variable, T-2–yeast ribosome binding and T-2–CHO ribosome binding were indistinguishable. Figure 3 shows the results of a binding isotherm experiment for T-2 and yeast ribosomes. As the concentration of added T-2 increased, so did the level of binding. At all concentrations examined, the addition of a 100-fold excess of unlabeled T-2 competed for 90% or more of the radiolabeled T-2–ribosome binding, indicating a specificity to the interaction. When analyzed by the method of Scatchard [10], the data described straight lines with high correlation coefficients (Fig. 3, inset). The slopes and x intercepts provided estimates for the dissociation constant and sites per ribosome as 2.7×10^{-8} M and 1.03, respectively. For five separate experiments with yeast ribosomes, the values for each parameter (mean ± SE) were $2.9 \pm 0.3 \times 10^{-8}$ M and 1.07 ± 0.02 sites/ribosome.

In another approach to determine the dissociation constant for T-2–ribosome binding, the amount of bound radiolabeled T-2 in the presence of various (competitive) amounts of unlabeled T-2 was determined. That concentration of unlabeled T-2 which blocked 50% of the binding should be an estimate of the dissociation constant. Figure 4 shows a representative experiment which gave a value of 6.4×10^{-8} M as the dissociation constant at both 4° and 37°. Although the binding assays were reproducible from the standpoint of replicate precision, there was some day-to-day variation in the position of a concentration–response curve. To make a reliable comparison of the competition of T-2 for [^{3}H]T-2 binding to CHO cells versus CHO cell ribosomes, we performed the experiment at the same time using the same dilution of competitor. The data (also shown in Fig. 4) presented a pattern in which competition curves with cells at 4° and ribosomes at both 4° and 37° were identical, whereas the curve for cells at 37° was positioned to the left of the other three.

Stereospecificity of T-2–ribosome binding. As detailed in the accompanying paper [9], an epimer of T-2 (termed β-T-2) was produced from the naturally occurring, stereochemically pure toxin (α-T-2) during the radiolabeling procedure. We found that β-T-2 was considerably less potent than α-T-2 at inhibiting cellular protein synthesis and also bound to cells at a much reduced level [9]. We compared the binding of α- and β-T-2 with isolated ribosomes and found that this interaction too was stereospecific; Fig. 5 shows representative data. Clearly, at similar concentrations, there was much more ribosomal binding by the α- compared to the β-isomer. At most concentrations, the ratio of bound α- to β-T-2 was 20:1.

In spite of the difference in levels of ribosomal binding by α- and β-T-2, both toxins appear to bind at the same site. Figure 5 (inset) shows the results of an experiment where the competition of unlabeled α-T-2 for ribosomal binding of radiolabeled α- or β-T-2 was compared. When plotted as a percentage of control (without competitor), the two curves were virtually superimposable. Although it is possible that the inhibition of β-T-2–ribosome binding is allosteric,

