

BIOLOGICAL ION EXCHANGER RESINS: VIII. A PRELIMINARY REPORT ON ACTIN-LIKE PROTEIN IN *E. COLI* AND THE CYTOTONUS CONCEPT

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(Received June 27, 1975)

We wish to report the isolation of an actin-like protein in *E. coli*. The protein was first extracted from *E. coli* strain CBH¹ by the acetone extraction technique used in the isolation of skeletal muscle actin. It reversibly polymerized, as evidenced by rapid sedimentation, on a sucrose density gradient (5%-15% sucrose) under the same catalytic conditions that cause polymerization of G to F actin. The protein fraction when isolated from a K⁺ transport deficient mutant of *E. coli* strain RD-2¹ exhibited abnormal to absent polymerization at low medium K⁺ concentrations, the concentrations at which the mutant failed to accumulate potassium. The protein from the parent strain polymerized normally in low K⁺ medium.

Coupled with this was the observation that *E. coli* increase their cell volume by 9-12% during K⁺ uptake,¹ as determined turbidimetrically, with the cytocrit technique of Schultz and Solomon² and in the Coulter Counter. The K⁺ deficient mutant failed to swell when it failed to accumulate potassium at low K⁺ concentrations.

The existence of a contractile-like protein in *E. coli* was further supported by sodium dodecyl sulfate (SDS) gel electrophoresis of the extracted protein (Fig. 1). A 45,000 molecular weight protein band, the characteristic weight of muscle actin, appeared on the gel (Gel A). Reaction of the acetone extracted protein fraction prior to electrophoresis with skeletal muscle myosin selectively removed this band at 45,000 molecular weight (Gel B). Myosin prior to reaction with the *E. coli* acetone extract did not exhibit the presence of this protein (Gel C). After reaction with the *E. coli* fraction, myosin exhibited the presence of a new band at 45,000 molecular weight. The removal of this protein by myosin was a specific absorption. Growth of *E. coli* in uniformly labeled ¹⁴C glucose (ICN, Irvine, California) to mark all cell proteins demonstrated that all of the radioactivity transferred to the myosin *E. coli* protein complex was concentrated in the 45,000 molecular weight band. Figure 2, a densitometer trace of the gel electrophoresis, demonstrates the selective appearance of a new 45,000 molecular weight protein on myosin after it has been reacted with fragmented preparations of *E. coli*.

The identification of an actin-like protein in *E. coli* stimulates curiosity about its role. Careful study of the organism from which it was extracted established that it could not

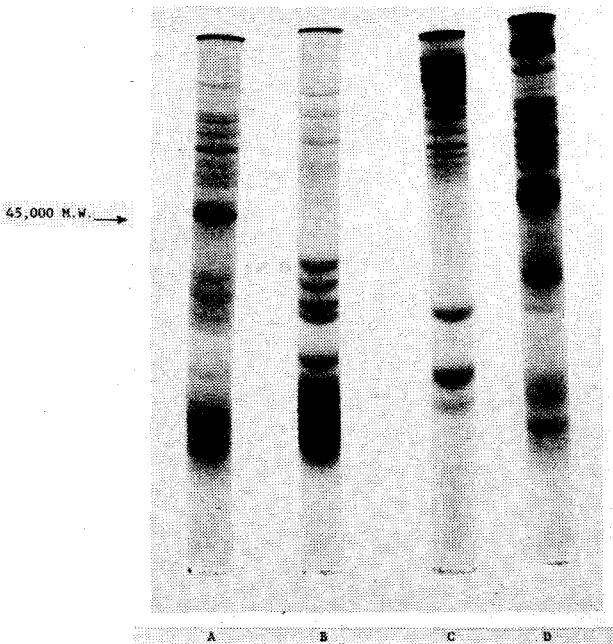


Figure 1. *E. coli* grown in KA medium¹ were suspended in the French press at 6,000 psi (4°C). The fragmented suspension was mixed for 1 hour at 4°C and centrifuged at 9,000 x g for 15 min. Skeletal muscle myosin prepared by the procedure of Dreizen, Hartshorne and Stracher¹² was then added to the soluble superantant, 1 g myosin to 10 g bacterial protein. Myosin containing bacterial extract was dialyzed overnight against a 25 mM KCl. A final concentration of 50 mM KCl in the protein compartment was achieved. The precipitate was collected by centrifugation at 9,000 x g for 15 min, washed twice in 0.05 M KCl, then washed in 0.4% NaHCO₃ and in distilled water. The washed protein precipitate was solubilized in 0.5 M KCl-12 mM imidazole buffer (pH 7.3) and analyzed by SDS gel electrophoresis.

be involved in locomotion since the organism was nonmotile.

The search for cellular contractile proteins began because of a need for a mechanism for regulating cellular hydration. We had shown from many different kinds of experimentation that cell hydration regulated alkali cation selectivity³⁻⁸ and was likely to be involved in the regulation of numerous other aspects of cellular physiology.

