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CHANGES IN ACTIN ASSOCIATED WITH THE CYTOSKELETON FOLLOWING CHEMOTACTIC STIMULATION OF DICTYOSTELIUM DISCOIDEUM

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SUMMARY: Chemotactic stimulation of Dictyostelium discoideum amoebae with pulses of cAMP or folate causes a series of rapid changes in the amount of actin protein associated with the Triton-insoluble cytoskeleton. The first of these changes occurs within 3 sec. of stimulation. The changes are dose-dependent and are within the physiological range of concentrations of cAMP or folate eliciting chemotaxis. These effects on the cytoskeleton show a pattern of regulation during development matching the respective chemotactic sensitivities of D.discoideum to cAMP (most sensitive at 4-8 hr of development) and to folate (rapidly decreasing sensitivity over 0-4 hr). At twelve hr, however, the responsiveness to folate unexpectedly reappears, suggesting a function of folate later in development than previously reported.

Much of the work on cell motility and its control has involved amoeboid cells (1). An amoeboid organism that has gained wide popularity is the cellular slime mould Dictyostelium discoideum (2).

D. discoideum exists as populations of free living amoebae which aggregate on starvation to form multicellular structures and undergo complex biochemical and morphological changes (3). Chemotaxis and hence cell motility are of importance to this organism during its vegetative, food-seeking phase when folic acid is the chemoattractant (4), and in the aggregation process

Abbreviations
cAMP : Adenosine 3',5'-cyclic monophosphate.
SDS-PAGE : sodium dodecyl sulphate polyacrylamide gel electrophoresis.
DTT : dithiothreitol.
EGTA : Ethyleneglycol-bis-(B-aminoethyl ether)N,N',-tetraacetic acid.
NEPHGE : nonequilibrium pH gradient electrophoresis.

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when cAMP is the signal involved in the movement of cells towards a central collecting point (5). Binding of the chemoattractants to specific receptors at the cell surface (6,7) is followed by a movement response with projection of a pseudopodium towards the incoming signal occurring within five seconds (8). The mechanism of coupling of receptors to the contractile apparatus is as yet unkown but several processes have a postulated involvement (9).

It is clear that chemotaxis and hence motility require the projection of pseudopodia and changes in shape (10,11), which are dynamic events demanding both a spatial and temporal control of actin assembly and disassembly. In this paper we present evidence for such temporal control of actin assembly in D. discoideum.

METHODS

Growth and development of cells

Amoebae of strain NC-4 were grown as mass plates on SM nutrient agar (12) in association with Klebsiella aerogenes and were harvested in 17mM phosphate buffer pH6.15 as they were clearing the bacterial lawn. Development was initiated by washing the amoebae free of bacteria by centrifugation at 190g for 2 min. The cells were resuspended at a density of 2×10^7 in the same buffer and shaken at 170 rpm at 22°C until the desired time of development was reached.

Isolation of cytoskeletal proteins

Cytoskeletal proteins were isolated as proteins insoluble in Triton X-100 (13,14) using a published method with minor modifications (15). Cells were harvested from shaking culture, centrifuged and resuspended at $10^8 ml^{-1}$. Typically 150ul aliquots were distributed to microcentrifuge tubes shaken at high speed on a Vibrax shaker (setting 1400rpm). The cells were then stimulated with a 10ul addition of cAMP or folic acid for a preset time before 150ul of 2 x Triton stock was added to terminate the process. In the case of both additions, mixing was apparently instantaneous, as shown by the use of dyes. Tubes were then placed on ice for 10min, then allowed to warm to room temperature for 10 min with occasional agitation. The samples were then spun down for 4 min in a Beckman Microfuge B at 11,000g. After decanting the supernatants, 300ul of 2 x Triton stock diluted 1:1 with 17mM phosphate buffer pH 6.15 was added and the tubes recentrifuged. Supernatants were discarded again and the tubes inverted to dry. The protein pellet was prepared for one-dimensional gel electrophoresis by heating to 80°C for 10 min in 50ul of sample buffer containing 2% SDS, 10mM DTT, 10% glycerol. Sample preparation for two-dimensional gel electrophoresis was by a modification of the method of Devine et al.(16), with DTT replacing 2-mercaptoethanol.

The 2 x Triton stock contained 2% Triton X-100, 20mM potassium chloride, 20mM imidazole, 20mM EGTA, 4mM sodium azide, pH 7.0.

The rationale for the composition of this solution is discussed in detail by Rosenberg et al.(17).