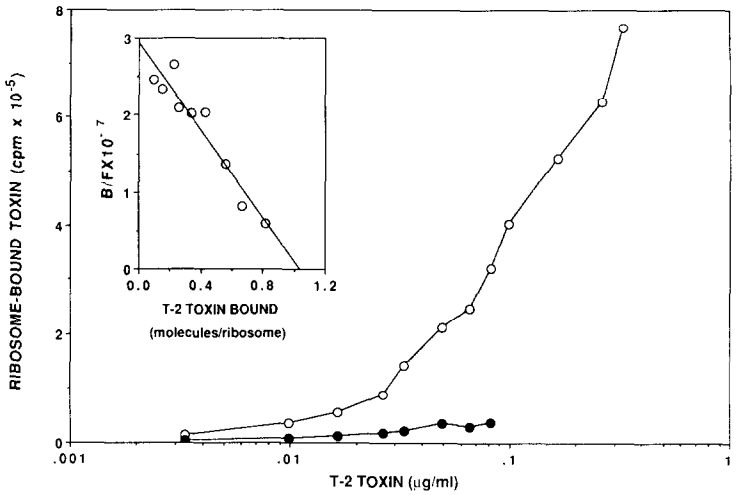

Fig. 3. Binding isotherm of T-2 toxin association with ribosomes. Yeast ribosomes were suspended in buffer D at 10 O.D.$_{260}$/ml, and 0.5 ml was introduced into one side of a dual-chambered dialysis apparatus. [^{3}H]T-2 toxin (14 Ci/mmol), at the indicated concentrations, with (●——●) or without (○——○) a 100-fold molar excess of non-labeled toxin, was introduced into the chambers opposite the ribosomes. The system was incubated at 4° with rotation until binding equilibrium was established (24–28 hr). Aliquots were removed from each chamber and counted with Aquasol 2 in a liquid scintillation counter. Bound cpm were determined by subtraction of free cpm (equilibrated across the dialysis membrane) from the total cpm obtained from the ribosome side of the chamber. Values plotted are the averages of duplicate determinations for each concentration of toxin. Inset: Scatchard plot of [^{3}H]T-2 toxin association with ribosomes. The specific ribosome-associated counts determined from the data in Fig. 3 were analyzed by the method of Scatchard and the line fit by linear regression methods.

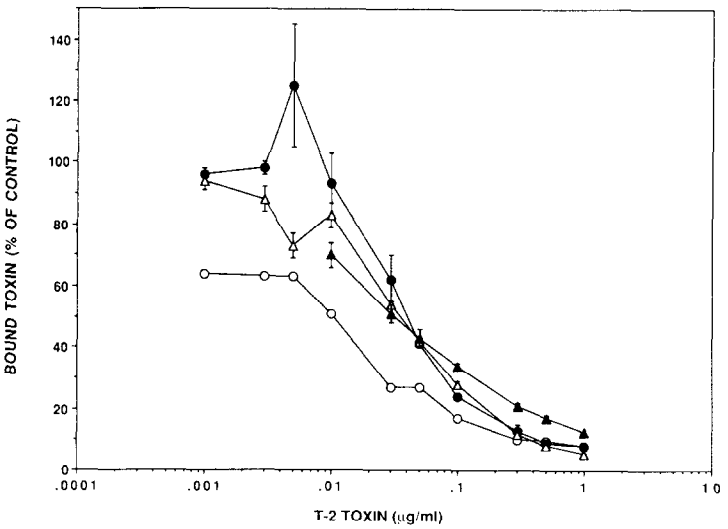

Fig. 4. Competition by non-labeled T-2 toxin for [^{3}H]T-2 toxin association with CHO cells and ribosomes at 4° and 37°. CHO cells were incubated with 0.025 μCi/ml [^{3}H]T-2 toxin (9.0 Ci/mmol) and the indicated concentration of non-labeled toxin for 1 hr at 37° (○——○) or 24 hr at 4° (●——●). CHO ribosomes were incubated with 0.1 μCi/ml [^{3}H]T-2 toxin and the indicated concentrations of non-labeled toxin for 45 min at 37° (△——△) or 24 hr at 4° (▲——▲). Cell experiments were terminated and processed for cell-associated counts as described [9]. Ribosome experiments were terminated by filtration, as described in Materials and Methods. Values are the means ±SE of triplicate determinations at each point. Control (non-competed) binding ranged from 1800 to 2000 cpm for both cells and ribosomes.

the similar shape and placement of the curves strongly suggest that both toxins bind to the same ribosomal site, albeit with different affinities.