The Cytotonus Concept

We have considered the role a contractile protein such as actin could play in living cells. It seemed to us that it might act to maintain a type of "tonus" at the microscopic level analogous to the well known tonus of macroscopic tissues. The tonus exhibited by gross organs and tissues in the living state should have its counterpart at the microscopic level. We have designated this tonus of the cellular world cytotonus. While the location

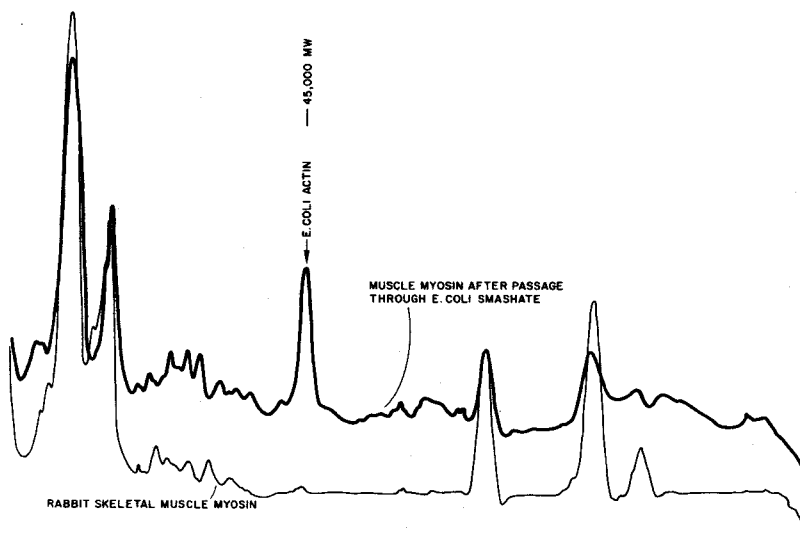


Figure 2. Densitometer tracing of SDS analytic gels made in a Gilford spectrophotometer model 240 equipped with the linear transport attachment (model 2410). SDS gel electrophoresis was performed on 9.25% acrylamide gels (bisacrylamide:acrylamide, 1:40) as described by Weber and Osborn¹⁰ with the modification of Abramowitz, Stracher and Detwiler.¹¹

of the contractile protein within the cell is not known exactly, it is likely from the work of Ambrose, Batzdorf, Osborn and Stuart⁹ that it has surface attachments.

Two possible arrangements among many whereby the contractile protein could act to regulate ion selectivity are illustrated in Figure 3. Ion selectivity would be achieved by an ATP-mediated contraction that would be followed by an extrusion of water. Reduced cell hydration would force the selection of the least hydrated ions both from thermodynamic and electrostatic considerations.^{3,5-7}

Cell contractile proteins and cytotonus would fulfill still another need. The role of intermediate metabolism in ion transport has never been well understood in our judgment. In the Biological Ion Exchanger Resin Model, we sought to understand what the intermediate metabolism achieved to permit the living ion exchanger resin to augment its selectivity many fold over what was possible in the resins nonmetabolizing cell and in synthetic and commercial ion exchanger resins. In light of the present observations, one role of intermediate metabolism would be to generate the ATP and other metabolites required to maintain cellular contraction at its proper tension.*

* We have also considered the possibility that cell proteins, perhaps not even contractile, could participate in the metabolism dependent contractility of cytotonus.

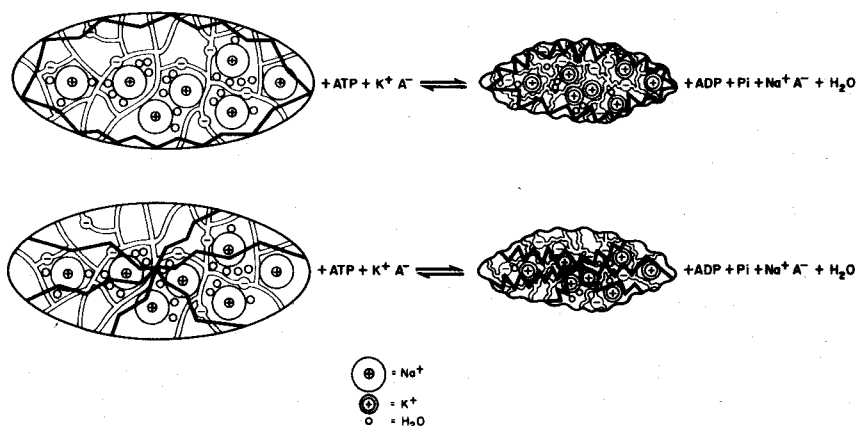


Figure 3. Schematic illustration of the regulation of cell hydration and ion selectivity by contractile proteins. The larger hydrated Na^+ ion is replaced by the less hydrated K^+ as cell contractile proteins activated by ATP establish cytotonus and extrude H_2O . Competition for limited space and limited solvent forces the replacement of Na^+ by K^+ .

Summary: Evidence has been accumulated suggesting the presence of an actin-like protein in *E. coli*. The protein was extracted by the procedure used to extract skeletal muscle actin, it polymerized reversibly under the same catalytic conditions that actin from skeletal muscle polymerized, and it exhibited a band on sodium dodecyl sulfate (SDS) gel electrophoresis at the characteristic molecular weight for actin.

Cross reaction of this extract with myosin from skeletal muscle, specifically removed the 45,000 band which then appeared in the gel electrophoretic patterns of the myosin that had been reacted with *E. coli* extract.

A role for contractile proteins in living cells has been proposed. It is suggested that they could serve to maintain a kind of "tonus" for the cell analogous to the "tonus" of macroscopic tissues and organs. This "tonus" or cytotonus, as we have called it, could limit the access of water to the Biological Ion Exchanger Resin and thereby control ion selectivity and other cell processes as well.

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