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Actin-Like Proteins From *Escherichia coli*: Concept of Cytotonus as the Missing Link Between Cell Metabolism and the Biological Ion-Exchange Resin

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A protein fraction (A-L fraction) with characteristics reminiscent of muscle actin has been isolated from *Escherichia coli*. The A-L fraction undergoes reversible aggregation under the same conditions in which actin is polymerized and depends primarily on potassium for its polymerization. This fraction, upon electrophoresis on acrylamide gels in the presence of sodium dodecyl sulfate, exhibits a distinct peak at the characteristic molecular weight of 45,000. Passage of skeletal muscle myosin through the A-L fraction specifically removes this 45,000-molecular weight peak. Examination of the myosin by sodium dodecyl sulfate electrophoresis after the passage reveals a new band at the proper molecular weight. The A-L fraction from wild-type *E. coli* is compared with the protein from a potassium transport mutant. Important catalytic differences exist between the A-L fractions of the two strains. The A-L fraction from the mutant fails to polymerize in low-K media in the K^+ concentration range in which the mutant fails to take up K^+. In low-K^+ media, the parent strain accumulates potassium and the A-L fraction from this organism polymerizes. The cell swelling reaction of both strains has been studied. Parent cells swell during low-K^+ uptake, whereas the mutant does not. It is construed from this that the differences in the characterization of the A-L fraction relative to that of the wild type are related to the loss of cell swelling in the mutant and hence to the loss in alkali cation selectivity. The possible role of contractile proteins in biological ion exchange is discussed.

Recently we described early results characterizing an actin-like protein from *Escherichia coli* (Fed. Proc. **33**:1332, 1975, abstract 616). We now describe more fully the isolation of this protein from *E. coli* by procedures similar to the method for isolating actin from skeletal muscle. Many such proteins have already been isolated from other nonmuscle sources (1, 4, 6, 28, 34). With crude actin-like fractions, we compared the characteristics of the proteins isolated from the wild-type *E. coli* with the same proteins isolated from a K^+ transport-deficient mutant.

MATERIALS AND METHODS

Bacteria. *E. coli* CBH, a nonmotile histidine auxotroph of *E. coli* B, and strain RD-2, a potassium accumulation mutant derived from CBH (nonmotile), were used exclusively throughout this study. Cells grown to stationary phase in KA medium (8) and collected in 2-ml volumes were maintained at −120 C in a Revco U.L.T. freezer. The bacteria were grown from these inocula in KA media (8). NaA medium was used as the low-potassium medium (8). Nonmotility in both strains was established by direct microscope examination of log-phase cells suspended in growth media (tryptic soy broth supplemented with 1% Casamino Acids). Motility in this strain of *E. coli* was searched for since one conceivable purpose for any contractile proteins isolated from this organism could be locomotion.

SDS-gel electrophoresis. Sodium dodecyl sulfate (SDS)-gel electrophoresis was performed on 9.25% acrylamide gels (bisacrylamide-acrylamide, 1:40) as described by Weber and Osborn (33) with the modifications of Abramowitz et al. (2). Densitometer tracings of the gels were made with a Gilford spectrophotometer model 240 equipped with the linear transport attachment (model 2410).

Protein concentrations. Protein concentrations were measured by the produce of Lowry et al. (24) as modified by Abramowitz et al. (2). This procedure was verified by comparison with a biuret analysis (21) of the proteins in question.

Measurement of glucose. Portions of bacterial suspension were taken, and the cells were removed by membrane filtration (Millipore Corp., 0.45 μm pore size). The media was assayed for glucose by a coupled enzyme colorimetric procedure (Glucostat, Worthington Biochemical Corp.).

Sucrose gradients. Gradients (5 to 20%, wt/vol) of

sucrose were obtained by layering 1.25-ml portions of 5, 10, 15, and 20% sucrose solutions on top of each other in order of decreasing sucrose concentration. Gradients were permitted to "age" for 8 h at 4 C. A portion (0.3 ml) of the A-L fraction protein in the appropriate buffer solution was layered on the gradient, which was then centrifuged at 300,000 × *g* for 1 to 2 h at 3 C in a Beckman SW50.1 rotor. Fractions were collected from the bottom of the gradient in 0.3-ml portions.

Sepharose column. A column (0.9 by 30 cm) containing Sepharose 6B pre-equilibrated with a solution of 0.2 mM adenosine 5'-triphosphate (ATP), 0.5 mM β-mercaptoethanol and 0.5 mM tris(hydroxymethyl)aminomethane (Tris)-hydrochloride (pH 7.6) was used for molecular weight analysis of protein isolated from *E. coli*. A 0.5-ml portion of protein solution in the same buffer was used for the analysis.

Cell volume measurement. Cell volume was measured in two ways. Relative changes in particle size were determined in accord with the relation of Koch (20) from changes in suspension optical density at 620 nm. A Gilford model 240 spectrophotometer equipped with a model 2453 linear potentiometer strip chart recorder was used. Continuous flow monitoring of suspension turbidity was possible in this way and permitted a recording of the entire time course of cell swelling during the K^+ accumulation reaction. Turbidity has been used widely to monitor swelling in many types of cells and subcellular organelles (3, 26, 27, 32).

Changes in volume were determined by an alternate method as well, utilizing a ZB Coulter counter equipped with a C100 channelizer (a multichannel analyzer) (Coulter Electronics Inc., Hialeah, Fla.). A 30-μm aperture was utilized with an aperture current of 1/2 at 1/2 amplification. Volume distributions were plotted directly from the multichannel analyzer.

Cell potassium and sodium. Cells for determination of intracellular potassium and sodium were collected on 25-mm membrane filters (Millipore Corp., 0.45 μm pore size) and washed with 5 ml of 400 mM sucrose solution. Cells were then treated with 2 drops of concentrated nitric acid. K^+ and Na^+ determinations were made on a Flame Photometer (Instrument Laboratory) utilizing methods previously described (8).

