Dexamethasone Induces Gelsolin Synthesis and Altered Morphology in L929 Cells

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ABSTRACT When L929 cells are exposed to 5 μg/ml dexamethasone, synthesis of a 90,000 M_r polypeptide is induced within 12 h. Flattening of the cells begins at about this time and progresses to become quite prominent after 48 h of exposure. Two-dimensional PAGE and partial proteolytic fingerprints identify the 90,000 M_r polypeptide as gelsolin, a Ca^{++}-dependent inhibitor of actin polymerization. Thus, this system provides evidence that gelsolin may have a role in regulating cell shape in response to physiological agents such as glucocorticoids.

We have shown that under conditions of glucose deprivation dexamethasone can modulate the synthesis of several heat shock and glucose-regulated proteins in murine L929 cells (1, 2). When glucose is present, however, the effects of the steroid on protein synthesis are much less dramatic, with only a few major SDS-polyacrylamide gel bands being affected (3). We now identify the 90,000 M_r polypeptide whose synthesis is induced by dexamethasone as the protein named "gelsolin" by

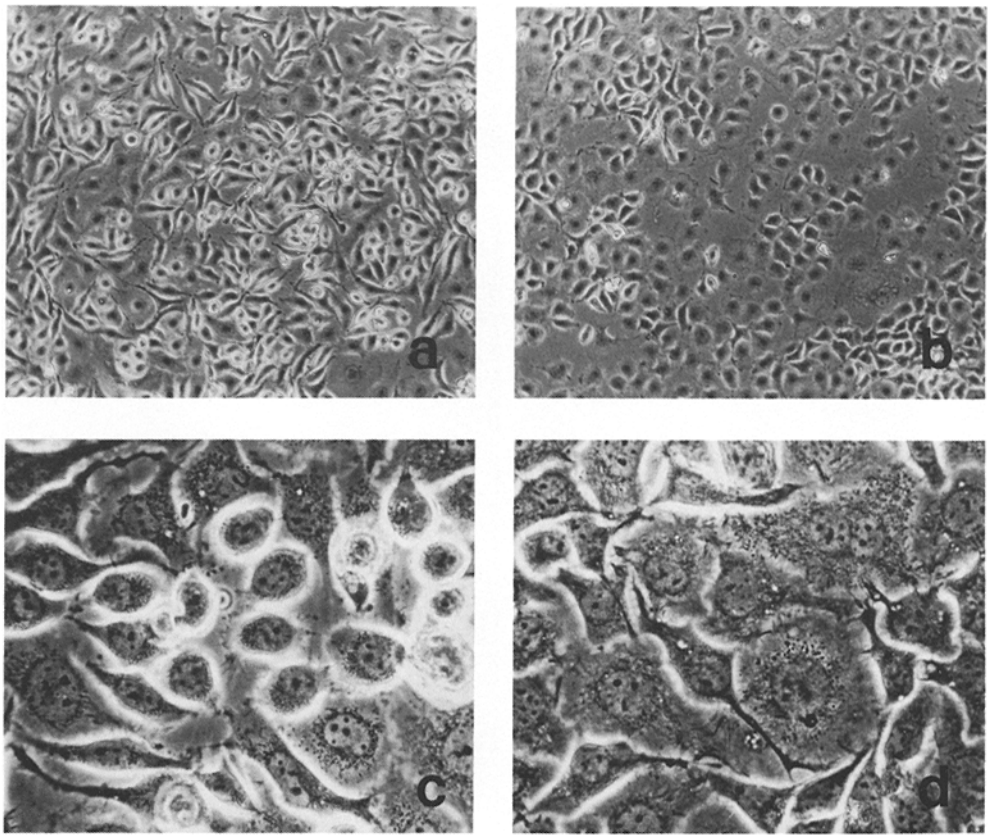

FIGURE 1 Phase contrast micrographs of L929 cells. (*a* and *c*) Control, × 51 and × 128, respectively; (*b* and *d*), after 48-h treatment with 5 μg/ml dexamethasone, × 51 and × 128, respectively.

Yin and Stossel (4). In the L929 system, dexamethasone produces a distinct morphological effect, i.e., cell flattening. Although gelsolin is known to be capable of affecting actin polymerization in vitro (4, 5), this appears to be the first demonstration that this protein is involved in regulating the morphology of intact cells.

