Effects of Emetine on the Specific Association of T-2 Toxin with Mammalian Cells

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ABSTRACT
The effects of emetine on the association of T-2 toxin with Chinese hamster ovary cells were examined. T-2 toxin-cell association at both 4°C and 37°C was reduced by up to 90% after preincubation of cells with emetine. Emetine-induced reduction in T-2 toxin-cell association was time-, temperature-, and concentration-dependent. A 4-min preincubation with emetine at physiological temperature was required to develop the maximum inhibitory effect. After brief exposures (≤5 min), emetine’s inhibitory effects on toxin-cell association were reversible. However, after longer exposure periods to emetine (50 min), toxin-cell association was irreversibly blocked. The addition of emetine to cells prebound with toxin resulted in dissociation at a rate 2 to 3 times slower than a competitive chase with nonlabeled toxin. Emetine did not compete directly for T-2 toxin binding to its receptor on isolated, purified, run-off ribosomes. However, the binding of toxin to purified ribosomes prepared from cells preincubated with emetine was markedly reduced. Scatchard analysis indicated that emetine’s inhibitory effects on T-2 toxin-cell association were mediated through mixed allosteric and competitive types of inhibition at specific, intracellular, T-2 toxin ribosomal binding sites.

Emetine is an alkaloid drug derived from the roots of ipecacuanha plants. (Brossi et al., 1971). Earlier investigations of emetine’s effects on mammalian cells demonstrated that emetine inhibited protein synthesis in Hela cells (Grollman, 1968) and CHO cells (Gupta and Siminovitch, 1976). The details of emetine’s effects on macromolecular synthesis were developed further in the investigations of Brinkerhoff and Lubin (1977) and Westwood and Wagenaar (1983). Studies on the mechanisms mediating the inhibition of polypeptide synthesis showed that emetine inhibits the translocation step in translation elongation. This results in an accumulation of messenger-bound (stabilized) polyribosomes with an associated depletion in the number of free ribosomes (Grollman, 1968).

T-2 toxin is a toxic metabolite produced by several species of the genus Fusarium and is a member of the sesquiterpenoid family of antibiotics. T-2 is also a potent inhibitor of eukaryotic protein synthesis (McLaughlin et al., 1977) and, in cell-free systems, it selectively inhibits peptide bond formation in the latter stage of translation initiation and early elongation (Cundliffe et al., 1974; Smith et al., 1975). Considerable data have been developed, primarily from cell-free studies with isolated subcellular systems, that indicate the toxin binds to a receptor on the 60S ribosome subunit at or around the catalytic center for peptidyltransferase activity (Barbacid and Vazquez, 1974; Wei et al., 1974, Cannon et al., 1976). This binding is thought to be a prerequisite for the inhibition of protein synthesis, and a correlation between the cellular accumulation of toxin, intracellular ribosomal binding and toxin-induced inhibition of protein synthesis has been demonstrated (Middlebrook and Leatherman, 1989a,b).

Although there have been numerous studies of trichothecene toxins using cell-free systems, few investigations into the mechanisms that mediate the inhibitory effects of T-2 on cellular protein synthesis have been reported. One mechanistic study, conducted with Hela cells, examined the effects of trichothecenes on intracellular ribosomal aggregation. These investigations established that, in contrast to emetine, T-2 toxin destabilizes polyribosomes with a resultant increase in the number of free ribosomes (Cundliffe et al., 1974). Apart from our studies (Middlebrook and Leatherman, 1989a,b,c), there have been no other investigations describing the intracellular interactions of T-2 with ribosomes, nor have there been reports of pharmacological agents that alter or disrupt those interactions.

Recently, we developed a cell culture system suitable for determining the biophysical parameters of T-2 toxin-cell association (Middlebrook and Leatherman, 1989a,b,c). We found that T-2 bound to CHO cells in a time-, temperature- and concentration-dependent manner. Toxin-cell association was specific, saturable and reversible. By comparing toxin binding to CHO cells and CHO cell-derived ribosomes, we obtained data consistent with the notion that T-2 freely and bidirectionally crosses the target cell plasma membrane to interact with...
the ribosomes. Furthermore, the interaction is not static, as it appears that cells continually bind, release and rebinding toxin. Because the 60S ribosome subunits continuously recycle through the various stages of translation, the intracellular T-2 receptor sites are not simultaneously homogeneous with respect to their structure, function or environment. To understand the association of T-2 with its ribosomal binding site, we used emetine as a probe to perturb T-2 ribosome binding as it occurs in the cell. Our results, reported here, indicate that emetine inhibits or reverses the association of T-2 with cells and can, as described in the accompanying manuscript, reduce the toxic effects of T-2 on CHO cells.

