Biochemical Studies on the Mode of Action of Cytochalasin B

PREPARATION OF [3H]CYTOCHALASIN B AND STUDIES ON ITS BINDING TO CELLS*

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SUMMARY
Tritium-labeled cytochalasin B of high specific activity (6 Ci per mmole) has been prepared by reduction of cytochalasin A with [3H]NaBH₄. The structure of the radioactive product was shown to be identical with that of authentic cytochalasin B by high resolution mass spectrometry, chromatographic analysis, and derivatization. The synthesized compound produced the same effects as authentic cytochalasin B in a number of biological systems. The [3H]cytochalasin B was found to bind rapidly and reversibly to HeLa cells and to bovine blood platelets at both high (10⁻¹ M) and low (10⁻⁷ M) drug concentrations. Scatchard plots of binding data indicated that there is a class of high affinity binding sites with dissociation constants of about 10⁻⁷ M (about 10⁶ sites per platelet and 10⁶ sites per HeLa cell) and a class of low affinity binding sites with dissociation constants of about 10⁻¹ M. Similar results were obtained with red blood cells and SV40 transformed mouse fibroblasts. Low affinity sites were also found in Dictyostelium discoideum amoebae, but high affinity sites were not detected. In comparison, bacterial cells bound relatively low levels of cytochalasin B at all drug concentrations.

Cytochalasin B, an alkaloid metabolite of the mold Helminthosporium dematoides (2) inhibits a wide variety of cellular movements such as cytokinesis, cell locomotion, cytoplasmic streaming, blood clot retraction, and movements associated with some developmental processes (3). In addition, it inhibits transport of hexoses across the cell membrane (4-6), and there is evidence that this effect is independent of the effects described above (7, 8). The mode of action of cytochalasin B at the molecular level is unknown. Several investigators have reported that inhibition of certain cellular movements by cytochalasin B is accompanied by disruption or disappearance of microfilaments (3) which, in some cases, have been shown to be similar to actin filaments from muscle (9, and for review, see Reference 10). Indeed, cytochalasin B causes a decrease in the intrinsic viscosity of actin (11), and, as judged by electron microscopy, the drug alters the morphology of actin filaments isolated from muscle and blood platelets (12). Thus, in some cases actin-like microfilaments of the cell may be the cytochalasin B receptor. However, such evidence is indirect, and it is possible that cytochalasin B inhibits movements by acting on some other component of the cell. The recent observations that cytochalasin B inhibits hexose transport (4-6) suggest that the cell membrane contains receptor(s) for the drug, and Blumenk (18), Estensen et al. (14), and Krishan (15) have suggested that the effects of the drug in general may be caused by its interaction with the plasma membrane. In order to identify and to characterize the cellular receptors of the drug, we have developed a facile procedure for preparing [3H]cytochalasin B of high specific activity. We report here initial studies on the binding of this [3H]cytochalasin B to a variety of cell types.

EXPERIMENTAL PROCEDURE

Materials
Authentic samples of cytochalasin A and cytochalasin B isolated from culture filtrates of H. dematioides were purchased from the Imperial Chemical Industries Ltd., Cheshire, England, and appeared to be homogeneous as judged by tlc. Dihydro-cytochalasin B was a generous gift of Dr. D. C. Aldridge, Imperial Chemical Industries Ltd. [3H]NaBH₄, specified as 22 Ci per mmole was obtained from International Chemical and Nuclear, [14C]inulin was from New England Nuclear, and activated MnO₂ was from Beacon Chemical Industries. Cytochalasin B treated by the Wilzbach labeling procedure by New England Nuclear was obtained from Dr. L. Wilson, Pharmacology Department, Stanford University. All organic solvents used were of spectral grade. Me₂SO was used to prepare all labeled and unlabeled cytochalasin B stock solutions used in the biological experiments and in the binding assays.

Many of the reactions described were utilized cytochalasin A prepared by a modification of the method of Aldridge (2). To a solution of 10 mg of cytochalasin B in 2 ml of chloroform were added 60 mg of activated MnO₂. The suspension was shaken at room temperature for 3 hours. After the MnO₂ was removed by centrifugation the chloroform solution was concentrated and chromatographed on thin layer sheets. Material having the same RF as authentic cytochalasin A was eluted from the sheets with ethyl alcohol (2).
acetate. The yield of cytochalasin A synthesized by this method was about 4 mg (40%).

