

CYTOCHALASIN B INHIBITS ACTIN-RELATED GELATION OF HeLa CELL EXTRACTS

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Cytochalasin B (CB)¹ can inhibit cell movements such as cytokinesis, pseudopod extension, locomotion, phagocytosis, and pinocytosis (25). These movements seem to be related to a membrane-associated microfilament system (14, 27, 28, 36, 38) that has frequently been shown to contain actin (2, 22, 24, 29, 30). Many morphological studies showing disruption of thin filament arrays (3, 6, 7, 18, 31, 32, 39, 40) and a few studies with purified actin (16, 21, 33, 34) or myosin (footnote 2 and reference 26) suggest that cytochalasins can interact directly with proteins of the microfilament system. However, because of the multiple and sometimes contradictory effects of cytochalasins (4, 5, 8, 12), no *in vivo* effect has yet been related unambiguously to direct interaction with the microfilament system.

Recently, several laboratories have discovered that gelation of suitable cell extracts can be related to actin (23), actin and a high molecular weight protein (HMWP) (35, 37) or actin, a HMWP, and a third protein (13). Knowing that CB disrupts the contractile ring (which contains actin) (28, 29) and binds to both high and low affinity sites in HeLa cells (15), it seemed of interest to test whether CB affected the formation and composition of gel in HeLa cell extracts.

MATERIALS AND METHODS

Extraction of HeLa cells basically followed Pollard's (23) modification of Kane's (13) method for preparing ex-

¹ *Abbreviations used in this paper:* CB, cytochalasin; DMSO, dimethylsulfoxide; DTE, dithioerythritol; EGTA, ethyleneglycol-bis [β -aminoethyl ether]-*N,N'*-tetraacetic acid; HMWP, high molecular weight protein.

² These workers investigated the interaction of myosin with cytochalasin D, which is structurally similar to CB (1) and which affects membrane-associated microfilaments in a manner similar to CB (19).

tracts from sea urchin eggs. HeLa cells, grown as described (10, 37), were collected at room temperature by low-speed centrifugation and washed once with 20 vol of cold 0.1 M NaCl. All subsequent operations were carried out at 0–4°C. Cells were suspended in 2 vol (based on the packed cell volume before washing) of 0.34 M sucrose, 0.001 M ethyleneglycol-bis [β -aminoethyl ether]-*N,N'*-tetraacetic acid (EGTA), 0.001 M ATP, 0.001 M dithioerythritol (DTE), 0.01 M imidazole-Cl, pH 7.0. To promote cell lysis in the concentrated sucrose solutions, sufficient 10% Triton X-100 (Rohm and Haas, Philadelphia, Pa.) was added to give a final concentration of 0.5%. The cells were then ruptured completely by 5–10 strokes of a tight Dounce homogenizer and extract was prepared by centrifuging 3-ml portions of the homogenate at 35,000 rpm, for 40 min, in a Spinco FA-40 rotor (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.). The clear supernatant fraction and floating turbid material were aspirated together from the pellets and used in subsequent tests of gelation.

To study the effect of CB on gelation, 1-ml portions of cold extract were mixed with 5 μ l of stock solutions (0.5 μ M, 5.0 μ M, 50 μ M, and 5000 μ M) of CB (Aldrich Chemical Company, Milwaukee, Wis.) in dimethylsulfoxide (DMSO) or with 5 μ l of DMSO or water in 10-ml Beckman centrifuge tubes and incubated at 25°C for 1 h. The extracts were then centrifuged at 35,000 rpm, for 30 min, at 22–24°C in a Beckman FA-40 rotor to collect the gel or other sedimentable proteins. Protein analysis was carried out by the method of Lowry et al. (17), using bovine serum albumin as a standard. Gel electrophoresis was done by the method of Neville (20), using the staining and destaining protocol of Fairbanks et al. (9).

Concentration of actin and HMWP (expressed as percent of total dye binding) was estimated by quantitative densitometry of fixed and stained gels, using a modified (37) Helena Quick Scan, Jr. All scans were done with the 570-nm filter, and the 100% transmittance was adjusted to the band of greatest intensity (usually actin). In all cases, however, the proteins that migrated to the tracking dye front were excluded from this adjustment

and from calculations. Recoveries of actin estimated in this way were usually $100\% \pm 15\%$, but recoveries of HMWP were much more variable, probably because of the difficulty in accurately measuring small amounts of protein close to the top of the gel.

RESULTS

Incubation of extract at 25°C caused formation of a gel plug (Fig. 1, tube *a*), but addition of 0.25 or $25\ \mu\text{M}$ CB inhibited gel formation (Fig. 1, tubes *e* and *f*). This is illustrated in Fig. 1, showing that when the tubes containing the ungelled extract are laid out horizontally, the extract flows horizontally as would be expected (tubes *e* and *f*), but that gelled extract retains its shape (tubes *a-d*). Visual inspection of extract treated with inhibiting concentrations of CB also did not show any small, clear lumps such as are formed when the protein concentration is not sufficient for plug formation. Thus, these concentrations of CB completely inhibited gel formation.