Electrophoresis of cytoskeletal proteins

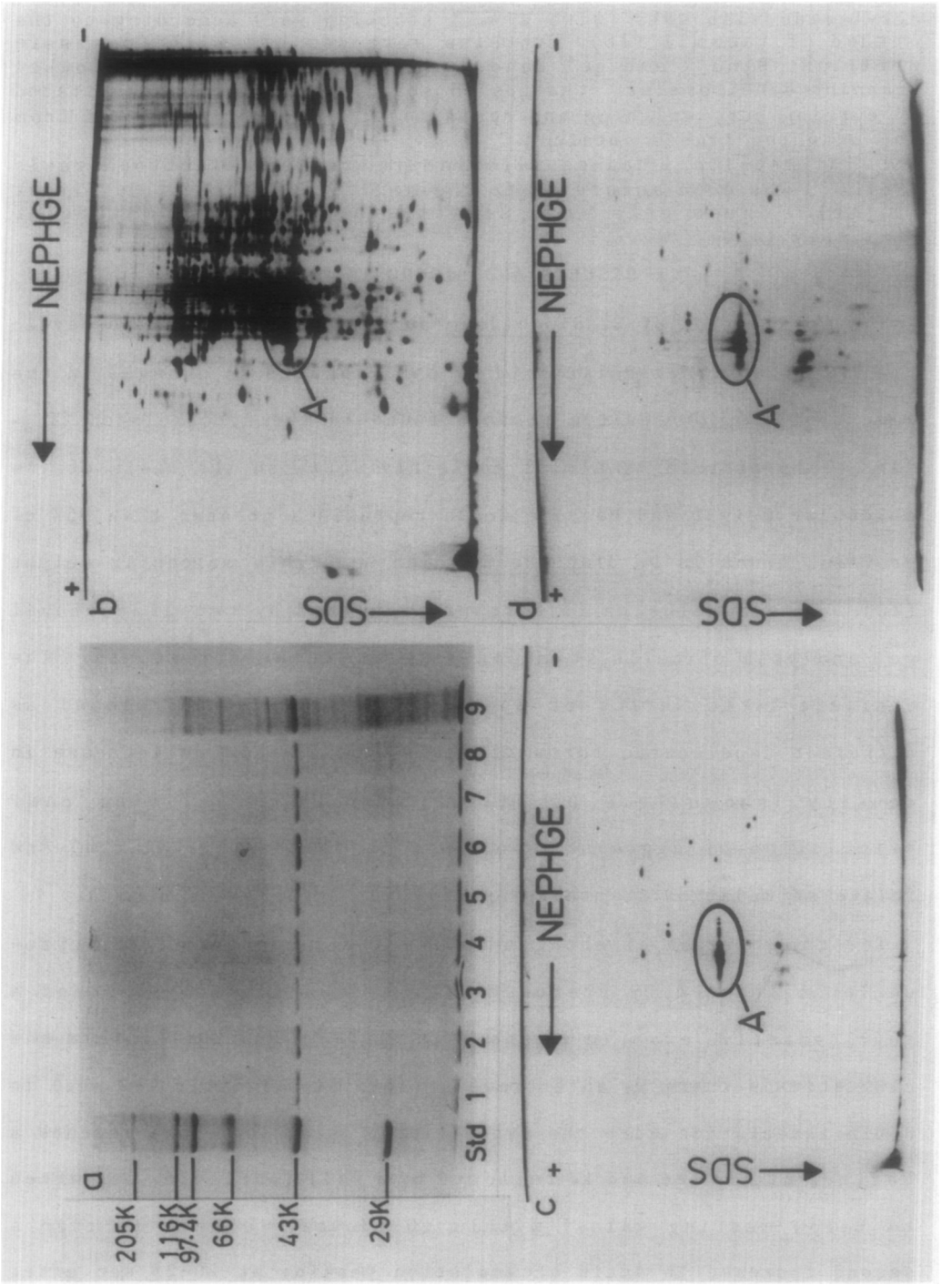
One-dimensional electrophoresis was carried out in 10% polyacrylamide slab gels (with a 4.5% stacking gel) according to the method of Laemmli (18). Proteins were stained with Coomassie Brilliant Blue R and gel bands were scanned on a Joyce-Loebel scanning densitometer. Changes in actin content were quantitated by cutting out, weighing and comparing the appropriate peaks from the scanning traces obtained.

Two-dimensional non-equilibrium pH gradient electrophoresis (NEPHGE) was done according to the modified method of Devine et al.(16). These gels were silver stained by the method of Morrissey (19).