Methods

Conversion of Cytochalasin A to Cytochalasin B-To a solution of 4 mg of cytochalasin A in 1 ml of isopropanol was added 0.4 ml of [3H] or [3H]NaBH4 solution (0.5 mg per ml) in isopropanol. After stirring at room temperature for 15 min, the reaction was quenched with 25 ml of 1 N HCl, concentrated by a thin stream of air and chromatographed on thin layer sheets. The material having the same Rf as authentic cytochalasin B was eluted with ethyl acetate and rechromatographed several times. Yields were typically about 20% (based on cytochalasin A) using unlabeled NaBH4 and 3% using tritiated reductant; the discrepancy was apparently due to decomposition of [3H]NaBH4 upon transport and storage.

Isolation of Cells—All operations were performed at 22°C. Bovine blood platelets and red blood cells were isolated from fresh blood containing 0.3 g of sodium citrate per liter. The whole blood was centrifuged at 900 × g for 20 min. The denser layer was centrifuged at 3000 × g for 10 min to sediment the red cells. These cells were washed repeatedly with Buffer W (0.1 M NaCl, 15 mM Tris-HCl, pH 7.0 at 22°C) and suspended in buffer 7. The less dense layer (platelet rich plasma) was centrifuged again at 900 × g for 15 min to sediment the platelets. The platelets were washed repeatedly with Buffer W containing 10 mM sodium citrate until they were free from leukocyte and red cell contamination as judged by phase contrast microscopy. The platelets were suspended in the same buffer at a concentration of about 1.5 × 107 cells per ml (absorbance at 660 nm of 20). Human red blood cells were isolated as described above for bovine red cells.

83 strain HeLa cells were provided by Dr. W. E. Levinson, Microbiology Department, University of California, San Francisco. These cells were grown in suspension in Eagle's minimal medium containing 10% fetal calf serum. Dictyostelium discoideum amoebae (strain A3, a gift of Dr. W. F. Loomis, Jr., Biology Department, University of California, San Diego) were grown in suspension in an axenic culture medium as described previously (18). Aerobacter aerogenes cultures were grown in the same medium. All three types of cells were collected by centrifugation, washed twice in Buffer W, and resuspended in Buffer W at a cell concentration giving an absorbance at 660 nm of 10 to 20 (HeLa cells; A660 = 10, 6 × 107 cells per ml; Dictyostelium, A660 = 15, 7 × 107 cells per ml; Aerobacter, A660 = 15, 7 × 107 cells per ml).

Assay for Cytochalasin B Binding to Cells—A cell suspension of 0.1 ml was added to 0.5 ml of Buffer W (with 0.1 M sodium citrate when platelets were used) containing 0.5% calf serum. Dicyctostelium discoideum cells were isolated as described above for bovine red cells.

Other Methods—The assays for blood clot retraction and determination of viscosity of actin solutions were performed as described previously (11, 12). Ultraviolet absorbance was determined with a Cary model 16 spectrophotometer. Radioactivity was determined with a Beckman LS-233 liquid scintillation counter. Samples were counted in a solution containing 4 g of Omnifluor (New England Nuclear) per liter of toluene. Protosol (New England Nuclear) was used to digest whole cells and to solubilize aqueous samples. Counting efficiencies were determined from a quench curve prepared with quenched standards (Nuclear Chicago) using Automatic Quench Compensation. High resolution mass spectra were obtained with an Associated Electrical Industries MS 902 spectrometer modified for chemical ionization. All thin layer chromatography was performed on Eastman chromatogram sheets, No. 6000 silica gel, with fluorescent indicator. Unless indicated otherwise, a chloroform-ethyl acetate (1:1) solvent system was used. In this system, cytochalasin A, cytochalasin B, and dihydro-cytochalasin B are clearly separated from each other (Rf values are 0.66, 0.56, and 0.48, respectively). Unlabeled compounds (1 to 10 μg) were detected by fluorescent quenching under shortwave (254 nm) ultraviolet light. Labeled compounds were detected by cutting the chromatograms into small sections and counting in a scintillation counter.

RESULTS

Preparation and Characterization of [3H]Cytochalasin B

Reduction of Cytochalasin A with [3H]NaBH4—Cytochalasin B differs in structure from cytochalasin A by having a hydroxyl group instead of a carbonyl group at the C-4 position (2) (Scheme 1). Reduction of cytochalasin A with a nearly equivalent amount of [3H]NaBH4 gave labeled products which moved on tle with the same mobility as cytochalasin B and dihydro-cytochalasin B, and some labeled material remained at the origin (Fig. 1). The labeled cytochalasin B was purified by repeated chromatography as described under “Experimental Procedure.” Tritium was incorporated into a nonexchangeable position of cytochalasin B, as shown by constancy of radioactivity after repeated evaporations from ethanol. In a typical preparation, 4 mg of cytochalasin A were reacted with 100 μCi of [3H]NaBH4 to yield 120 μg of labeled cytochalasin B.