Competition by other toxins for T-2–ribosomal binding. Further competition studies were carried out with several other trichothecenes (Fig. 6). Generally speaking, those toxins equal to or greater than T-2 at inhibiting protein synthesis competed very

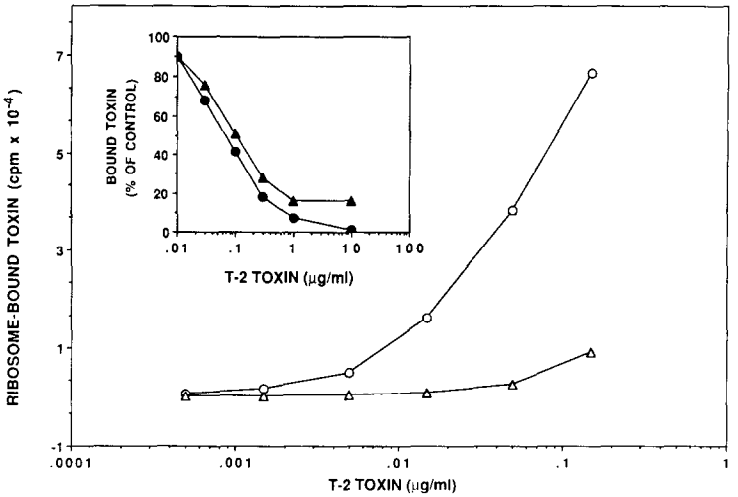

Fig. 5. Association of [^{3}H]α- and [^{3}H]β-T-2 toxins with ribosomes. Yeast ribosomes were suspended in buffer D at 10 O.D.$_{260}$/ml and incubated in 100-μl aliquots with the indicated concentrations of [^{3}H]α-T-2 toxin (○——○) or [^{3}H]β-T-2 toxin (△——△) for 24 hr at 4° (both toxins, 9.0 Ci/mmol). At equilibrium, ribosome-associated counts were determined by filtration, as described in Materials and Methods. Values are the averages of duplicate determinations at each point. Inset: Competition for [^{3}H]α- and [^{3}H]β-T-2 toxin association with yeast ribosomes by non-labeled α-T-2 toxin. Yeast ribosomes were suspended in buffer D at 10 O.D.$_{260}$/ml and incubated with either 0.1 μCi/ml [^{3}H]α-T-2 toxin (●——●) or 2.0 μCi/ml [^{3}H]β-T-2 toxin (▲——▲) for 24 hr at 4° and the indicated concentrations of non-labeled α-T-2 toxin. Ribosome-associated counts were then determined by filtration, as described in Materials and Methods. Control (non-competed) [^{3}H]α-T-2 and [^{3}H]β-T-2 binding were 4700 and 4300 cpm, respectively. Values are the averages of duplicate determinations at each point.

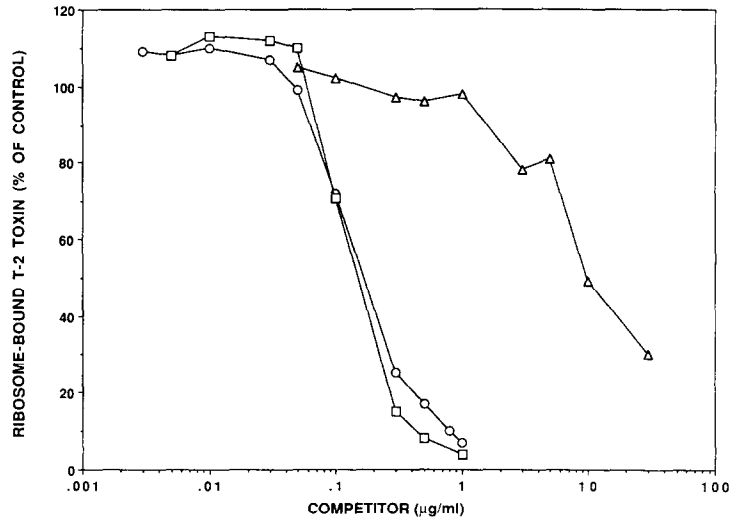

Fig. 6. Competition for [^{3}H]T-2 toxin association with ribosomes by other trichothecene toxins. Yeast ribosomes were suspended in buffer D at 10 O.D.$_{260}$/ml and incubated at 4° with 0.2 μCi/ml [^{3}H]T-2 toxin (14 Ci/mmol) in the presence of the specified concentrations of non-labeled T-2 toxin (○——○), verrucarin A (□——□), or deoxynivalenol (△——△). After 24 hr at 4°, ribosome-associated counts were determined by filtration, as described in Materials and Methods. Values are the averages of duplicate determinations. Control (non-competed) binding values ranged from 10,000 to 12,000 cpm.