**Methods**

**Cell and cell culture.** Seed stock for the CHO cell line was obtained from the American Type Culture Collection (number CCL-61, Rockville, MD). Cells were maintained in 75- or 150-cm² T-flasks (Costar no. 3075) with Earle's minimal essential medium, 10% fetal bovine serum and 50 µg/ml of gentamycin. For experiments, cells were seeded into 24-well tissue culture plates and cultured at 37°C under an atmosphere of 5% CO₂ for 48 to 72 hr. On the day of experimentation, the growth medium was replaced with 0.25 to 1.0 ml/well of H-199 medium supplemented with 10% fetal bovine serum, 50 µg/ml of gentamycin and 25 mM HEPES buffer titrated to pH 7.4 with 10 N NaOH (complete H-199). For isolation and purification of ribosomes, CHO cells were seeded into 4- or 6-well tissue culture plates and cultured with a minimal essential medium supplemented with 10% fetal bovine serum, 50 µg/ml of gentamycin and 10 mM HEPES, pH 7.4.

**Media and sera.** All media, vitamins, antibiotics and amino acids were obtained from Grand Island Biological Co. (Grand Island, NY). Fetal bovine serum was obtained from Armour Pharmaceutical (Kankakee, IL).

**Toxin and drug.** T-2 and emetine were purchased from Sigma Chemical Co. (St. Louis, MO). T-2 was labeled with tritium by New England Nuclear (Boston, MA), Amersham/Searle (Arlington Heights, IL) or Dr. F. Chu (University of Wisconsin, Madison, W1) using a previously published procedure (Wallace et al., 1977). The toxin preparations had specific activities from 9.0 to 19.5 Ci/mmol and were equipotent to unlabeled T-2 in a protein synthesis inhibition assay. Toxin was prepared in methanol and diluted so that the maximal alcohol concentration exposure to cells was 0.1%. This concentration of alcohol had no measurable effect on protein synthesis or toxin-cell association.

**Toxin-cell association assay.** Assays were performed with 2.5 to 5.0 × 10⁵ cells/well. Radiolabeled T-2 was added to cells in 25- or 50-µl aliquots and incubation carried out under the conditions and for the times stipulated in the figure legends. Assays were terminated by washing three times with ice-cold HBSS. The cells were then solubilized in 0.5 ml of 0.1 M NaOH, titrated with 50 µl of 1.0 M HCl, transferred into 7-ml scintillation vials with 5 ml of Aquasol-2 (New England Nuclear) and counted in a Beckman model 5801 liquid scintillation spectrophotometer.

**Protein synthesis assay.** Protein synthesis was measured by adding 1 to 2 µCi/well of [³H]leucine (New England Nuclear, 110-150 Ci/mmol) and incubating at 37°C for the times indicated. The pulse was terminated by rinsing the cells twice with HBSS and adding 0.1 ml of 0.1 M NaOH. After 5 to 10 min at 37°C, a prenumbered 11-mm disc (Schleicher and Schuell 740-E, Thomas Scientific, Swedesboro, NJ) was added to each well to absorb the solubilized cells. Each disc was then transferred to a bottle of 10⁻⁴ M trichloroacetic acid and the entire experiment processed in mass as follows: two rinses with 5% trichloroacetic acid, two rinses with 50:50 ethanol:acetone and one rinse with acetone. After drying, each disc was assayed for radioactivity in 2.0 ml of Liquanfluorotoluene (New England Nuclear).

**Filtration of ribosomes.** Ribosomes from CHO cells were purified as described by Gupta and Simunovich (1976). Toxin-ribosome binding assay. A previously described filter assay was used (Middlebrook and Leatherman, 1980b). Usually, 10 µl of toxin were added to 10 µl of ribosomal suspension (absorbance of 4.0 at 280 nm) in buffer D (20 mM HEPES, pH 7.5; 120 mM KCl; 1.5 mM Mg acetate; 6 mM 2-mercaptoethanol. Incubation was then 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 with buffer D. Each filter was then transferred to 20 ml of cold ethanol and solubilized in 1.0 ml of 1 N NaOH for 30 min. Aquasol 2 (10.0 ml) was added to the vials and after overnight equilibration, the samples were counted in a Beckman model 5801 liquid scintillation spectrophotometer.