MATERIALS AND METHODS

L929 cells were maintained in suspension culture as previously described (6). Cells were plated at a density of $5 \times 10^4/cm^2$ in 35-mm tissue culture polystyrene dishes and cultured overnight before use. The cultures were then changed to serum-free medium to which dexamethasone had been added from a concentrated stock solution in ethanol. The ethanol concentration never exceeded 0.1%, a concentration that was without effect on protein synthesis pattern or morphology. The dexamethasone concentration used (5 μg/ml) did not reduce viability. Since proliferation was already inhibited in serum-free medium, the effects of dexamethasone on this parameter were not observed. High magnification photomicrographs were taken using a Zeiss 40X water immersion phase contrast objective.

Cultures were labeled with [^{35}S]-methionine (100 μCi/ml; 1.4 Ci/mmol; Amersham Corp., Arlington Heights, IL) for 1.5 h at 37°C in medium lacking serum, unlabeled methionine and glucose. Monolayers were then washed and scraped into O'Farrell lysis buffer (7). Aliquots containing 600,000 TCA-precipitable CPM were either directly analyzed by two-dimensional PAGE or adjusted to 1% SDS and subjected to electrophoresis in 5–15% SDS-polyacrylamide gels as previously described (6).

One-dimensional peptide mapping by limited proteolysis was performed in 15% SDS-polyacrylamide gels by the method of Cleveland et al. (8) using *Staphylococcus aureus* V8 protease (Miles Laboratories, Inc., Elkhart, IN). Unlabeled polypeptide bands were cut from briefly stained preparative gels as previously described (9). The bands generated by unlabeled peptide fragments were detected using the silver stain of Oakley et al. (10) including the initial prefixing step. After staining, gels were immersed in Kodak rapid fixer and lightly wiped to remove surface precipitates.

Purified macrophage gelsolin for use as a standard in the various electrophoretic and mapping techniques was generously supplied by Dr. Helen Yin (Massachusetts General Hospital, Boston, MA). Other proteins used as molecular weight standards included phosphorylase a (92,000), transferrin (81,000), bovine serum albumin (68,000), catalase (60,000), and ovalbumin (43,000).

RESULTS

Our attention was drawn to this phenomenon by two, initially uncorrelated observations. On one hand, dexamethasone was

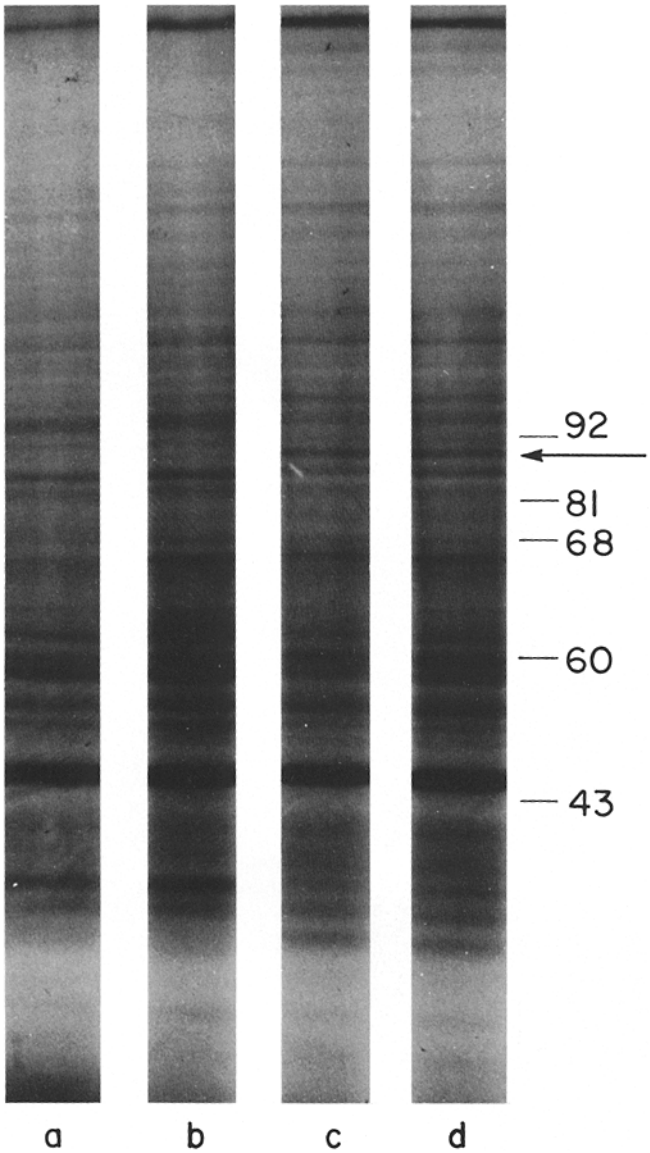

FIGURE 2 SDS polyacrylamide gel analysis of [^{35}S]-methionine-labeled L929 cell proteins. Cultures were labeled after exposure to medium containing dexamethasone (5 μg/ml) for (*a*) 0 h, (*b*) 12 h, (*c*) 24 h, or (*d*) 48 h. The arrow indicates the position of the 90,000 M_r band and the molecular weights of the protein standards are given in kilodaltons.