RESULTS AND DISCUSSION

The stimulation of D. discoideum at appropriate times of development with either folic acid or cAMP results in changes in the amount of a 43,000 dalton protein found in the cytoskeleton (fig. 1a). This protein is almost certainly actin on the basis of its molecular weight and because actin represents greater than 95% of proteins found in D. discoideum which have this molecular weight (20). This conclusion is further supported by two-dimensional gel analysis of cells stimulated by the chemoattractants. The characteristic series of spots which have been proposed as different isoelectric forms of actin (21,22) show an increase in density (shown here by silver staining) following cAMP stimulation of aggregation competant cells (fig. 1b,c,d) and folate stimulation of preaggregative cells (data not shown).

The time course of changes in the actin content of the cytoskeleton induced by chemoattractants was investigated and a representative example is shown in fig.2. For both folate and cAMP stimuli there is an extremely rapid rise (within 1-3 sec) in actin association with the cytoskeleton (peak I). This reaches a maximum at 3-7 sec and is followed by a fall (variable, but often to below resting value) within 10-13 sec. There is then a second increase in actin accumulation peaking at 20-25 sec after stimulation (peak II) and this again is followed by a fall. This in turn is succeeded by a third increase in actin content which


a
205K
116K
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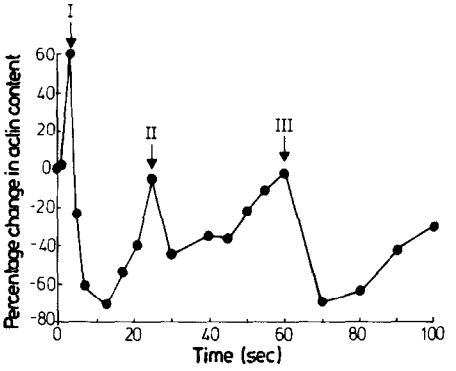

Figure 2. Time course of actin association with the cytoskeleton following stimulation of 0hr (preaggregation) cells with 5 x 10^{-5}M folic acid. This is a representative example of twelve experiments.

peaks at 60-70 sec (peak III). The third peak is often difficult to define since it is commonly part of a series of oscillations lasting at least 3 min following the initial stimlus.

The timing of the first peak of actin association is similar to that of pseudopodium formation and may reflect a process necessary for shape change and motility (8). It is also interesting that the timing of the second and third peaks corresponds to the two peaks of reduced optical density observed during measurement of the light scattering properties of cell

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Figure 1. (a) SDS-PAGE of control cells and cytoskeletons from chemoattractant-treated cells. Lanes 1-4 were cytoskeletons from 0hr (preaggregation) cells treated with 5 x 10^{-5}M folic acid as follows: lane 1, cytoskeleton extract prior to stimulation; lane 2, three sec after stimulation; lane 3, seventeen sec after stimulation; lane 4, twenty one sec after stimulation. Lanes 5-8 were cytoskeletons from 8hr (aggregation competent) cells treated with 10^{-7}M cAMP as follows: lane 5, cytoskeleton extract prior to stimulation; lane 6, five sec after stimulation; lane 7, thirteen sec after stimulation; lane 8, twenty one sec after stimulation; lane 9 is whole cell extract applied at a third of the cell number used for cytoskeletal extracts. Molecular weight standards were (a) rabbit muscle myosin, 205,000 daltons; (b) B-galactosidase, 116,000 daltons; (c) phosphorylase B, 97,400 daltons; (d) bovine serum albumen, 66,000 daltons; (e) rabbit muscle actin, 43,000 daltons; (f) carbonic anhydrase, 29,000 daltons.

(b) Two dimensional gel of a whole cell lysate of 2.25 x 10^6 unstimulated 8hr cells. "A" marks the position of actin.

(c) Two dimensional gel of 2.25 x 10^6 cytoskeletons from 8hr cells prior to stimulation.

(d) Two dimensional gel of 2.25 x 10^6 cytoskeletons from 8hr cells, five seconds after stimulation with 10^{-7}M cAMP.

All three 2D gels were stained together in the same tank for exactly the same amount of time.

suspensions following cAMP (23) or folate (24) stimulation. These optical density changes have since been correlated with shape changes and motility (10,25).