Isolation of the A-L fraction. The bacteria were grown by continuous culture in a New Brunswick fermentor (MicroFerm 114) in KA medium. Bacteria were harvested in a Sharples continuous flow centrifuge equipped with refrigeration cooling coils that were maintained at approximately 10 C. Bacterial suspensions in distilled water at approximately 20 mg of dried bacteria/ml were fragmented in a French press (6,000 lb/in² at 0 C), and the fragmented suspension was diluted in 5 volumes of cold acetone and stirred continuously for 1 h at 4 C. After filtering the suspension through Whatman paper no. 3 and air drying the acetone precipitate, it was suspended in a solution containing 0.2 mM ATP, 0.5 mM β-mercaptoethanol, and 0.5 mM Tris-hydrochloride (pH 7.6), hereafter called "depolymerizing solution," by analogy to its action in muscle actin preparations. One volume of the final solution was dialyzed against 50 volumes of depolymerizing solution to remove potassium. The preparation was then centrifuged in a Sorvall refrigerated centrifuge (4 C, 48,000 × *g* for 2 h) to remove rapidly sedimenting cellular debris. An additional centrifugation for 2 h in a Beckman preparative ultracentrifuge 60 Ti fixed angle titanium rotor at 120,000 × *g* removed all remaining insoluble material.

The protein was then "polymerized" (or aggregated) by increasing the salt concentration of the final supernatant solution to 50 mM KCl and 0.2 mM $MgSO_4$. (A solution containing 50 mM KCl, 0.2 mM $MgSO_4$, 0.2 mM ATP, 0.5 mM β-mercaptoethanol, and 0.5 mM Tris (pH 7.6) is termed "polymerizing solution." The resulting solution was mixed for 2 h at 4 C and then centrifuged for 2 h at 120,000 × *g* in a Beckman type 60 Ti rotor. The pellet was then suspended in depolymerizing solution and dialyzed overnight against 400 volumes of depolymerizing solution. The final solubilized protein that survived two or more repetitions of the "polymerization-depolymerization" cycle was termed the A-L fraction. It was found subsequently that a third repetition of the polymerizing-depolymerizing cycle did not alter the purity of the A-L fraction and decreased protein yield. It was thus discontinued.

Myosin preparation. Myosin from rabbit skeletal muscle was prepared by the procedure of Szent-Gyorgyi (31) as modified by Dreizen et al. (16). Myosin prepared in this way was stored for continued use in 0.5 M KCl-5 mM $NaHCO_3$-0.02% NaN_3-0.1 mM dithiothreitol (pH 7.0) at 4 C.

Reaction of E. coli with myosin from rabbit skeletal muscle. *E. coli*, grown in 2 liters of KA medium (8) in a reciprocating shaker and harvested by centrifugation, were suspended in a buffered solution containing 0.6 M KCl and 12 mM imidazole (pH 7.3, 4 C) and fractured in a French press (Aminco, Silver Spring, Md.) at 6,000 lb/in² at 4 C. The resulting fragmented suspension was stirred for 1 h at 4 C and then centrifuged at 9,000 × *g* for 15 min to remove particulates. Skeletal muscle myosin was then added to the soluble supernatant to achieve a final mass ratio of 1 g of myosin to 10 g of bacterial protein. The myosin-containing bacterial extract was dialyzed overnight against a 0.025 M KCl concentration until a final concentration of 0.05 M KCl was achieved. The precipitate was collected by centrifugation at 9,000 × *g* for 15 min, washed twice in 0.05 M KCl, and then washed in 0.4% $NaHCO_3$ and in distilled water. The washed protein precipitate was then solubilized in 0.5 M KCl-12 mM imidazole buffer (pH 7.3) and analyzed by SDS-gel electrophoresis.

Reaction of the A-L fraction from E. coli with skeletal muscle myosin. The A-L fraction was suspended in 0.5 M KCl-12 mM imidazole buffer, pH 7.4. Myosin in KCl-bicarbonate stock solution was added to the A-L fraction until a final mixture of 1 part myosin to 10 parts bacterial protein (wt/wt) was achieved. Myosin precipitate was then formed by dilution of the final suspension KCl concentration to 0.05 M by equilibrium dialysis. Dialysis was performed at 4 C for 12 h with an external solution containing 25 mM KCl. Myosin precipitate was collected by centrifugation at 9,000 × *g* for 15 min and

washed twice with 0.05 M KCl, then with 0.4% $NaHCO_3$, and finally with distilled water. The resultant protein was then suspended in 0.5 M KCl-5 mM $NaHCO_3$-0.02% NaN_3-0.1 mM dithiothreitol (pH 7.0) and analyzed by SDS-gel electrophoresis.

RESULTS

Characterization of the A-L fraction. A protein fraction (called the A-L fraction) that polymerized and depolymerized reversibly in the solvents used to obtain this result with skeletal muscle actin was isolated from *E. coli*. Demonstration of the reversible polymerization of the A-L fraction in 50 mM KCl polymerizing solution was provided by density gradient analysis in continuous gradients of aqueous sucrose (Fig. 1). Centrifugation of "depolymerized protein" for 2 h on sucrose gradients at 300,000 × *g* yielded a large fraction at the top of the gradient ($s_{20,w}$ = 11.6) that accounted for 75% of the protein initially applied (curve A). Centrifugation of polymerized A-L fraction deposited 90% of the protein at the bottom of the tube after 1 h of centrifugation at the same centrifugal force (curve B). The total amount of the protein applied to both gradients in curve A and B was equivalent. Polymerized protein packed at the bottom of the centrifuge tube in curve B after centrifugation was not disturbed in the aqueous sucrose of the gradient and therefore does not appear in curve B. Ninety percent of the depolymerized A-L fraction eluted in the 7-ml void volume when subjected to gel filtration on a 19-ml Sepharose 6B column. Exclusion of solubilized protein from Sepharose 6B (exclusion molecule weight, 10^6 to 4 × 10^6) suggested a large molecule.

SDS-acrylamide gel electrophoresis of the A-L fraction demonstrated a complex protein fraction composed of subunits ranging in weight from 131,000 to 16,500 daltons (Fig. 2). Repeated polymerization solubilization cycles aimed at improving purity did not alter the SDS profile representing the subunit composition of the A-L fraction and indicated that individual peaks could not be attributed to extraneous impurities that had survived the initial isolation. It was therefore concluded that the individual bands on SDS-gel electrophoresis were an integral part of a reversibly polymerizing system that polymerized in the presence of 50 mM KCl and came apart into small monomers in its absence.