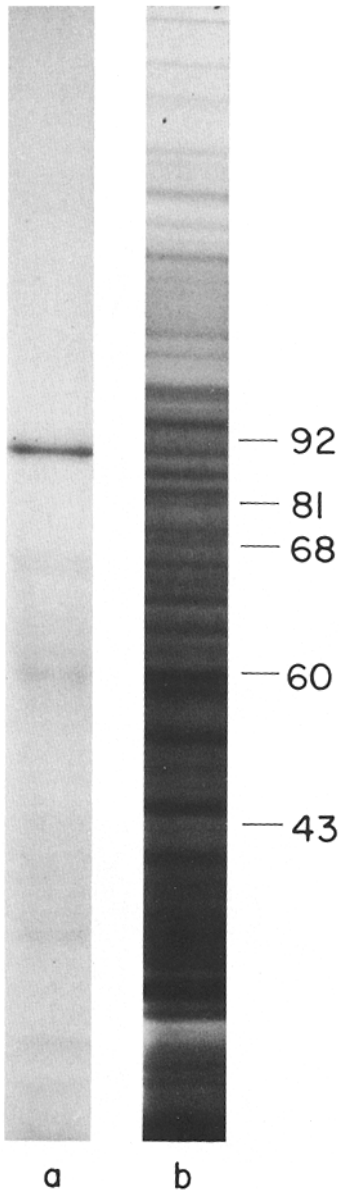

FIGURE 3 Coomassie Blue-stained SDS polyacrylamide gels of (*left*) 5 μg purified gelsolin and (*right*) 125 μg total L929 cell protein from cultures treated with dexamethasone (5 μg/ml) for 48 h.

seen to cause marked flattening of L929 cells within 24 to 48 h of exposure (Fig. 1). This process occurred in both the presence and absence of glucose, even though, in the latter case, the untreated cells began to round up and detach by the end of the incubation period. On the other hand, dexamethasone treatment induced synthesis of a 90,000 M_r polypeptide within the same time frame (Fig. 2). It is noteworthy that even though synthesis of a number of other polypeptides is altered by dexamethasone, the glucose-regulated proteins of 95,000 M_r, 85,000 M_r and 82,000 M_r are not affected. Induction of the 90,000 M_r polypeptide also occurred in the absence of glucose (data not shown), but synthesis of the glucose-regulated proteins was also altered under these conditions (1). We estimate approximately 50-fold induction of synthesis on the basis of densitometric scanning of the autoradiograms depicted in Fig. 2. It should be kept in mind that precise quantitation was not possible because the labeled 90,000 M_r band was almost undetectable in untreated cells. Densitometry of Coomassie Blue-stained gels (not shown) indicated that the amount of the 90,000 M_r polypeptide relative to total cell protein was 0.067% in untreated cultures and 0.32% after 48 h of dexamethasone treatment, an increase of 4.8-fold.

A number of proteins with reported M_r of ~90,000 were examined using one-dimensional SDS PAGE to determine whether their mobilities were similar to that of the dexamethasone-induced band. These included phosphorylase a, transferrin, glycogen synthase, α-actinin and gelsolin. Of these, only gelsolin co-migrated with the 90,000 M_r band (Fig. 3). The identity of the M_r 90,000 band with gelsolin was further established by peptide mapping and two-dimensional polyacrylamide gel electrophoresis. Fig. 4 illustrates the one-dimensional partial proteolytic peptide maps obtained from purified gelsolin and from the four major SDS-polyacrylamide gel bands of M_r 95,000, 90,000, 85,000, and 82,000 present in dexamethasone-treated L929 cells. Even though some differences in the partial proteolytic peptide maps are to be expected since the 90,000 M_r band is from murine cells whereas the gelsolin is from rabbit macrophages, it can be seen that the patterns produced by these two proteins are very similar and not obviously related to those produced by any of the other major bands in the M_r region.