**Data analysis.** The IC₅₀ were determined by conversion of the binding data obtained at eight inhibitor concentrations to logit with linear regression against the log of the inhibitor concentration, as described by Rodbard and Frazier (1975). In saturation experiments, nonspecifically associated toxin was determined in the presence of a 100-fold molar excess of nonlabeled T-2. Specifically associated toxin was computed by subtracting nonspecific from total cell-associated toxin. The equilibrium data were analyzed using the Scatchard (1949) equation (1) and preliminary values for the equilibrium binding constants Kᵣ and Bₘₗ were determined by linear regression techniques. These values were provided as the initial estimates for nonlinear, least-squares regression analysis using the computerized, iterative, curve-fitting program Enzfitter (Leatherbarrow, 1987).

\[
B = B_{\text{sat}} + B_{\text{max}} K_r
\]  

Where Bₘₗ is the maximum binding capacity in moles cell⁻¹. [F] is the concentration of free ligand in moles per liter defined as the difference between added and bound (labeled) ligand and Kᵣ is the molar equilibrium dissociation constant. Hill coefficients were determined by linear regression after transformation of the data using equation 2 (Rodbard and Frazier, 1975).

\[
\log[B/(B_{\text{max}} - B)] = n\log[F] - \log K_r
\]  

Where nᵣ is the Hill coefficient defined as the slope of the line at B = 0.33 Bₘₗ, [F] is the molar concentration of free ligand and B is bound ligand in moles per cell. The differences between inhibited and uninhibited values for Kᵣ, Bₘₗ, nᵣ and IC₅₀ were statistically analyzed using the Student's t-test.

**Results**

The effects of emetine on T-2 toxin-CHO cell association at 37°C are illustrated in figure 1 using 10 ng/ml of toxin. Incubation of CHO cells with increasing concentrations of emetine produced a concentration-dependent decrease in the association of T-2 with cells. A nearly identical dose-response relationship was observed if cells were incubated with emetine for 60 min at 37°C, washed with chilled medium and incubated with radiolabeled T-2 toxin at 4°C (fig. 1). In contrast, incubation of cells with emetine at 4°C, followed by incubation with T-2 at 4°C resulted in only small decreases in toxin-cell association (fig. 1). The effect of emetine-induced inhibition of toxin-cell association varied from 70 to 90%, whereas the maximum concentration of emetine required to produce the maximum effect was 0.1 to 0.5 µg/ml.

To determine whether emetine's effects on T-2 toxin-cell association were a result of a direct competition at the intracellular T-2 ribosome receptor, ribosomes were prepared from CHO cells. T-2 binding to isolated, purified ribosomes was then
Emetine's effect on toxin-cell association was then examined using different concentrations of toxin. Cells were preincubated at 37°C with a maximally effective concentration of emetine and cell-associated toxin was measured over a 2-log range of toxin concentrations at 37°C and a 3-log range at 4°C (fig. 2). At 37°C, inhibition of toxin-cell association was ≥70% at toxin concentrations from 2 to 40 ng/ml. As the toxin concentration was increased beyond 40 ng/ml, the levels of inhibition dropped to approximately 3 to 5% at 200 ng/ml. When toxin-cell association was measured at 4°C, inhibition was ≥70% from 1 to 100 ng/ml, beyond which emetine's inhibitory effects declined to about 40% at 1,000 ng/ml toxin.

Because T-2 toxin, in sufficient concentrations, inhibits protein synthesis, it was important to determine whether emetine maintained its inhibitory effects on toxin-cell association under conditions where the toxin was pharmacologically active. This was determined by parallel experiments measuring protein synthesis and toxin-cell association. As previously reported (Middlebrook and Leatherman, 1989a), incubation of CHO cells with radiolabeled T-2 toxin resulted in a dose-dependent inhibition of protein synthesis, with 20 ng/ml of toxin producing a 95% inhibition within 1 hr (fig. 3A). The same solutions of radiolabeled toxin were used to measure T-2 cell association after preincubation with emetine (fig. 3B). Over the toxin's linear dose-response range, emetine reduced toxin-cell association by 75% at equilibrium (1-1.5 hr) and maintained its inhibitory effect for an additional 1.5 hr.