The effect of varying chemoattractant concentration on the magnitude of the first and second peaks of actin incorporation was also investigated. The third peak was eliminated from the present work due to the difficulty of obtaining a clear response. As shown in fig.3, the changes in actin content of the cytoskeleton are clearly dose-dependent for both cAMP and folate. For cAMP the most effective concentrations for the first and second peaks are 10^{-7} and 10^{-8}M respectively (fig.3a) and for folate the value for the first two peaks are 10^{-4} and 10^{-8}M respectively (fig 3b). These values are similar to the threshold values for chemotactic reponses to cAMP (5) and folate (4) seen with amoebae on agar plates and are within the range of concentrations required for optimal binding of the chemoattractants to their specific receptors (6,7). This indicates that the increase of actin associated with the cytoskeleton may be of physiological importance in chemotaxis. The differing dose-

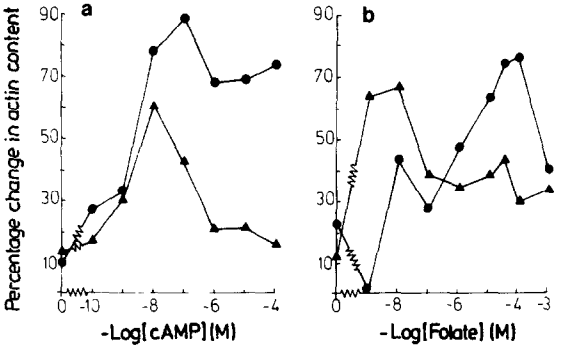

Figure 3. Dose response curves for the association of actin with the cytoskeleton. (a) 8hr cells were stimulated with varying concentrations of cAMP and the changes in actin content of cytoskeletons were mea ıred for the 3-7 sec (●) and 20-25 sec (▲) peaks of actin incorporation.
(b) 0hr cells were stimulated with varying concentrations of folate and the changes in actin content were measured for the 3-7 sec (●) and 20-25 sec (▲) peaks of actin incorporation. These results are the means of at least three separate experiments.

effect relationships for the first and second actin association peaks for folate stimulation (fig.3b) may indicate some divergence of function in folate-induced cytoskeletal changes.

Another important aspect of the control of chemotactic motility in D. discoideum is its developmental regulation. The chemotactic response to folate is present in vegetative and preaggregative cells but disappears during the aggregation phase (4). During aggregation the cells become responsive to cAMP which has little or no effect at the earlier stages of development (26). Actin incorporation in the cytoskeleton appears to show a similar developmental regulation in its responses to the two chemoattractants (fig.4). The response to cAMP is clearly at its greatest during the peak time (4-8hr) of aggregative chemo-

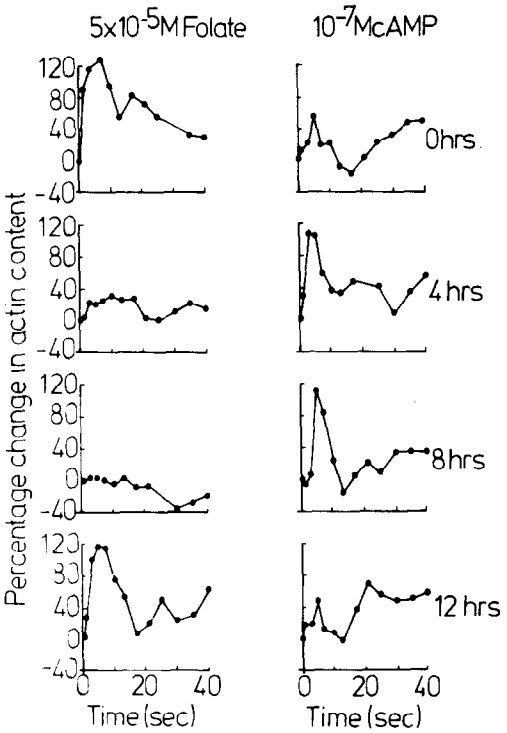

Figure 4. Developmental regulation of the actin association response following stimulation of 0,4,8 and 12 hr cells with 5 x 10^{-5}M folate or 10^{-7}M cAMP. Results are displayed as time courses of actin association with the cytoskeleton and are a representative example of four separate experiments.

tactic activity before declining again by 12hr when the motile events of aggregation are complete. In contrast the response to folate shows a peak during the preaggregative stage (0hr) then declines during aggregation. An unexpected but potentially important finding, however, is that the response to folate reappears at 12hr. This may indicate a role for folate in the later stages of development.

We conclude that chemotactic stimulation causes an increase in actin association with the cytoskeleton of D. discoideum. Similar changes in cytoskeletal composition have been reported for other motile cellular systems (15,27). The association is concentration dependent, shows temporal control, and is also developmentally regulated. The patterns of these responses fit closely to other responses reported to be involved in chemotaxis (9). Work is at present in progress to elucidate the mechanism of these changes in cytoskeletal structure and their regulation.

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