The location of purified gelsolin in the two-dimensional gel pattern was established by coelectrophoresis of the purified protein with an aliquot of L929 cell lysate (Fig. 5). The cell lysate showed two closely spaced spots, one at pI 6.17 (major) and one at pI 6.14 (minor), corresponding to two spots of similar relative intensity in the purified gelsolin preparation. The effect of dexamethasone is to markedly increase the [^{35}S]-methionine-labeling of these gelsolin species (Fig. 5). As in the case of the one-dimensional gels, it should be noted that the

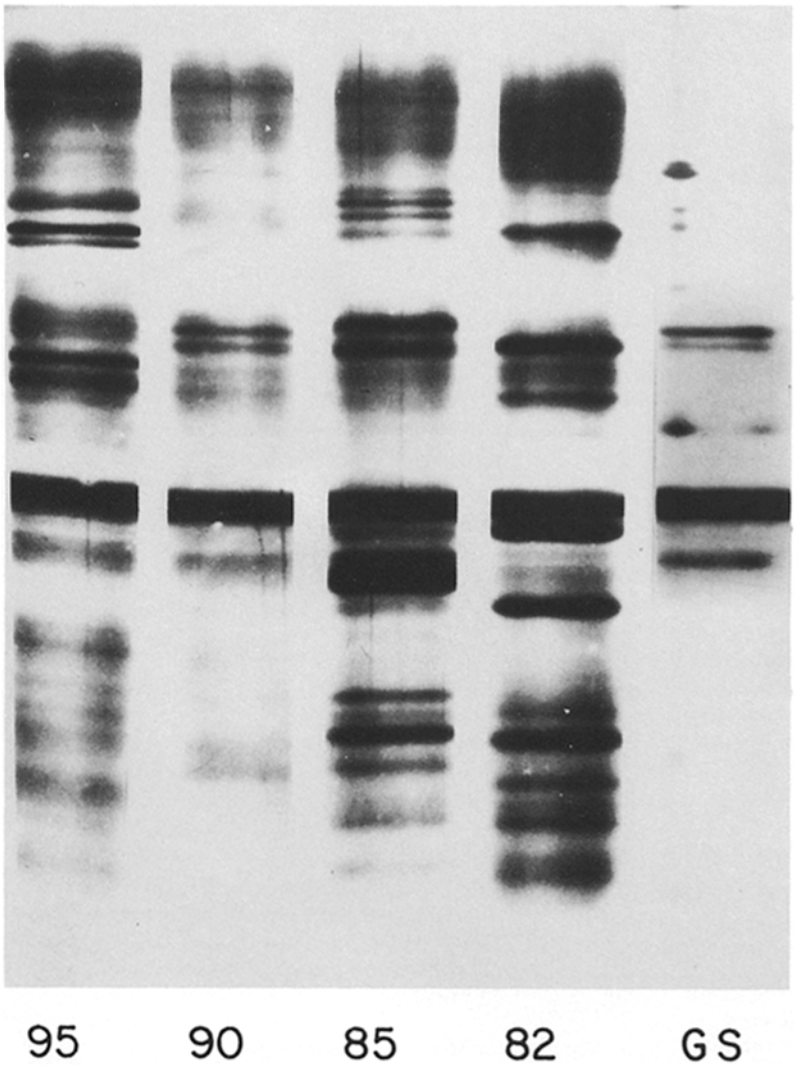

FIGURE 4 Peptide maps obtained by limited proteolysis and separation in a 15% SDS polyacrylamide slab gel. 95, 90, 85, 82, and GS refer to L929 cell polypeptides of the indicated M_r ($\times 10^3$) and gelsolin, respectively.