The inhibitory effects of emetine on the cellular association of T-2 toxin developed quite rapidly at physiological temperature. To measure the kinetics, cells were exposed to emetine at 37°C, and toxin-cell association was subsequently measured at 4°C (fig. 4). Preincubation of cells with emetine for only 30 sec produced substantial inhibition at higher emetine concentrations (>1.0 µg/ml). At lower emetine concentrations (0.3-0.5 µg/ml), inhibition of toxin-cell association was measurable within 30 sec and reached maximum levels within 4 to 5 min. After a 5-min preincubation, emetine produced maximum reductions in toxin-cell association which were statistically equivalent for all emetine concentrations >0.1 µg/ml.

![Graph showing concentration- and temperature-dependent effects of emetine upon the association of T-2 toxin with CHO cells and CHO cell-derived ribosomes](image-url)
Emetine's effect on toxin-cell association could be reversed by washing the cells and reincubating at physiological temperature. However, recovery depended upon the length of emetine preincubation. Figure 5 shows the results of an experiment where cells were preincubated with a constant concentration of emetine for increasing periods of time, then washed and reincubated under drug-free conditions for up to 1 hr at 37°C. Toxin-cell association was then measured at 4°C. After a 5-min emetine preincubation, T-2 cell association recovered completely within 45 to 60 min after washout of the drug. As the emetine preincubation interval increased, recovery of toxin-cell association diminished until, by 30 min, the effect was completely irreversible (fig. 5).

Previous studies (Middlebrook and Leatherman, 1989b) demonstrated that T-2 cell association is a dynamic process and that it is possible to demonstrate dissociation by addition of a toxin "chase." To determine whether emetine would produce such an effect, we performed several experiments such as that depicted in figure 6. Cells were prebound to a steady-state association level with radiolabeled T-2 toxin. Then, either excess unlabeled T-2 or emetine was added and toxin-cell association was measured over the next 3 hr. A chase with emetine eliminated labeled toxin from the cells slower than a chase with nonlabeled toxin. Semilogarithmic plots of the data indicated that both dissociative processes were first order, with linear coefficients of correlation of 0.98 and 0.96 for T-2 and emetine, respectively. The slope-derived dissociation rate constants were 0.48 hr⁻¹ for T-2 and 0.18 hr⁻¹ for emetine with computed elimination half-times of 1.4 hr (T-2) and 3.9 hr (emetine).

To evaluate further the biophysical parameters of toxin association with emetine-treated cells, binding isotherms were developed at both 4°C and 37°C. Both control- and emetine-treated cells exhibited saturation at 37°C (fig. 7A). At lower concentrations of toxin, the number of toxin molecules associ-
Scatchard analysis of the equilibrium binding data is shown in figures 8 (37°C) and 9 (4°C). At 37°C, discontinuous, nonlinear Scatchard plots were obtained for both control and emetine-treated cells. With control cells, the data linearized at toxin concentrations ≥20 ng/ml and could be extrapolated to an intercept of 2.9 × 10^6 sites/cell with a slope-derived K_d = 4.7 × 10^-9 M (linear correlation r = 0.91). A similar linearization occurred with the emetine data, but at toxin concentrations ≥60 ng/ml with an identical intercept at 2.9 × 10^6 sites/cell and a slope-derived K_d = 1.59 × 10^-9 M (linear correlation r = 0.96). Over the pharmacologically active range of toxin concentrations (2 to 20 ng/ml, fig. 3A), the Scatchard data described lines with negligible slope and poor linear correlation (r < 0.01 for both control and emetine-pretreated cells). In contrast, at 4°C, the Scatchard data for control cells were linear over the entire range of toxin concentrations from 1 to 1,000 ng/ml (fig. 9B). The linear coefficient of correlation was 0.93 ± 0.01; B_max = 2.0 × 10^6 ± 0.3 sites/cell and K_d = 2.4 ± 0.2 × 10^-9 M (mean values from three experiments). After preincubation of cells with emetine, the data at 4°C were distinctly and consistently nonlinear and concave (fig. 9, inset). At lower concentrations (<50 ng/ml), the data linearized and extrapolated as a class of homogeneous binding sites with B_max = 0.6 ± 0.1 × 10^6 sites/cell; K_d = 5.5 ± 1.8 × 10^-9 M, linear correlation r = 0.90 and Hill coefficient n_H = 0.91 ± 0.02 (means from three experiments). At higher toxin concentrations (≥50 ng/ml), the emetine data did not linearize by Scatchard transformation, and iterative, nonlinear regression defined a line with continuously variable K_d which became asymptotic with the x axis. For the homogeneous class of sites, Student's t tests on the control and inhibited values for the binding constants showed significant differences in B_max at the P < .05 probability level and differences in K_d were significant at the P < .1 probability level.