Lower concentrations of cytochalasin (0.0025 or $0.025\ \mu\text{M}$) or 0.5% DMSO (the solvent used to add cytochalasin to the extract) do not inhibit gel formation (Fig. 1, tubes *a-d*). Thus, CB, not DMSO, inhibited gelation.

To discover whether CB was affecting any particular protein or set of proteins, we incubated the cell extract in the presence and absence of CB for

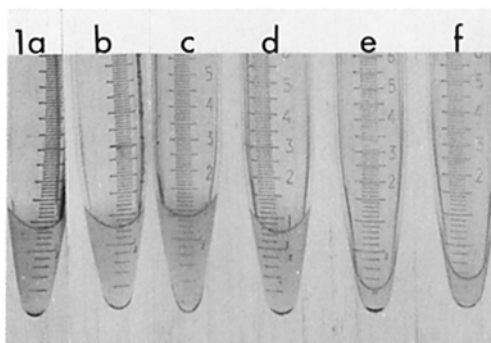


FIGURE 1 Effect of CB on gel plug formation. 1-ml aliquots of HeLa cell extract in 12-ml conical centrifuge tubes were mixed with: (a) $5\ \mu\text{l}$ of H_2O ; (b) $5\ \mu\text{l}$ of DMSO; (c-f) $5\ \mu\text{l}$ of: (c) $0.5\ \mu\text{M}$ CB²; (d) $5.0\ \mu\text{M}$ CB; (e) $50\ \mu\text{M}$ CB; (f) $5000\ \mu\text{M}$ CB in DMSO. After incubating the mixtures at 25°C for 1 h to induce gelation, they were laid out horizontally on a light box and photographed. The final concentrations of reagents are as follows: (a) no DMSO or cytochalasin B; (b-f) 0.5% DMSO; (c) $0.0025\ \mu\text{M}$ CB; (d) $0.025\ \mu\text{M}$ CB; (e) $0.25\ \mu\text{M}$ CB; (f) $25\ \mu\text{M}$ CB.

1 h, collected the proteins which could be sedimented at $100,000\ g$, and analyzed these proteins by polyacrylamide gel electrophoresis.

For control gels formed after adding water or DMSO alone, we found, as expected, that actin (identified by comigration with authentic rabbit striated muscle actin) and the HMWP were enriched in the pelleted gel relative to the extract from which the gel was formed (Fig. 2, compare gels *a* and *g*; Fig. 3; and reference 37). Low concentrations of CB (0.0025 or $0.025\ \mu\text{M}$) which did not inhibit gelation also did not significantly change the concentration of actin in the pelleted gel (Fig. 2, gels *d* and *e*; and Fig. 3). At the lowest concentration of CB, there was a tendency for the HMWP to be more concentrated in the pelleted gel than under any other condition (Fig. 3), but difficulties in reproducibly scanning small amounts of protein at the top of the gel preclude a firm decision about the significance of this observation.

Concentrations of CB (0.25 or $25\ \mu\text{M}$) which inhibited gel formation also consistently lowered the concentration of actin and of HMWP in the protein collected from incubated extracts (Fig. 2, gels *f* and *g*; and Fig. 3). Because no other changes in the gel electrophoretic patterns of pelleted gel or pelleted proteins were apparent by inspection of the gels (Fig. 2), and because the fraction of extract protein which could be pelleted after incubation did not change significantly as a result of these treatments (Fig. 3), we conclude that concentrations of CB which inhibit gelation also lower specifically the amount of actin and HMWP which can be pelleted under these conditions.

DISCUSSION

Gelation of cytoplasmic extracts has been related to actin (23) or interactions of actin and other proteins (13, 35, 37) in at least four different systems. In the macrophage system (35), gelation is also inhibited by $>1\ \mu\text{M}$ CB³ (11). The present observations, showing a concomitant inhibition of gelation and specific disaggregation of both actin and HMWP by CB, provide additional evidence for the specific role of actin and HMWP in gelation of cytoplasmic extracts.

³ Inhibition of gelation of extracts of rabbit alveolar macrophages has been observed (Hartwig, J. H. and T. P. Stossel. 1976. Interactions of actin, myosin, and an actin-binding protein of rabbit pulmonary macrophages. III. Effects of cytochalasin B. *J. Cell Biol.* **71**:295-303.)