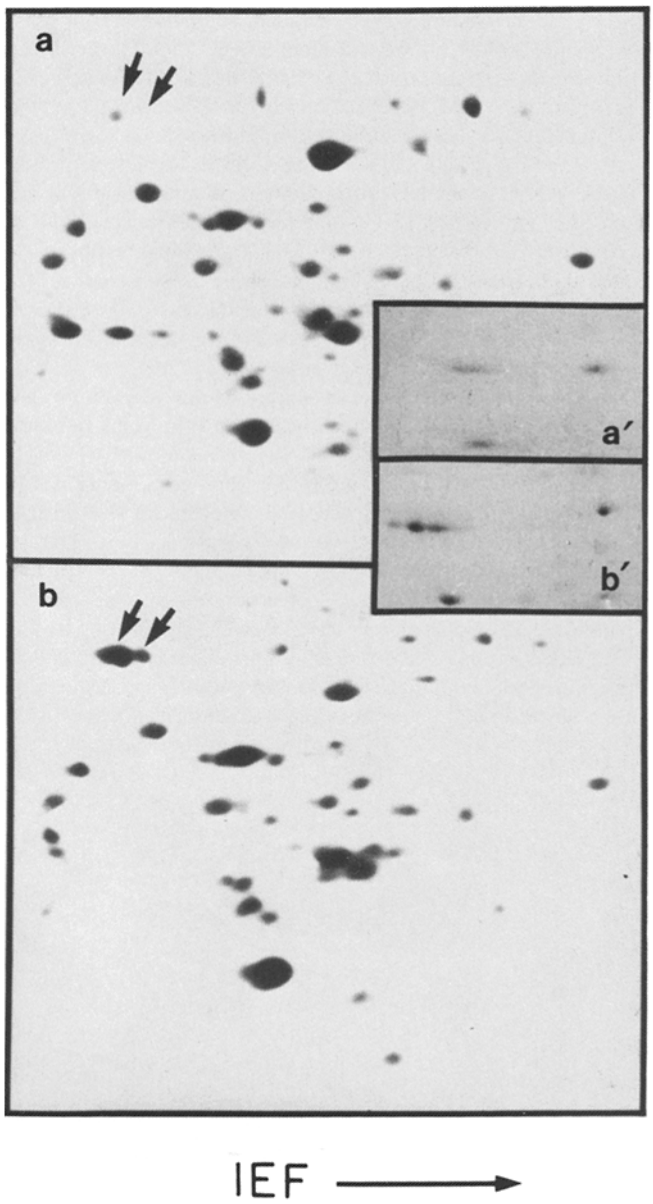

FIGURE 5 Two-dimensional polyacrylamide gel electrophoresis of [^{35}S]-methionine-labeled L929 cell polypeptides from (*upper panel*) untreated culture and (*lower panel*) culture treated with 5 μg/ml dexamethasone for 48 h. The pI range is from 6.0 (*left*) to 5.0 (*right*). The gelsolin species are indicated by *arrows*. The *inset* shows Coomassie Blue-stained two-dimensional polyacrylamide gel patterns in the vicinity of the gelsolin spots, (*a′*) L929 cell lysate and (*b′*) L929 cell lysate with 7.5 μg purified gelsolin.

intensities of several other as yet unidentified spots are also affected by dexamethasone treatment. Thus, peptide mapping and two-dimensional SDS PAGE confirm that the 90,000 M_r one-dimensional SDS-polyacrylamide gel band is gelsolin.

DISCUSSION

Two major findings emerge from the present study: first, that the steroid dexamethasone induces gelsolin synthesis in L929 cells and, second, that it also induces a defined morphological effect, i.e., cell flattening. In the course of demonstrating these points, it was established that the protein is present in this system, thereby supporting the conclusion of Yin et al. (11) based on immunofluorescence data that it is ubiquitous in distribution. In addition, the location of two gelsolin isoelectric species was determined in two-dimensional gels. Although the basis for the existence of these multiple isoelectric species is presently unknown, their identification *per se* is of interest.

Since gelsolin is known to be a calcium-dependent inhibitor of actin polymerization (4), it is tempting to speculate that the increases in its abundance and rate of synthesis are related in some manner to the morphological change occurring at approximately the same time. The most straightforward relationship would assign the increased gelsolin concentration a direct role in causing cell flattening. Although this effect is not consistent with the known ability of gelsolin to shorten actin filaments, thereby promoting gel-sol transformation, present understanding of cell shape regulation does not preclude such a role. This explanation would require increased intracellular Ca^{++} concentrations to promote both the flattening and the increased gelsolin synthesis. On the other hand, there are no data indicating that dexamethasone affects intracellular Ca^{++} or that if such changes did occur they would affect gelsolin synthesis.

Indirect mechanisms could also be invoked to involve gelsolin in the dexamethasone-induced morphological effect. Both one- and two-dimensional polyacrylamide gel analyses show that synthesis of a number of proteins in addition to gelsolin is induced by dexamethasone. Although these proteins have not

yet been identified, they may be involved in the morphological effect either directly or as modifiers of cytoskeletal organization, with the effects on gelsolin being secondary. Involvement of extracellular proteins appears less likely. For example, dexamethasone has also been reported to induce the formation of an extracellular fibronectin matrix by rat hepatocytes in vitro (12) and to reverse the loss of the fibronectin and procollagen matrix around transformed human cells (13). Although this effect could cause cell flattening, our L929 cells synthesize little fibronectin in either the presence or absence of dexamethasone, and that which can be demonstrated, e.g., by lactoperoxidase-catalyzed radioiodination, is preferentially associated with the cell surface rather than with the substratum (6). Thus, while this intriguing area appears to require further study, it is clear from the limited data now available that gelsolin very likely participates in cell-shape regulation under physiological conditions.

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