The A-L fraction was further dialyzed extensively against 0.2 mM ATP–0.5 mM β-mercaptoethanol–0.5 mM Tris (pH 7.6) to further remove cofactors. Strong ultraviolet absorption was noted at 260 nm in preparations that had been dialyzed for 3 to 4 days. Ribonucleic acid and deoxyribonucleic acid were measured

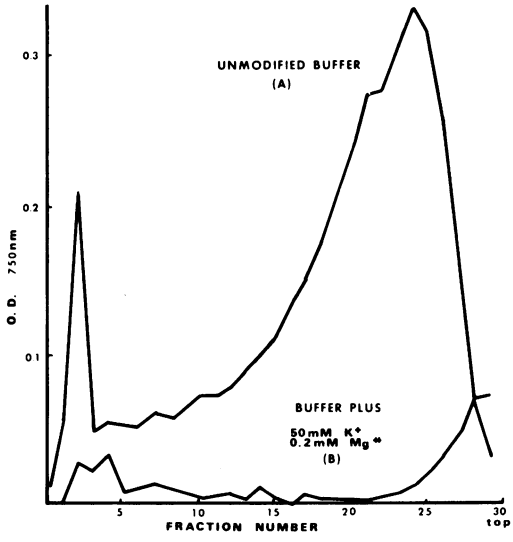

FIG. 1. *Sucrose gradient analysis of the A-L fractions with protein determined by the method of Lowry et al. (24) as modified by Abramowitz et al. (2). (A) Approximately 2.5 mg of protein/ml of A-L fraction dissolved in depolymerizing solution (0.2 mM ATP–0.5 mM β-mercaptoethanol–0.5 mM Tris, pH 7.6) was layered on a 5 to 20% sucrose gradient and centrifuged for 2 h at 300,000 × g in a Beckman SW50.1 rotor. (B) Sucrose gradient centrifugation of the A-L fraction (2.5 mg/ml) in polymerizing solution (50 mM KCl–0.2 mM $MgSO_4$–0.2 mM ATP–0.5 mM β-mercaptoethanol–0.5 mM Tris, pH 7.6) centrifuged for 1 h as above. Protein was determined by the modified procedure of Lowry (2) on each fraction.*

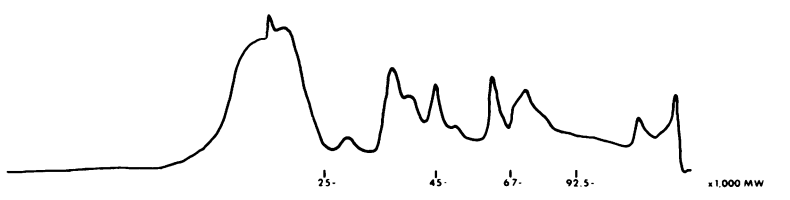

FIG. 2. *Densitometric scan of SDS-electrophoresis of the A-L fraction from E. coli. Molecular weight markers run on separate gels during the same electrophoresis were: phosphorylase a, 92,500; bovine serum albumin, 67,000; ovalbumin, 45,000; chromotrypsinogen, 25,000. Fractions (0.02 ml) containing 1.5 mg of A-L protein per ml in depolymerizing buffer were applied to polyacrylamide gels.*

by the method of Schneider (29). Only trace amounts of deoxyribonucleic acid were present, but 30% of the A-L fraction (by weight) proved to be ribonucleic acid.

Potassium catalyzed the polymerization more efficiently than sodium (Fig. 3).

Reaction of skeletal muscle myosin with E. coli. Figure 4 compares the densitometer trace

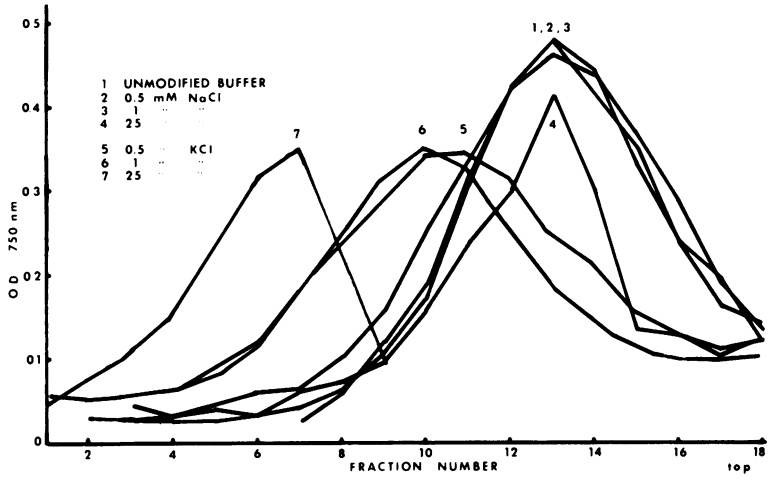

FIG. 3. *Polymerizing effects of Na^+ and K^+ on E. coli actin-like protein by sucrose gradient analysis. Fractions (0.3 ml) containing 7 mg of A-L protein per ml in 0.2 mM ATP–0.5 mM β-mercaptoethanol–0.5 mM Tris (pH 7.6) were applied to the gradient. Salts of the alkali cations were added as indicated. All samples contained an additional 4 mM Na^+ contributed by the salt of ATP. The protein used on all the gradients was from the same preparation of A-L fraction. Gradient centrifugations were carried out for 2 h at 300,000 × g in an SW50.1 rotor.*