Although cytochalasin B does not show any maxima in the accessible range of its ultraviolet spectrum, its end absorption measured at 200 nm was found to be proportional to its concentration over the range of 1 to 20 μg (Fig. 2). With this method of quantitation, an extinction coefficient of 4 × 104 cm−1 M−1 was found, and the synthesized [3H]cytochalasin B was found to have a specific activity of 6 Ci per mmole. This value is in excellent agreement with the theoretical value of 5.4 Ci per mmole calculated from the specific activity of the [3H]NaBH4 used (22 Ci per mmole or 5.4 Ci per mmol of hydride).

2 Although not established, NaBH4 reduction of cytochalasin B should not proceed stereospecifically and, in all likelihood, the product we obtained is a mixture of the two possible diastereoisomers.
RELATIVE MOBILITY

FIG. 1. Thin layer chromatography of a reaction mixture after reduction of excess cytochalasin A (CA) by \(^{3}H\)NaBH\(_4\). An aliquot (2 \(\times\) 10\(^{5}\) cpm) of the reaction mixture was chromatographed on tlc using unlabeled cytochalasin A, cytochalasin B (CB), and dihydro-cytochalasin B as markers. After development the chromatogram was cut into 12 small sections and their radioactivity was determined.

RELATIVE MOBILITY

FIG. 2. Spectrophotometric determination of the concentration of cytochalasin B in solution. Solutions of varying cytochalasin B concentrations were prepared by dilution of a stock solution of cytochalasin B (0.10 mg per ml in 1% ethanol) with water. The absorbance at 200 nm was measured against blanks containing equal amounts of ethanol.

RELATIVE MOBILITY

FIG. 3. Thin layer chromatography of purified \(^{3}H\)cytochalasin B. An aliquot of \(^{3}H\)cytochalasin B (4 \(\times\) 10\(^{4}\) cpm) purified three times on tlc was diluted with unlabeled cytochalasin B and chromatographed. CB, cytochalasin B; CA, cytochalasin A.

RELATIVE MOBILITY

FIG. 4. A, thin layer chromatography before (-----) and after (---) reaction of \(^{3}H\)cytochalasin B with acetic anhydride. The reaction was carried out by adding a small amount of \(^{3}H\)cytochalasin B (4 \(\times\) 10\(^{4}\) cpm) diluted with 2 mg of unlabeled cytochalasin B to 50 \(\mu\)l of pyridine and 200 \(\mu\)l of acetic anhydride. After 90 min at room temperature the reaction was quenched with 250 \(\mu\)l of ethanol. The reaction mixture was concentrated to a small volume with a stream of air, redissolved in ethanol, and an aliquot was chromatographed. B, thin layer chromatography before (-----) and after (---) oxidation of \(^{3}H\)cytochalasin B by activated MnO\(_2\). The reaction was carried out by the addition of 10 mg of activated MnO\(_2\) to a solution of \(^{3}H\)cytochalasin B (2 \(\times\) 10\(^{4}\) cpm) in 200 \(\mu\)l of chloroform and the reaction mixture was stirred overnight at room temperature. The mixture was centrifuged and an aliquot of the supernatant chromatographed. CA, cytochalasin A; CB, cytochalasin B.

Identity and Purity of \(^{3}H\)Cytochalasin B—More than 99% of the radioactivity of the purified \(^{3}H\)cytochalasin B comigrated with authentic cytochalasin B on tlc in the chloroform-ethyl acetate solvent system (Fig. 3). Similar results were obtained with four other solvent systems (ethyl acetate-hexane, 10:1; toluene-acetone, 2:1; benzene-methanol, 5:1; chloroform-methanol-formic acid, 18:1:1).

When cytochalasin B was reacted with acetic anhydride, the diacetate derivative obtained was clearly separated from the reactant by tlc. Similarly, when \(^{3}H\)cytochalasin B was reacted with acetic anhydride and the mixture was analyzed by tlc, all of the radioactivity was found to comigrate with the diacetate derivative (Fig. 4A).