well for [^{3}H]T-2 binding to ribosomes. Those trichothecenes less potent than T-2 were correspondingly weaker at blocking T-2–ribosome binding. For example, we found the macrocyclic trichothecene verrucarin A was slighly more potent at inhibiting protein synthesis in CHO cells than was T-2 [9]. Figure 6 shows that verrucarin A was as efficient as T-2 at competing for [^{3}H]T-2–ribosomal binding. An example of the converse was deoxynivalanol. That trichothecene was ≤100-fold less potent at inhibiting protein synthesis than T-2 [9]. Correspondingly, about 100-fold more deoxy-

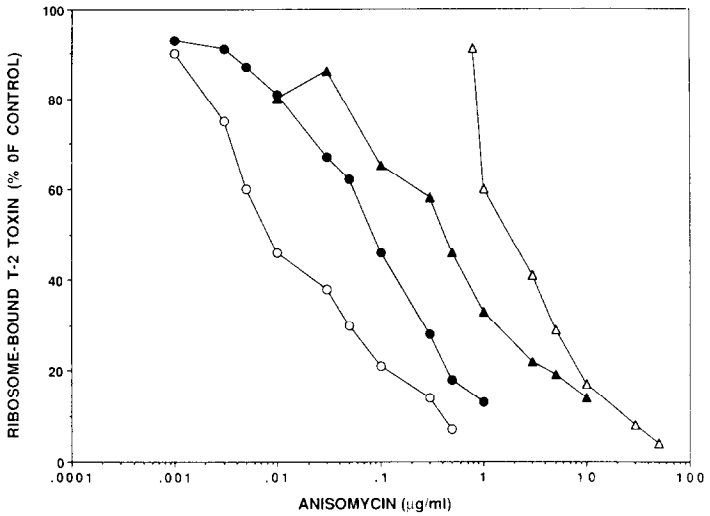

Fig. 7. Effect of anisomycin upon the association of [^{3}H]T-2 toxin with CHO cells and ribosomes. CHO cells were incubated at 0.1 μCi/ml [^{3}H]T-2 toxin (14 Ci/mmol) with the indicated concentrations of anisomycin for 1 hr at 37° (○——○) or for 24 hr at 4° (●——●). CHO ribosomes were incubated at 0.1 μCi/ml [^{3}H]T-2 toxin for 45 min at 37° (△——△) or for 24 hr at 4° (▲——▲). Cell experiments were terminated and processed for cell-associated counts as described [9]. Ribosome experiments were terminated by filtration, as described in Materials and Methods. Values are the averages of duplicate determinations at each point. Values for control (non-competed) association of labeled toxin were: 37° cells, 2600 cpm; 4° cells, 7500 cpm; 37° ribosomes, 3300 cpm; 4° ribosomes, 1100 cpm.

nivalenol than T-2 was required to block 50% of the [^{3}H]T-2–ribosome binding (Fig. 6).

Several other protein synthesis inhibitors examined, including cycloheximide, puromycin, oligomycin and chloramphenicol, had no detectable effect on T-2–ribosome binding. However, anisomycin and emetine, structurally unrelated inhibitors of eukaryotic protein synthesis, both altered T-2 binding in curious patterns. Anisomycin competed for the binding of T-2 to ribosomes and, in agreement with the literature [11], the apparent binding of anisomycin for ribosomes was tighter at reduced temperature (Fig. 7). Anisomycin also competed for the association of T-2 with CHO cells, but with a strikingly different pattern. First, the competition was more effective at 37° than 4°, exactly the opposite of the interaction with isolated ribosomes (Fig. 7). This pattern was also unlike the competition pattern of T-2 for itself (Fig. 4). Second, the concentration for 50% competition was much less (*ca.* 1/200) with cells than ribosomes at physiological temperature.