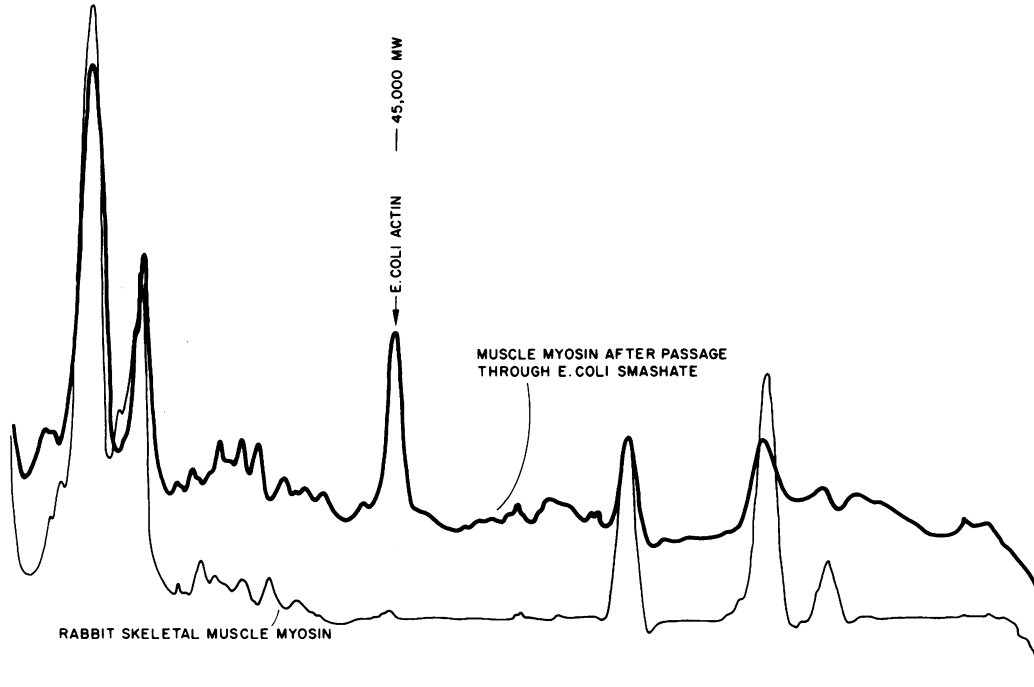

FIG. 4. *Densitometer trace of SDS-electrophoresis of skeletal myosin before and after it was reacted with the soluble supernate of fragmented E. coli. Forty milligrams of protein was applied to the gel column.*

of the SDS-gel electrophoresis of myosin from rabbit muscle (lower profile) with the trace of the myosin that had been incubated with a fragmented preparation of *E. coli* and then removed as described above. The two differ by the presence of an additional peak that was removed from the soluble supernatant of fragmented *E. coli* after it was incubated with skeletal muscle myosin and that survived the four washing cycles (see above) of the centrifuged myosin pellet with KCl, buffer, and then distilled water. The new peak was at 45,000 molecular weight, the characteristic weight of actin. A control experiment with *E. coli* grown in medium KA with uniformly labeled [^{14}C]glucose (ICN, Irvine, Calif.) as the only carbon source established that virtually all of the radioactivity in the electrophoresis was concentrated in the 45,000-molecular-weight peak.

Reaction of skeletal muscle myosin with the proteins of the A-L fraction of E. coli. Since the A-L fraction exhibited behavior similar to that of muscle actin, we were interested in determining if the 45,000-molecular-weight protein of this fraction was actin. The results of absorbing the A-L fraction with muscle myosin are shown in Fig. 5. Gel A contains the SDS-polyacrylamide gel electrophoretic pattern of the A-L fraction. Gel B contains the A-L fraction after treatment with myosin, demonstrating the total removal of the 45,000-molecular-weight band by myosin treatment. Gels C and D compare myosin before passage through the A-L fraction with myosin after passage. Gel C demonstrates the absence of the 45,000-molecular weight band in native myosin prior to passage through the A-L fraction. Gel D demonstrates the appearance of the new band at 45,000 molecular weight after passage. Because of the presence of an actin-like protein both in crude extracts of *E. coli* and in a fraction (A-L fraction) extracted by the same solvents used to extract skeletal muscle actin, we have called it *E. coli* actin-like protein.

Physiological characterization of differences between the parent strain and the K^+ transport mutant (RD-2). We briefly summarize here the phenotype differences between K^+ transport mutant RD-2 and the parent *E. coli*. The A-L fractions from both these organisms were investigated for dissimilarities that could connect A-L protein with potassium transport. The mutant, unlike the parent, fails to accumulate potassium at concentrations below 100 μm (8).

Figure 6 demonstrates glucose consumption in the mutant and parent strains during potassium uptake in K^+-deficient medium. Glucose consumption and potassium accumulation were studied simultaneously in these experiments. The potassium uptake reaction, which occurred simultaneously, is shown in the figure inset. It can be seen that glucose consumption proceeded normally in the K^+ transport mutant (RD-2) even through it failed to accumulate potassium.

Cell swelling during K^+ accumulation. Cell swelling has previously been demonstrated as a concomitant of the K^+ accumulation reaction in *E. coli* (Table 13 of reference 8). During uptake of 85 mmoles of potassium/1 liter of cell water, the volume of the cell cytocrit increased 12%. The change in the packed cell volume of potassium-accumulating *E. coli* was measured in cytocrit tubes with the aid of an optical comparator for pellet volume determinations. The method used was the one devised by Schultz and Solomon (30), which possessed a high degree of precision, as demonstrated by the deviations from the mean.

The results of optically monitored swelling experiments are shown in Figure 7. Parent strain and mutant that were depleted of cell potassium by methods described elsewhere (8) were suspended in NaA medium (8) (approximately 20 μM K^+). At the addition of glucose, the K uptake reaction was initiated in the parent strain but failed to occur in the mutant RD-2. A decrease in optical density or particle swelling occurred in the parent as it accumulated potassium (Fig. 7), but in the mutant absence of the K^+ accumulation reaction at potassium concentrations below 33 μm was accompanied by a lack of swelling. Medium K^+ concentration was then increased in two increments, first to one (33 μM) which was known to be below the K accumulation threshold for the mutant (100 μM), and then to one which was high enough to stimulate the potassium uptake reaction. Particle swelling in the mutant occurred only at the higher concentration, where the K^+ uptake reaction was occurring. This last experiment demonstrated that the swelling reaction existed in the mutant but was K^+ concentration dependent and failed in the same region of the K^+ concentration curve in which K^+ transport failed. Glucose consumption proceeded normally in both strains from the time of glucose addition to the culture (Fig. 6).

The swelling phenomenon is a reversible event as evidenced by the return of the optical density to its initial value over a period of 30 to 40 min. We therefore refer to the entire process as a "swelling-contraction" cycle.