We previously reported that actin is present and HMWP is concentrated in the plasma membrane fraction of HeLa cells (10), and that both actin and the HMWP can be enriched in a precipitate fraction of a suitable extract of the plasma membrane (37). These results indicate that actin and HMWP from plasma membrane or whole cell extracts undergo similar interactions. In view of reports that cytochalasins can interact with cytoplasmic actin (16, 21, 33, 34) or myosin (26), it will be of interest to discover whether the *in vivo* effects of CB can be related to its binding to proteins forming a plasma membrane-associated gel.

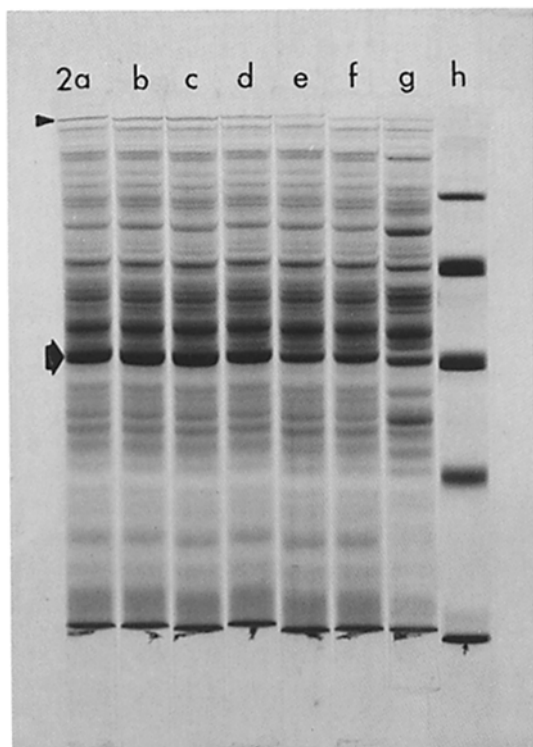


FIGURE 2 Effect of CB on the gel electrophoretic patterns of protein centrifuged from incubated extract. 1-ml aliquots of extract were mixed with: (a-f) reagents as described for Fig. 1. Then, incubation and isolation and analysis of protein fractions were carried out as described in Materials and Methods. Gel (g) is of unincubated extract. Gel (h) is of standard proteins; from top to bottom, these are: β -galactosidase, bovine serum albumin, rabbit striated muscle actin, and α -chymotrypsinogen. The small arrowhead points to the HMWP; the large one to actin.

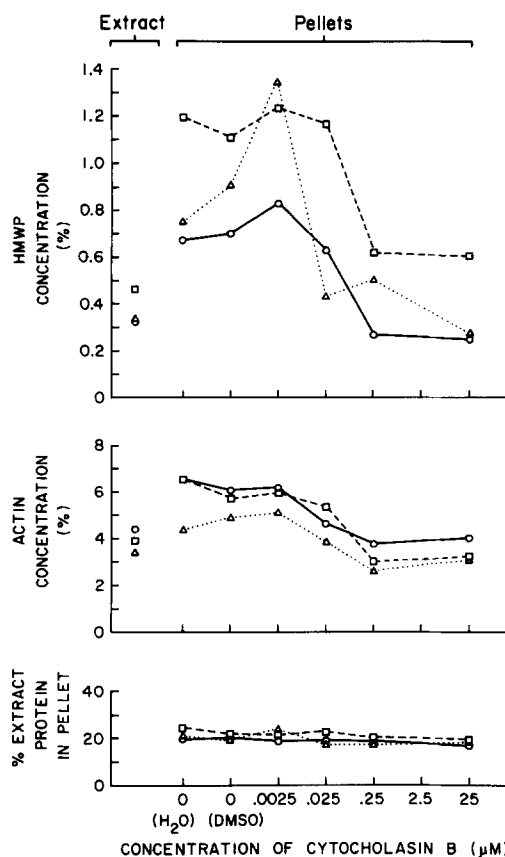


FIGURE 3 Effect of CB on the concentration of actin and high molecular weight protein in protein centrifuged from incubated extract. Extract, pelleted gel, and pelleted protein were prepared as described in the text and Fig. 1. Each symbol (O, Δ , and \square) denotes data from a separate experiment.

SUMMARY

When the 100,000 g supernatant fraction (extract) of HeLa cells lysed in a buffer containing sucrose, ATP, DTE, EGTA, imidazole, and Triton X-100 is incubated at 25°C, it gels, and actin and a HMWP are progressively enriched in the extract and in gel isolated from extract. CB ($\geq 0.25 \mu\text{M}$) inhibits gelation and specifically lowers the concentration of actin and the HMWP in the fraction which sediments at 100,000 g after incubation. These results indicate that actin and HMWP are partly disaggregated by cytochalasin treatment, and thus that their aggregation is related to gelation. Inasmuch as previous results showed that actin is present and HMWP is enriched in the plasma membrane fraction of HeLa cells, the re-

sults also point to a possible relation between plasma membrane-associated gel and in vivo effects of CB.

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