Other interesting results were provided by competition experiments with emetine. As demonstrated by the data depicted in Fig. 8, emetine competed for [^{3}H]T-2–CHO cell association, whereas no block of labeled T-2–ribosome binding was observed over the entire concentration range. This lack of competition with ribosomes was confirmed by other experiments where up to 1 mg/ml of emetine was used (data not shown). Emetine blocked [^{3}H]T-2–cell association more efficiently at 37° than at 4°, although the two curves appeared to have the same general shape.

DISCUSSION

It is well established that trichothecene toxins inhibit protein synthesis and do so by binding to eukaryotic ribosomes [1]. There is also good evidence that the trichothecenes fall functionally into one of two classes, one whose members inhibit protein synthesis at the stage of initiation and a second whose members inhibit protein synthesis at the stage of elongation or termination [1, 3]. A limited amount of direct information is available on the ribosomal binding by the latter class of trichothecenes and none on the former. As determined in three separate studies, the dissociation constant for an elongation/termination inhibitor–ribosome (trichodermin) binding is approximately 1 μM [4–6]. The stoichiometry for the interaction is 0.4 to 1 toxin molecules per ribosome. We, too, obtained a ratio of one toxin molecule bound per ribosome, but calculated a binding affinity about 100-fold tighter (*ca.* 0.03 μM) for T-2 than that for trichodermin. If T-2 is representative of the initiation inhibitor trichothecenes, this observation might explain, in part, the fact that initiation-inhibiting trichothecenes are considerably more potent than elongation/termination inhibitors [1]. However, we also have developed evidence that another important property determining potency is the ability of a given trichothecene to enter the target cell and that all trichothecene toxins do not cross the plasma membrane at similar rates (Middlebrook and Leatherman, *J Pharm Exp Ther*, in press).

The primary purpose of this study was to obtain the same toxin-binding parameters with isolated ribosomes that we had determined for intact cells [9]. The cell studies produced data consistent with either of two general models: (a) T-2 interacts with the cell membrane in a manner which determines whether the toxin enters the cell to bind to ribosomes or (b) the cell membrane is essentially an inert "sack"