A third technique (Fig. 8) was utilized to

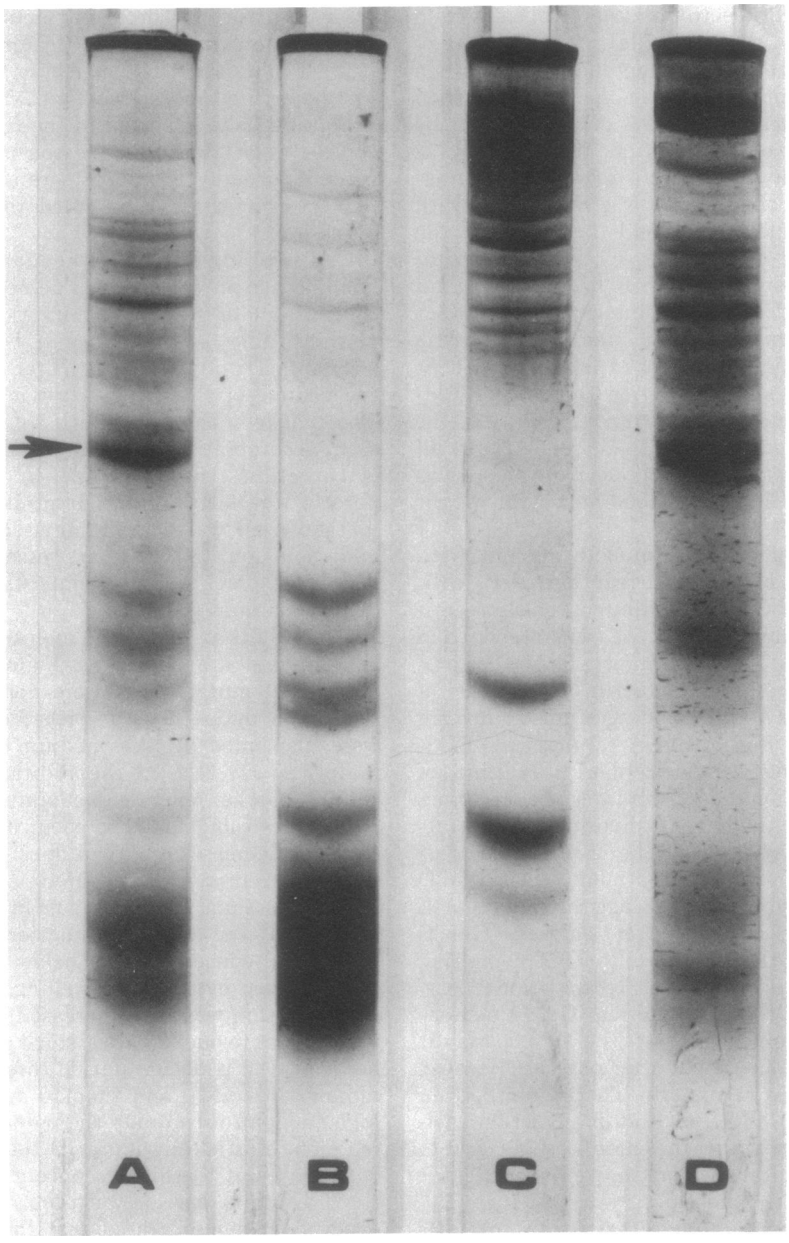

FIG. 5. *SDS-polyacrylamide electrophoresis of the A-L fraction before and after myosin passage through the extract. (A) A-L fraction prior to myosin passage; (B) A-L fraction after passage; (C) rabbit skeletal muscle myosin prior to passage; (D) myosin after passage. Arrow indicates the 45,000-molecular weight protein.*

corroborate that the observed optical density changes represented cell swelling. Measurements were made in a Coulter counter size distribution analyzer before and after the addition of glucose in the experiment shown in Fig. 7. The curve at 0 min corresponds to the initial cell size prior to the onset of the optical density change, and the dashed curve corresponds to the size distribution after 5 min of the swelling reaction. The observed difference in the Coulter counter sizing spectrum represents an increase of 9.5% in cell volume after 5 min of swelling, in reasonable agreement with the cytocrit determination that showed a 12% increase in cell volume after K uptake.

Comparison of the A-L fractions from the

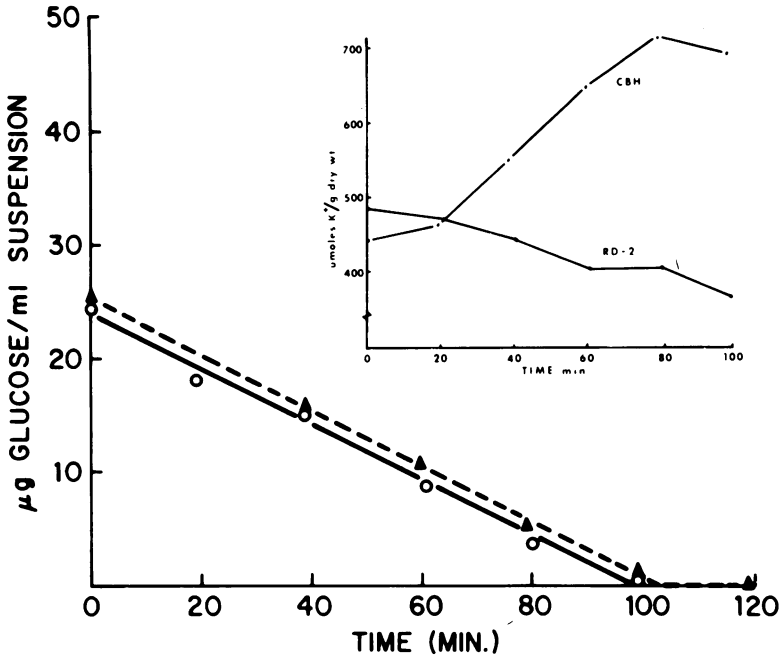

FIG. 6. *Glucose consumption during K^+ accumulation in low K^+ media. Symbols: ▲, Mutant RD-2; O, parent strain CBH. Inset shows the K^+ accumulation reaction for the experiment.*

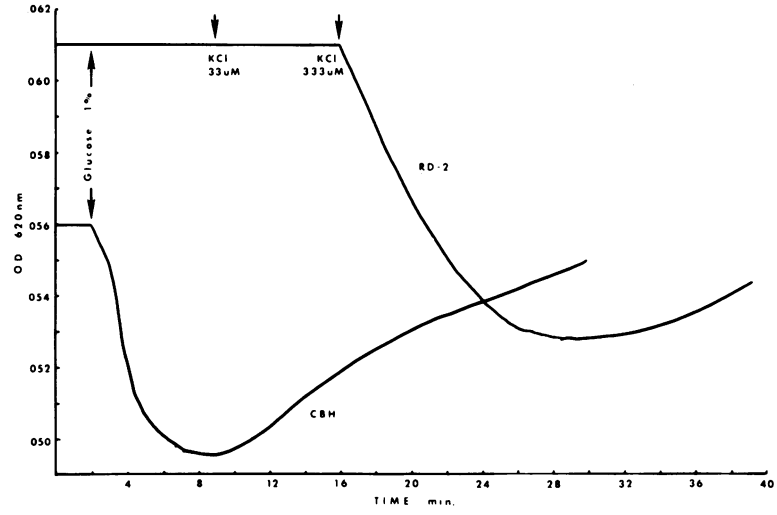

FIG. 7. *Cell swelling in parent strain (CBH) and mutant (RD-2) as measured by changes in suspension optical density at an observing wavelength of 620 nm.*

parent strain and the K^+-deficient mutant. Because of the importance of swelling in the K^+ accumulation phenomenon, we were interested in the prospect that the defect in the potassium transport-deficient mutant (RD-2) could be related to an abnormality in its contractility at low potassium concentrations, i.e., an abnormality in the cell's contractile system at low K^+. We therefore compared the behavior of the A-L fractions isolated from the two strains. We looked for differences in this protein fraction that would be manifest in the same potassium concentration range in which the K^+ accumulation phenotypes of the two strains differed.