Discussion

The mechanism of action of T-2 and other trichothecene toxins has been extensively investigated in cell-free systems. All trichothecene toxins appear to bind to a common site on the 60S ribosome subunit and inhibit the catalytic activity of peptidyl transferase. Some inhibit translation-elongation (E-type) whereas others, such as T-2, selectively inhibit the formation of the first peptide bond in translation initiation (I-type). One model for explaining these selective inhibition patterns proposes that E-type inhibitors bind preferentially to polyribosomes and I-type inhibitors, such as T-2, bind preferentially to free ribosomes or newly initiated polyribosomes. This model is supported by a considerable body of evidence which was thoroughly reviewed by McLaughlin et al. (1977).

As demonstrated by Grollman (1968), emetine acts intracellularly to stabilize polyribosomes and reduce the cellular content of free ribosomes. Because cell-free studies provide evidence for the preferential binding of T-2 to free ribosomes, we have examined the effect of emetine on T-2 toxin-cell association. Our data demonstrated that emetine significantly reduced toxin-cell association in a time- and concentration-dependent manner (figs. 1 and 4). Maximum inhibition required preincubation of cells with emetine at physiological temperature (fig. 1). At high toxin concentrations, emetine was more effective when toxin-cell association was measured at reduced temperature (fig. 2). Nevertheless, its effect on T-2 cell association was

![Graph showing time versus toxin-associated toxin concentration](image-url)

Fig. 4. Kinetics of emetine's effect on T-2 toxin-cell association. Cells were incubated with the following concentrations of emetine at 37°C: 0.1 μg/ml (○), 0.3 μg/ml (●), 0.5 μg/ml (▲), 1.0 μg/ml (■), 3.0 μg/ml (△), or 10.0 μg/ml (▲). At the specified times, cells were transferred to ice and the medium was immediately removed. The cells were washed once with ice-cold HBSS and cold complete medium was added. After a 30-min equilibration on ice, the cells were transferred to 4°C and incubated with 3.0 ng/ml of [3H]T-2 toxin. After 24 hr, cell-associated toxin was measured as described under "Methods." Data points are the means of triplicate determinations with S.E.
clearly evident at 37°C using concentrations of toxin that were pharmacologically active (fig. 3, A and B).

In earlier investigations, data were presented that indicated that nearly all specific cell-associated T-2 toxin was associated with ribosomes (Middlebrook and Leatherman, 1989c). We are unaware of any evidence for the binding of toxin to other subcellular structures or for cell-surface or membrane-bound receptor interactions. All data are consistent with a view that the cell membrane functions as a permeable, passive barrier to toxin entry into the cell (Middlebrook and Leatherman, 1989a,b,c). Because cell-associated T-2 represents intracellular ribosome-bound toxin, emetine induced reduction of toxin-cell association could result from direct competition at the ribosome. That possibility is unlikely, as suggested by several results. First, although we did observe a chase of cellular associated T-2 (fig. 6), the dissociation after the addition of emetine occurred at a different rate than that induced by a chase with a true competitive inhibitor, unlabeled T-2. More importantly, emetine did not compete for T-2 binding to its ribosome receptor with purified, isolated, run-off ribosomes, even at very high emetine concentrations (fig. 1). However, binding of T-2 toxin to ribosomes prepared from cells preincubated with emetine was much lower than the binding to ribosomes isolated from untreated cells (fig. 1, inset). Considered collectively, these data suggest that emetine’s inhibition of T-2 cell association is mediated at the ribosomal level, but only after some drug-induced action in the natural, intracellular environment of the T-2 receptor.
There is evidence to indicate that multiple steps or stages are involved in the development of emetine's depressant effects on T-2 toxin-cell association. The kinetics of the effect were rapid, with maximum inhibition of toxin-cell association observed within 4 to 5 min of incubation with the drug (fig. 4). However, an additional 30 to 60 min exposure to emetine was required before the maximum effect became completely irreversible. These data suggest that additional drug-induced events (with apparently slower kinetics) are required for the complete development of the irreversible effect.