Reduction of cytochalasin A with \(^{3}H\)NaBH\(_4\) should result in the incorporation of tritium at the C-4 position which would be released when cytochalasin B is oxidized back to cytochalasin A. In contrast, other possible reduction products such as the dihydro derivatives of cytochalasin A and cytochalasin B cannot be converted back to cytochalasin A by this reaction. The position of the label was confirmed to be at C-4 by demonstrating that oxidation of \(^{3}H\)cytochalasin B with activated MnO\(_2\) resulted in over 90% loss of the radioactivity (Fig. 4B).

The molecular weight of the \(^{3}H\)cytochalasin B preparation was determined by high resolution mass spectrometry using
chemical ionization. The value obtained was 479.2669, corresponding to the empirical formula of cytochalasin B (C_{39}H_{48}N_{10}O_{14}).

[3H]Cytochalasin B stored at 0°C in ethyl acetate or MeSO for at least 6 months showed no changes in its chromatographic behavior or its binding properties to cells.

Effects of Synthesized Cytochalasin B on Biological Systems—In all cases, the effect produced by cytochalasin B synthesized by reduction of cytochalasin A was indistinguishable from that produced by an equivalent amount of authentic cytochalasin B.

Human neoplastic glial cells cultured in monolayers were treated with medium containing 10^{-5} M synthesized cytochalasin B. Within an hour, the cells changed from their normal form to a stellate shape typical of cytochalasin B-treated cells (17). This effect was fully reversible; the glial cells returned to their normal condition within an hour after removal of the cytochalasin B from the medium.

An Elodea leaf was suspended in water and cytoplasmic streaming, as indicated by the movement of chloroplasts, was observed with a phase-contrast microscope. When synthesized cytochalasin B (5 × 10^{-3} M) was added to the water, cytoplasmic streaming stopped completely within 15 min. Cytoplastic streaming resumed within 15 min after the leaf was washed and resuspended in water without cytochalasin B.

Platelet rich plasma was prepared from fresh bovine blood containing 0.63% sodium citrate. Clotting was initiated by addition of 25 μl of 2 M CaCl₂ to 1 ml of the platelet rich plasma, and the clot formed was allowed to retract at 37°C for 3 hours. Under normal circumstances, the clot retracted to about 10% of its original size. In the presence of 5 × 10^{-5} M synthesized cytochalasin B, clot retraction was completely inhibited.

Cytochalasin B interacts with highly purified muscle actin, resulting in a decrease of its viscosity (11). The reduced viscosity of a 0.5 mg per ml of actin solution decreased the same amount (from 0.90 to 0.63 ml per mg) with either synthesized cytochalasin B or authentic cytochalasin B at a concentration of 7 × 10^{-5} M.

Binding of [3H]Cytochalasin B to Cells

Time Course of Interaction of [3H]Cytochalasin B with Platelets and HeLa Cells—Samples of platelets and of HeLa cells were incubated in Buffer W containing 10^{-5} M cytochalasin B for time periods ranging from 30 s to 2 hours. As shown in Fig. 5A, the amount of cytochalasin B bound to both types of cells reached a plateau level within minutes and longer incubation times up to 2 hours did not result in significant changes of this level. To determine the reversibility of cytochalasin B binding, samples of platelets and of HeLa cells incubated in 10^{-5} M cytochalasin B for 2 hours were pelleted as before and diluted by suspending in Buffer W containing no cytochalasin B. After shaking for various lengths of time, the cells were again pelleted and the amount of cytochalasin B bound to them determined. It was found that within minutes, new equilibria were established and the amount of cytochalasin B bound to the two types of cells dropped to lower levels. Similar results were obtained when experiments were repeated with 10^{-5} M cytochalasin B (Fig. 5B).