The A-L fraction, in the absence of salts that cause muscle actin to polymerize, remains on a

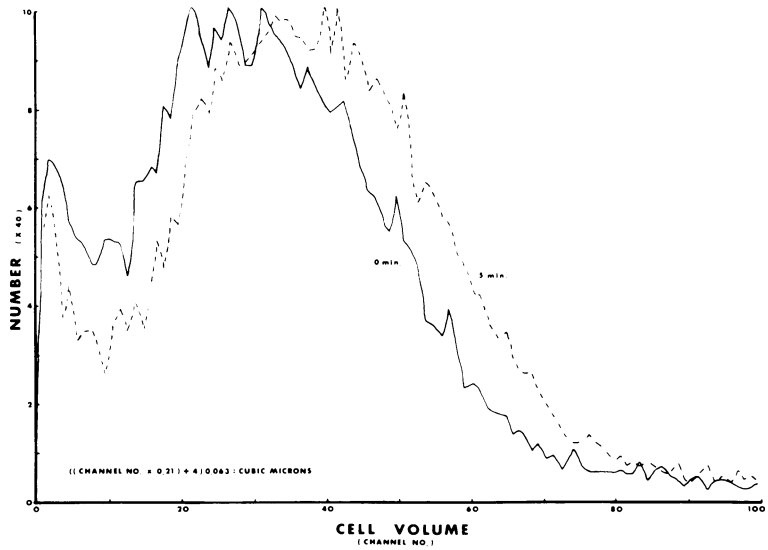

FIG. 8. *Measurements of cell size in the Coulter counter of the parent strain E. coli (CBH) prior to (0 min) and at the height of the swelling reaction (5 min) shown in Fig. 7.*

5 to 20% sucrose gradient after 120 min of centrifugation at 300,000 × *g*. In the presence of polymerizing reagents (50 mM KCl, 0.2 mM $MgSO_4$), the A-L fraction goes to the bottom of the gradient during a centrifugation of the same duration, signifying an increase in molecular weight.

The difference between the polymerizing tendency of the A-L fraction from the parent strain from that of the mutant is shown in Fig. 9. In the low K^+ concentration ranges, where potassium accumulation failed in the mutant (<100 μM K^+), no evidence of polymerization was exhibited on sucrose density analysis. The A-L fraction from the mutant ran without added potassium (KCl) or at 0.5 mM KCl (solid lines 1 and 2) had not left the top of the gradient after 2 h of centrifugation. The A-L fraction from the parent strain (dashed curves 4 and 5), on the other hand, gave evidence of polymerization at the low K^+ concentrations. It exhibited sedimentation at low K^+ concentration in contrast to the A-L fraction from the mutant, which did not. Evidence for the A-L fraction polymerization in the mutant did not appear until a KCl concentration of 1 mM (curve 3) was reached, a concentration at which the mutant cell accumulates potassium. The differences in the polymerization (or aggregation) of actin-like protein in these two strains occurs in the same K^+ concentration range in which the intact cells differ in their phenotype.

DISCUSSION

Possible function of a contractile protein in a nonmotile bacteria. In a recent paper, we investigated the availability of ATP for supplying the energy requirements of pump-mediated transport (25). ATP is generally regarded as the energy currency for maintaining thermodynamic gradients of solutes across biological interfaces. The expectation was that energy from the terminal phosphate bonds of ATP, liberated by the membrane ATPase, could compensate directly the thermodynamic energy required by pumps to maintain two compartments that are in contact through a semipermeable membrane at different concentration. The energy available from ATP fell far short of the amount required and could supply only 14% of the energy needed for the five solutes studied.

Whereas the energy available from ATP did not quantitatively match the energy requirements of the existing concentration gradients, the empiric association of ATP and cellular ATPase activity with ion transport is well substantiated by the literature and our experience. We were, therefore, sensitive to the possibility that ATP might have another role in ion transport apart from the direct conversion of energy from the terminal bond of ATP to the maintenance of concentration gradients. We found that most of the necessary energy for concentration gradients could be accounted for

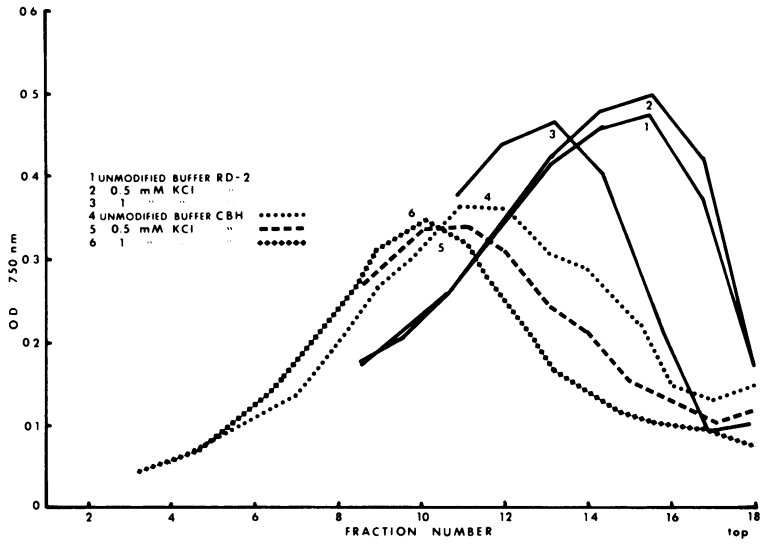

FIG. 9. *Polymerizing differences of the A-L fractions from the parent strain (CBH) and mutant (RD-2) at different K^+ concentrations. Determinations of protein concentration on fractions from 5 to 20% sucrose gradients were after 120 min of centrifugation at 300,000 × g. Portions (0.3 ml) of the A-L fraction (7.6 mg of protein/ml) from fragmented suspensions of whole bacteria were applied to the gradient in the buffer indicated.*