The equilibrium binding constants reported here are in agreement with those reported earlier (Middlebrook and Leatherman, 1989a). In the present investigation, we conducted a more detailed examination of toxin-cell association at T-2 concentrations which define the linear region of the toxin's response curve for the inhibition of protein synthesis. The 37°C association isotherm (fig. 7A) over this range was steep, and after Scatchard transformation, the data defined a region of apparently increasing $K_d$ (fig. 8). This steep isotherm and convex Scatchard plot were not observed when toxin-cell association was measured at 4°C. The shallow slope ($n_H = .93$) of the 4°C isotherm (fig. 7B), together with homogeneous Scatchard data (fig. 9), indicated that at 4°C, T-2 bound to a single class of noninteracting recognition sites. Similar data were obtained for T-2 binding to isolated ribosomes. As previously reported (Middlebrook and Leatherman, 1989b), the binding of T-2 to isolated, purified ribosomes was homogeneous and subsequent analysis of that data produced a Hill slope of 1.04 ± .05. Based on these observations, we conclude that the conditions resulting in the steep association isotherm and convex Scatchard plot occur only in physiologically active cells.

One possible explanation for this complex Scatchard plot can be derived from a consideration of the toxin's pharmacological activity. At 37°C, T-2 inhibits protein synthesis by establishing a selective blockade at initiation and early elongation. This results in a "run-off" of downstream messenger-bound ribosomes (Cundliffe et al., 1974) and predicts that, at maximally effective T-2 concentrations, most ribosomes will be present inside the cell as free ribosomes. Thus, increasing T-2 concentrations from 2 to 20 ng/ml should produce an incremental redistribution of the ribosome population from primarily polysomes to predominantly free ribosomes. Because cell-free studies have shown preferential binding of T-2 to free ribosomes, a conversion of polysomes to free ribosomes should
be accompanied by corresponding changes in T-2 cell association. Binding of toxin to cells would become homogeneous only at toxin concentrations sufficient for total inhibition of protein synthesis and, hence, complete polysome runoff to a structurally homogeneous pool of free ribosomes. We cannot assume that all of the mechanisms defined from cell-free studies are necessarily operative in intact cells. Nevertheless, the discontinuous nature of the 37°C Scatchard plot is consistent with the notion that, inside cells, T-2 toxin receptor interactions were heterogeneous at toxin concentrations insufficient to produce complete inhibition of protein synthesis (fig. 3A).

After preincubation with emetine, the association of T-2 toxin with cells was substantially different at 4°C vs. 37°C. At 37°C, emetine produced a "competitive-type" of inhibition, with a shift to the right in the association response curve and no apparent decrease in $B_{\text{max}}$ (fig. 7A). The linearization of the Scatchard data for emetine-preincubated cells occurred at toxin concentrations $>60$ ng/ml, which was 3-fold higher than that measured in control cells (fig. 8). Interpretation of these effects through Scatchard analysis was problematic due to the nonlinearity. If interpreted literally, the affinity of the toxin for its receptor after preincubation with emetine was 3.4-fold lower than that measured in controls, as determined from the slopes of the linear regions of the Scatchard plot (fig. 8). In contrast, when toxin-cell association was measured at 4°C, emetine preincubation (37°C) produced a shift to the right in the association isotherm with an apparent reduction in $B_{\text{max}}$. Scatchard analysis indicated an allosteric type of inhibition with a 75% reduction in the number of binding sites and no (statistically significant) change in $K_d$ (fig. 9).

These complex effects of emetine on T-2 cell association isotherms most likely result from a drug-induced shift in the intracellular ribosome/polysome ratio. As mentioned in the introduction, emetine's action on cultured cells produces an accumulation of polysomes with associated depletion in the number of free ribosomes (Grollman, 1968). Sucrose density gradient profiles of emetine-treated CHO cells produce data consistent with that report (Leatherman and Middlebrook, unpublished data). Because Cannon et al. (1976) demonstrated that T-2 does not bind efficiently to polysomes, it follows that cells treated with emetine and chilled to a temperature where physiology essentially ceases (4°C) should have substantially fewer T-2 binding sites. This is exactly what we observed. Alternatively, preincubation with emetine at 37°C, followed by incubation with T-2 at physiological temperature apparently allows additional cellular events to occur which we are unable to explain without further investigation.

References


Middlebrook, J. L. and Leatherman, D. L. Differential association of T-2

Fig. 9. Scatchard analysis of 4°C binding isotherm data. The data presented in figure 7B were analyzed by the method of Scatchard (1949). No drug, ( ), emetine (●). Inset: for better visualization, the emetine data from three independent experiments were plotted on an expanded scale.


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