Concentration Dependence of Binding of Cytochalasin B to Different Types of Cells—Binding of cytochalasin B to several cell types was determined over a wide range of drug concentrations. In these experiments, it was necessary to dilute the labeled cytochalasin B preparation with unlabeled cytochalasin B. The following experiments were performed to demonstrate that the labeled material is indistinguishable from unlabeled cytochalasin B in its binding properties and that there are no contaminants present in the labeled cytochalasin B preparation which interfere with the binding of cytochalasin B. Human red blood cells were incubated in buffers containing different concentrations of an undiluted [3H]cytochalasin B preparation, and the amount of cytochalasin B bound was determined as described under “Methods.” This binding experiment was then repeated using [3H]cytochalasin B that was diluted with unlabeled cytochalasin B. If the binding properties of the labeled and unlabeled cytochalasin B were identical, isotope dilution with unlabeled cytochalasin B would not change the total number of cytochalasin B molecules bound to the cells at any given free cytochalasin B concentration. On the other hand, if their binding properties were different, this number would change depending on the amount of isotope dilu-
extrapolation of the high affinity portions of the curves to the intercepts at the abscissa gives values corresponding to about 10^4 of the high affinity binding sites per platelet and about 10^6 sites per HeLa cell. The low affinity components of the curves have slopes corresponding to K_d \approx 10^{-3} M. Similar binding experiments using human red blood cells and SVT2 cells (SV40 transformed 3T3 mouse fibroblasts) also revealed 10^5 to 10^6 high affinity sites (K_d \approx 10^{-7} M) per cell as well as a class of low affinity sites.

The binding curve for amoebae of D. discoideum is essentially linear in the range shown, and indicates that K_d \approx 10^{-5} M. The high affinity binding sites found in the three mammalian cell types appear to be absent in these cells. The binding curve for the bacterial cells is close to the abscissa with essentially a slope of zero, indicating a very low affinity for cytochalasin B.

Effect of Serum on Cytochalasin B Binding to Cells—All the binding experiments described above were performed in a simple isotonic buffer. Although studies on the effect of cytochalasin B on hexose transport in mammalian cells were performed in the absence of serum (4-6), studies on the effect of cytochalasin B on blood clot retraction and on the motility of mammalian cells were done in serum (3, 19) and media containing serum (3, 17, 19, 20), respectively. Thus, in order to compare the binding data described here with the biological effects of the drug reported elsewhere, we examined the effect of serum on the cytochalasin B binding experiment. As shown in Fig. 6, the number of cytochalasin B molecules bound at a given concentration of free cytochalasin B was the same regardless of whether undiluted [3H]cytochalasin B preparation (about 6 Ci per mmole) (○), [3H]cytochalasin B diluted 4-fold with unlabeled cytochalasin B (CB) (●), or [3H]cytochalasin B diluted 8-fold with unlabeled cytochalasin B (Δ).

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As shown in Fig. 6, for the three types of mammalian cells (HeLa cells, bovine platelets, and bovine red blood cells), the binding curves are not linear, suggesting that in each type of cell there are different types of binding sites showing different affinities for cytochalasin B.4 The high affinity components of the curves for the three mammalian cell types tested correspond to K_d \approx 10^{-7} M. Extrapolation of the high affinity portions of the curves to the intercepts at the abscissa gives values corresponding to about 10^4 of the high affinity binding sites per platelet and about 10^6 sites per HeLa cell. The low affinity components of the curves have slopes corresponding to K_d \approx 10^{-3} M. Similar binding experiments using human red blood cells and SVT2 cells (SV40 transformed 3T3 mouse fibroblasts) also revealed 10^5 to 10^6 high affinity sites (K_d \approx 10^{-7} M) per cell as well as a class of low affinity sites.

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4 This kind of nonlinearity of the binding curve can also be explained by negative cooperativity in binding to only one type of binding site. However, we have preliminary evidence showing that the high and low affinity sites can be separated by fractionation of the cells.
binding to cells. When Buffer W was replaced by serum in the binding assay, the amount of cytochalasin B bound to bovine platelets at cytochalasin B concentrations ranging from $10^{-3}$ to $10^{-5}$ M was decreased by 80 to 90% (Fig. 8). Scatchard plots of these data showed that the apparent $K_D$ values obtained, as compared with those determined in buffer, increase from $10^{-7}$ to $10^{-6}$ M for the high affinity sites and from $7 \times 10^{-8}$ M to $2 \times 10^{-7}$ M for the low affinity sites. The decrease in the levels of bound cytochalasin B in the presence of serum is probably due to non-specific binding by serum proteins. Experiments using equilibrium dialysis to measure binding showed that serum albumin has a high capacity for very low affinity binding of cytochalasin B ($K_D \geq 10^{-7}$ M).