by treating the cell as a biological ion-exchange resin (9–14) where mobile ions inside the cell were associated with "fixed charges" on cell polyelectrolytes and therefore did not require energy in the form of direct contributions from ATP. The concentration gradient of ions were maintained instead by the Coulomb attractions of oppositely charged ions that themselves were part of the macromolecule fabric of the cell. Some metabolic energy, however, was required for ion selectivity, the choice of the biological resin between ions of like charge. Synthetic ion-exchange resins, with a few exceptions, are not capable of the degree of selectivity possessed by living cells. We have observed this selectivity to be dependent on ATP (25), as has Ling (22, 23). Ling has observed that muscle ion composition is unchanged so long as 15 to 20% (2.5 μmol/g of fresh tissue) of the normal pool of muscle ATP remains. We made a similar observation in *E. coli*. Cell cation content remained largely unchanged so long as 25% of the bacterial ATP pool remained (2.7 μmol/g of dried bacteria). When this ATP was depleted, potassium selectivity collapsed (25).

Because of the acknowledged importance of swelling in ion-exchange equilibria in resin beads (18, 19), we were attracted by repeated reports of swelling phenomena in cells and subcellular organelles and by our own experience with the swelling reaction of potassium-accumulating *E. coli* (8).

"Limited Water Hypothesis" of Cells and the Control of Ion Transport by Contractile Proteins That Regulate Cell Water Content: a Concept of Cytotonus

The role of water in biological ion exchange can be viewed in two ways. In Gregor's model of ion-exchange equilibria, which we call the macroscopic view, the ion-exchange resin can be visualized as an elastic matrix (Fig. 10). Access of water to the inside of the resin for the purposes of hydrating fixed charges and mobile counter-ions is limited ultimately by the expansibility of the bead. In a cell it would be limited by the expansibility of the cytoplasm and cell surface layer. The pressure developed by the mechanical resistance of the bead becomes an energy for selecting between ions of equal charge but unequal hydrated radius. The ion of smallest hydrated radius is selected for by the relation of Gregor:

$$RT \cdot \ln K_{A/B} = \Pi(\nu_A - \nu_B) \qquad \text{(i)}$$

where $K_{A/B}$ is the selectivity coefficient for completing counter-ions A and B, Π is the osmotic pressure, and ν_A and ν_B are the partial molar volumes of ions *A* and *B*, respectively. Competition among mobile ions for internal solvent that has been restricted by the expan-

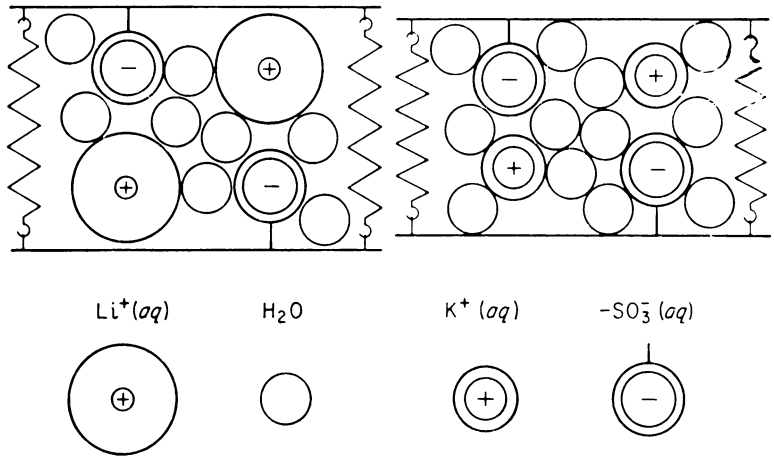

FIG. 10. *Swelling and selectivity of the ion-exchange resin. Hydration of the resin is resisted by the expansibility of the bead. Ions of lesser hydration such as K^+ are therefore preferred over larger hydrated ions like Li^+ (7).*

sivity of the resin bead energetically favors a choice for the ion of smallest hydrated radius.

The more microscopic view is represented by the following equation:

$$F = \frac{1}{4\pi\epsilon} \cdot \frac{q_1 q_2}{r^2} \qquad \text{(ii)}$$

where the Coulomb force (F) between a fixed charge and its counter-ion partner is 1 over the medium dielectric ϵ, q_1 and q_2 are the charge on fixed charge and counter-ion, respectively, and r is the distance between their atomic centers. Increased availability of water favors an increase in the medium dielectric, thereby reducing the pairing force. One can see the influence of water on the difference in the pairing force between counter-ions A and B if the ratio of r_A/r_B is constant, a reasonable case since the hydration numbers of two ions such as Na^+ and K^+ can be expected to remain constant over a significant range of concentration. For the exchange of counter-ion species A and B, the force differential between the two competing species (Fig. 10) and the fixed charge over equal distance is minimized when cell water is abundant.

$$\Delta F = \frac{1}{4\pi\epsilon} \cdot \left(\frac{q_1 q_A}{r_A^2} - \frac{q_1 q_B}{r_B^2} \right) \qquad \text{(iii)}$$

$$(r_A \neq r_B)$$

Integrated over distance, this implies the difference in energy between a fixed charge pairing with A or B is minimized when ϵ is maximal, i.e., when intracellular water content is elevated sufficiently for its dielectric constant to approach 80, the value of ϵ for bulk water. Accordingly, the greatest differences in the pairing energies of the two competing species will favor the greatest degree of ion selectivity and will occur at the reduced dielectric of limited intracellular water.

Experimental evidence for the role of water in biological ion selectivity has been previously reported (14). Ion selectivity in *E. coli* was shown to vary with the osmotic pressure of the medium. This was interpreted to mean that excess cell hydration, occurring when a cell is immersed in dilute bathing medium (<0.4 osmolar), lessens alkali cation selectivity. Selectivity was eliminated altogether ($K_{K/Na} = 1$) when cell hydration was maximum during suspension in distilled water. It was also shown to vary in the same fashion with two other solutes and with temperature. In the case of temperature, selectivity transitions occurred in the region characteristic of the typical anomaly of water structure, 4 C (14). Added evidence of changes in cell water with ion composition was provided by a nuclear magnetic resonance study of the bacterial water signal. The nuclear magnetic resonance line width was shown to vary with the alkali cation composition of the cell. Finally, calculations from measurements of the water content of *E. coli* and its intracellular charges (10) demonstrated that at a maximum, only 55 water molecules were available to each cell ionic group for solvation. This permitted a mean hydration atmosphere for each ion 2.5 water molecules thick. The actual hydration of each ion is considerably less. No account of cell polar groups other than ions was considered in this calculation (13, 14). Cell sugars, for example, with their polyhydric composition, were not

allowed for in the calculation and would very likely further reduce the solvent insulation available to cell ions. A value of 2.5 water molecules is well within polarizing range of the central charge, as has been pointed out by Debye (15) and more recently confirmed by Grahame (17). Consequently the cell exists with an internal environment in which the solvent dielectric insulating the Coulomb pairing between fixed charge and mobile counter-ion varies. It depends upon the solvent content of the cell and is critical in establishing the final equilibrium that is achieved between competing counter-ions and the biological resin.