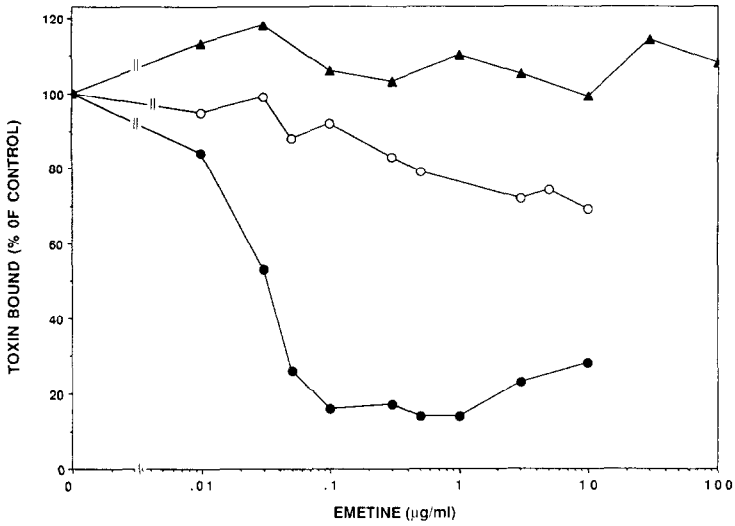

Fig. 8. Effect of emetine upon the association of [^{3}H]T-2 toxin with CHO cells and ribosomes. CHO ribosomes (▲——▲) were suspended in buffer D at 5 O.D.$_{260}$/ml and incubated with 0.1 μCi/ml [^{3}H]T-2 toxin (8.6 Ci/mmol) and the specified concentrations of emetine. Incubation was at 37° for 45 min in a final reaction volume of 50 μl. CHO cells were incubated with 0.05 μCi/ml [^{3}H]T-2 toxin and the indicated concentrations of emetine for 1 hr at 37° (●——●) or for 24 hr at 4° (○——○). At the end of the incubation periods, ribosomes were processed by filtration for ribosome-associated counts, as described in Materials and Methods. CHO cell incubations were terminated and processed for cell-associated counts [9]. All values are the means from triplicate determinations. Control (non-drug treated) [^{3}H]T-2 toxin association was: CHO ribosomes, 2200 cpm; CHO cells 4°, 2400 cpm; CHO cells 37°, 3000 cpm.

in which the ribosomes are contained and plays no role in the flow of T-2 in and out of the cell. Nearly all features of the ribosomal binding suggested that the latter model is correct. The initial rates of association and their temperature-dependence were similar for T-2 binding to cells and isolated ribosomes. Likewise, the dissociation rates for T-2–cell (half-time 1.7 to 2.0 hr) and T-2–ribosome (half-time 2.5 hr) binding at physiological temperature were essentially the same. Furthermore, if the temperature was lowered to 0–4°, T-2 dissociation from either cells or ribosomes was prevented totally. The binding affinities for T-2–cell or –ribosome association at 4° were remarkably similar, 3.6×10^{-8} and 2.9×10^{-8} M, respectively. Stereospecificity was the same in both systems, with the more biologically potent α-isomer binding better than the β-isomer to apparently the same site. Finally, the pattern of competition by other trichothecenes for radiolabeled T-2 association with either cells or ribosomes was qualitatively and quantitatively the same.

An interesting phenomenon unconvered in this study was the apparent increased thermal stability of the T-2 binding site when it is occupied. Just how extensive this effect is with the organelle was not clear. It is hard to imagine that the entire ribosome is stabilized by binding a molecule as small as T-2. Indeed, we have no evidence that any other ribosomal structure or function is stablized by binding T-2. However, the fact that some increased thermal stability was observed may suggest that the binding site for T-2 undergoes some conformational or other alteration upon ligand association. In one sense, the enhanced stability is reminiscent of the stabilization apparently brought about by steroids binding to their receptors [12].

Apparently there are mechanisms other than direct competition by which certain drugs can block the association of T-2 with cells; the results with emetine serve as an example. Emetine blocked T-2–cell association approximately 80% in the concentration range 0.1 to 0.5 μg/ml, while no block of T-2–ribosome binding was observed at 1 mg/ml of the drug. We initially interpreted these results to suggest the presence of a binding site on (or in) cells other than the ribosome. On further investigation, we obtained data which indicate that emetine treatment of cells converted their ribosomes to a state wherein they cannot bind T-2 (Middlebrook and Leatherman, unpublished data).

Another anomaly was presented by the results with anisomycin. It was shown previously that anisomycin would compete for the binding of an elongation/termination inhibitor, trichodermin [4]. However, because of the uncertainty that one trichothecene toxin will compete for the binding of another to ribosomes [6], it is not a foregone conclusion that anisomycin would compete for T-2–ribosome binding. Furthermore, the lack of cross-resistance to anisomycin by T-2-resistant CHO cells (Middlebrook and Leatherman, unpublished data) suggested that T-2 and anisomycin may not interact with ribosomes at the same site. Our results show that anisomycin does block the binding of T-2 to isolated ribosomes. In addition, the concentration/temperature pattern of the competition is consistent with previous observations [10] indicating a higher affinity of anisomycin for ribosomes at lower tem-

peratures, i.e. 4° vs 37°. However, we were unable to explain why the drug was 100-fold more effective in blocking the binding of T-2 to cells than to ribosomes. Using radiolabeled anisomycin, we found that CHO cells did not transport or in some other way concentrate the drug intracellularly (data not shown), one obvious explanation for the data in Fig. 7. Whether anisomycin acts by the same general mechanism as emetine remains to be determined.

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