**DISCUSSION**

Treatment of cytochalasin B with excess NaBH₄ has been reported to result in saturation of the 2,3-double bond to provide 2,3-dihydro-cytochalasin B in high yield (2). Predictably, a similar reduction of cytochalasin A should also proceed to dihydro-cytochalasin B via the intermediacy of cytochalasin B. We reasoned that if the rate of reduction of cytochalasin B did not greatly exceed that for the initial conversion of cytochalasin A to cytochalasin B, utilization of limiting or near equivalent quantities of NaBH₄ in the reduction of cytochalasin A should provide isolable amounts of cytochalasin B. Indeed, we found that when cytochalasin A was allowed to react with about 2 hydride eq of NaBH₄, cytochalasin B was obtained in 20% yield along with lesser amounts of dihydro-cytochalasin B. The yield of cytochalasin B was considerably lower when [3H]NaBH₄ was used. Since the specific activity of [3H]cytochalasin B obtained was identical to that of the radioactive reductant used, and since most of the reactant cytochalasin A was recovered unchanged in the reaction, the low recovery is undoubtedly a result of impurities in the commercial preparation of [3H]NaBH₄ used. Thus, in this experiment the reductant was present as the limiting reagent. Nevertheless, the reaction provided sufficient quantities of [3H]cytochalasin B of high specific activity (6 Ci per mmole) for numerous biological experiments.

The method described here represents the first reported procedure for preparation of [3H]cytochalasin B, and has several advantages over other possible methods. First, the procedure involves only a single reaction using commercially available materials and a relatively simple purification by tlc. Second, the availability of [3H]NaBH₄ of high specific activity allows [3H]cytochalasin B to be prepared with a specific activity sufficiently high to detect biological receptors present in low concentrations. Third, the reduction produces only a limited number of predicted products which are easily separated, whereas a general labeling procedure such as that of Wilzbach produces a heterogeneous mixture as analyzed by tlc. Finally, the method provides access to [3H]dihydro-cytochalasin B and other radioactive derivatives of cytochalasin B which should be useful in further studies of its mechanism of action.

The rapid and reversible binding of cytochalasin B at $10^{-7}$ M and $10^{-5}$ M to platelets and HeLa cells is consistent with the rapid and reversible effects of the drug in this concentration range observed in numerous biological systems. The concentration dependence of binding of cytochalasin B to different types of cells revealed that the five types of mammalian cells tested have more than one type of binding site for cytochalasin B. Although the exact number of different sites present in each type of cell cannot be determined at this time, it is apparent that there are at least two classes, the high and low affinity binding sites. In contrast, amoebae of the slime mold D. discoideum show only low affinity binding. With bacterial cells of A. aerogenes the affinity for cytochalasin B was even lower. As is shown with platelets, the $K_D$ values of the different binding sites can vary significantly if the assay buffer is replaced by serum. Because previously reported studies on the effect of cytochalasin B on blood clot retraction and on mammalian cell motility were performed in serum or in media containing serum (3, 12, 19, 20), the apparent $K_D$ values of the cytochalasin B

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**Fig. 8.** Effect of serum on the binding of cytochalasin B (CB) to bovine platelets. **A,** samples containing $1.5 \times 10^9$ platelets were incubated in Buffer W (●) or bovine serum (○) containing low concentrations of cytochalasin B. **B,** platelets were incubated in high concentrations of cytochalasin B.
binding sites in these experiments may vary somewhat from the values obtained here. Moreover, in most of the biological studies, the drug induced effect is a qualitative one, making determinations of parameters such as $K_i$ of the drug impossible. Despite these complications, the binding data reported here may be related to the reported biological actions of the drug. In mammalian cells, cytochalasin B inhibits hexose transport across the plasma membrane with a $K_i$ of about $10^{-7}$ M as determined in the absence of serum (4), in good agreement with the high affinity binding we observe. Cytochalasin B does not inhibit hexose transport in bacteria (5) and it appears that there is no hexose transport in cellular slime molds (21), consistent with our inability to detect high affinity binding sites in these cells. From these data, it is reasonable to assume that some, if not all, of the high affinity binding sites ($K_D \approx 10^{-7}$ M) found in mammalian cells are hexose transport sites. Higher concentrations of cytochalasin B ($10^{-4}$ to $10^{-3}$ M) appear to be required to inhibit motile events such as cytokinesis, cell locomotion, and blood clot retraction and to alter the general morphology of cells (12, 19, 20). Thus, the low affinity binding sites present in mammalian cells and in slime mold amoebae ($K_D \approx 10^{-3}$ M) may correspond to components of the cell responsible for motility and general morphology. We cannot, of course, rule out the possibility that binding sites with intermediate affinities for cytochalasin B are also present in the mammalian cells and that these or even some high affinity binding sites may be essential components for cell motility. A better correlation of cytochalasin B binding to the biological effects of the drug must await the isolation and characterization of the different binding sites from the cells.

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