Contractile proteins in E. coli and the regulation of ion transport. The experiments with *E. coli* demonstrate the existence of an actin-like protein in a nonmotile *E. coli*. In addition, it is shown that swelling contraction cycles accompany the K^+ uptake reaction in *E. coli*. The two are presumably related insofar as the K^+ transport-deficient mutant RD-2 fails to exhibit a swelling-contraction cycle and likewise exhibits a contractile protein fraction, the A-L fraction, that is deficient in the same region of the K^+ concentration curve in which the intact organism fails to accumulate potassium. The A-L fraction from the mutant requires a higher K^+ concentration for polymerization than the normal strain.

The existence of a contractile protein in *E. coli* and its relation to selective ion accumulation as demonstrated in the K^+-transport-deficient mutant provides the possibility of a control mechanism for the regulation of ion accumulation in living cells. Such a protein would provide, through its constrictions, the necessary mechanism for regulating cell hydration (Fig. 11), contracting to force out water when it was in excess and relaxing to permit its access when it was deficient. It would maintain a type of cell "tonus," which we have termed cytotonus, analogous to the more macroscopic tonus of living tissues such as skeletal muscle. This seems reasonable to us since it is plausible that the well recognized tonus of the macroscopic organs has its counterpart at the microscopic level. Cytotonus would set the limits on expansibility of a living cell, with death ensuing with a relaxation of the cell and an influx of water. Some mechanism for the regulation of cell water content is imperative to the maintenance of cell function in our judgement. In addition, it would supply the "missing link" in ion transport since energy metabolism would be required to generate the ATP needed in contraction. The regulation of ion composition of the biological resin would therefore be achieved by cellular contractile proteins that prevent overhydration of the cell and maintain it in the "water limited state" required for alkali cation selectivity. Some mechanism for the control of cell hydration is imperative for the maintenance of cell function.

The location of the contractile protein within the cell is not known, but the work of Ambrose

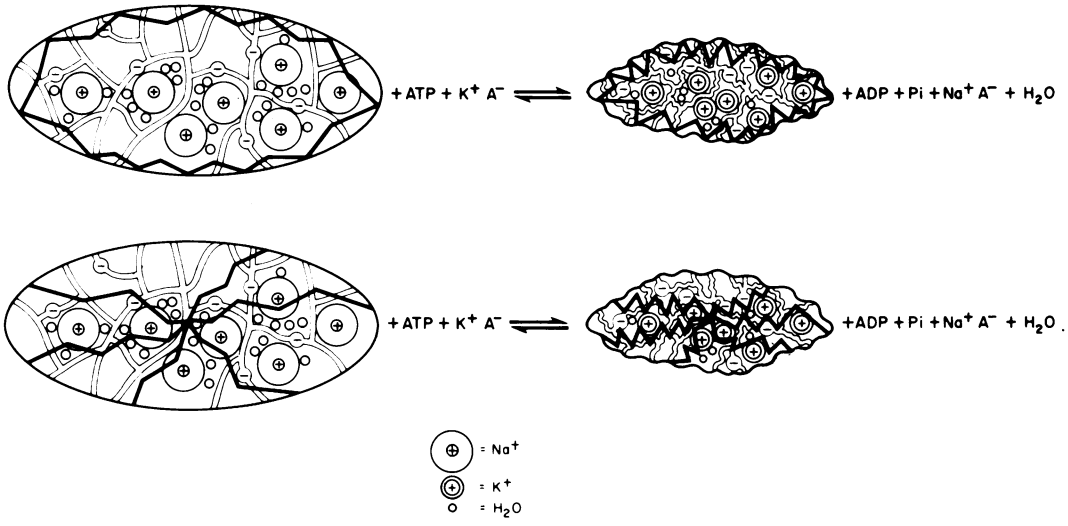

FIG. 11. *Schematic illustration of the regulation of cell hydration and ion selectivity by contractile proteins. The larger hydrated ion Na is replaced by the less hydrated K^+ as cell contractile proteins activated by ATP establish cytotonus and extrude water. Competition for limited space and limited solvent forces the replacement of Na^+ by K^+.*

et al. (5) is suggestive that it may be close to the cell surface. Two possible arrangements among many, whereby the contractile protein could act to regulate selectivity, are as follows.

With either of the two arrangements shown in Fig. 11, selectivity is achieved by a contraction of the intact cell mediated by the constriction of ATP-dependent contractile protein. In both cases, attachment to the cell surface would probably be useful. In the first case, cell contraction is accomplished by a concentric arrangement of contractile proteins (corrugated element in schematic) in close proximity with the cell surface. In the second case, the contractile element has a transcytoplasmic arrangement to effect cellular contraction. In either case, the contraction brought about by the hydrolysis of ATP expels water from the cell and creates the required limited water state for ion selectivity and other cell functions. The reduced cell water content imposes a demand for ions of least hydration among the counterions that would neutralize cell anions. An exchange of the cell Na^+ for the less hydrated K^+ ion is forced, with consequent enhancement of biological alkali cation selectivity. Cellular cytotonus maintained analogous to the more macroscopic tonus of mature muscular tissues would serve the purpose of maintaining the limited water state and keeping alkali cation electivity maximal. Perhaps even periodic rhythmic contractions would allow for freshening of the chemistry of the cellular interior by periodic rinsing of the cell with fresh solvent. We have considered the possibility that proteins other than the one we have isolated might cooperate in the maintenance of cytotonus and that the phenomenon may not be restricted to the traditional contractile proteins. Ion selectivity is therefore achieved through the regulation of cell water content by the molecular mechanics of a cell in a limited water state, namely, the variation is caused by changes in cell hydration or the competition of ions for limited solvent in the elastic spring model of Gregor.

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