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COMMUNICATIONS

A Binding Protein for L-Glutamine and Its Relation to Active Transport in *E. coli*[1]

In recent years a number of amino acid-binding proteins released by osmotic shock (1) have been identified (2). Evidence has accumulated to implicate these proteins in the active transport of amino acids. In this paper we report that osmotic shock causes a 90% decrease in the initial rate of L-glutamine transport and this is associated with the release of a glutamine-binding protein. The protein has been purified to homogeneity by several criteria, and evidence for its involvement in active transport is presented.

Binding was measured by equilibrium dialysis in Plexiglas cells containing two wells separated by dialysis tubing, as described previously (3). Side A contained protein and Side B contained 2×10^{-7} M glutamine (subsaturating) or 2×10^{-6} M glutamine (saturating). Both sides were made up to 0.1 ml in 0.05 M NaCl and 0.01 M phosphate buffer, pH 7.0. Subsaturating levels were used for assay of column fractions while for more quantitative studies a saturating concentration was used. Transport was measured as follows (all operations at 23°). Cells harvested in midexponential growth phase were twice washed with minimal medium (5) and incubated for 5 min in minimal medium supplemented with 10 mM glucose and 80 μg/ml of chloramphenicol. A fraction of the suspension was added to the reaction mixture which contained 10 mM glucose, 80 μg/ml of chloramphenicol, 3×10^{-6} M ^{14}C-labeled L-glutamine, and minimal medium to a volume of 0.5 ml. After 15 and 30 sec, samples were filtered on Millipore filters (0.45-μ pore size), washed with 10 ml of 0.01 M Tris–HCl, pH 7.3, containing 0.15 M NaCl and 0.5 mM $MgCl_2$, and counted as described previously (3). The number of cells was adjusted so that less than 10% of the radioactive substrate was removed. Points taken at 7, 15, 22, 30, and 60 seconds showed an uptake of L-glutamine that was linear with time.

E. coli Strain 7 (derived from K10, obtained from Dr. E. C. C. Lin (4)) were grown to stationary phase in minimal medium (5) supplemented with 1% succinic acid (Baker and Adamson). The cells were harvested and osmotically shocked as described previously (3).

Radioactive glutamine, obtained from New England Nuclear, had a specific activity of 212 mCi/mmole and was diluted to one-tenth this specific activity for most work. Protein was determined by a modification of the method of Lowry *et al.* (6) with bovine serum albumin as standard.

Shock fluid was concentrated to about 6 mg protein per ml in an Amicon Model 401 ultrafiltration cell fitted with a UM-10 filter, and passed through a Bio-Gel P10 column, equilibrated with deionized water. The protein peak (1700 mg) was applied to a 2.5 × 54-cm column of DEAE-cellulose (Brown and Co.) which had been prepared by the usual washing procedure (7) followed by equilibration with 5 mM Tris–HCl buffer, pH 7.6. The glutamine-binding protein did not adsorb and appeared in the flow-through fraction. The glutamine-binding fraction was electrofocused, using a pH gradient of 7–9 (8) (Fig. 1). The peak fractions from the electrofocusing step were dialyzed against 0.1 M NaCl and then against deionized water, in order primarily to remove ampholytes.

Overall yield and fold purification are difficult to assess because binding cannot be measured in crude shock fluid, due to an interfering glutaminase. In the DEAE-cellulose step 90 mg of protein were obtained that bound 19 mμmoles glutamine per mg. The electrofocusing step gave, in 44% yield after dialysis, material that bound 30 mμmoles glutamine per mg protein.

The protein from the electrofocusing column was felt to be pure because, within experimental limits, constant specific activity was observed over the peak, and the protein migrated as a single band in polyacrylamide disc-gel electrophoresis at pH 10.1 and 4.2. At pH 10.1 and at 4° a modified Tris–glycine system (9) was used. A β-alanine buffer system (10) was used at pH 4.3. As further evidence of homogeneity, the protein migrated as a single band in gels that varied from 5 to 15% acrylamide.

The Sephadex gel-filtration method of Andrews (11) with appropriate standards indicated a molec-

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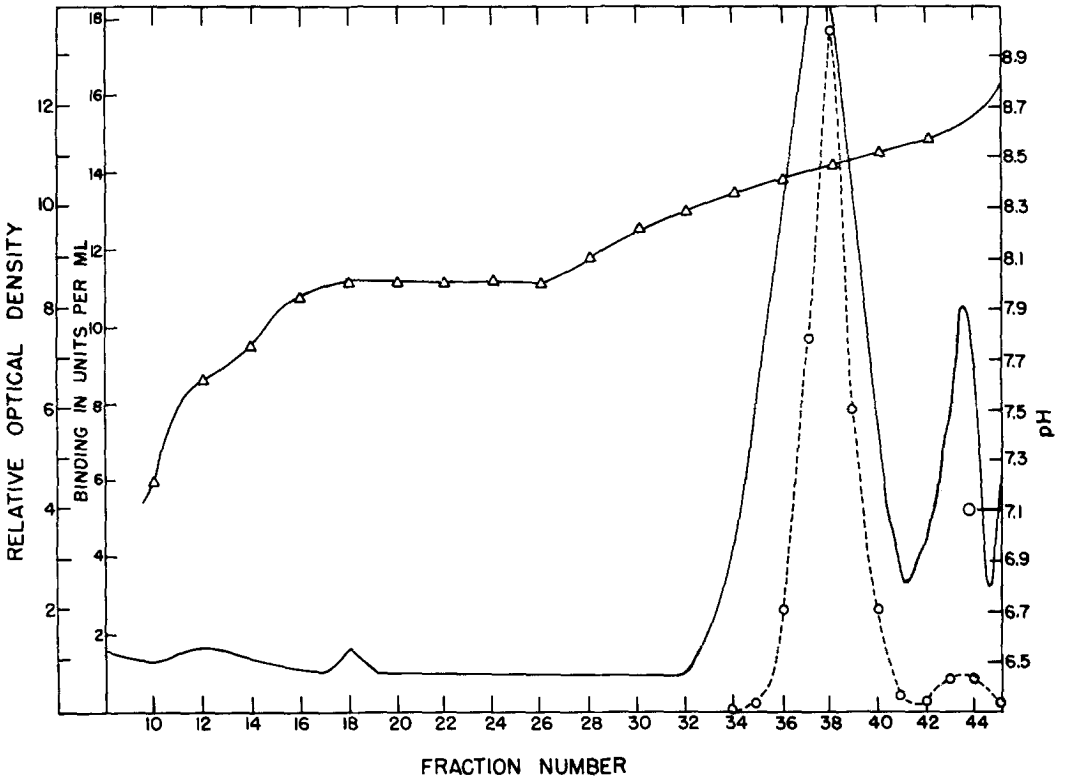

FIG. 1. Isoelectric focusing pattern of the glutamine-binding protein in a pH gradient of 6 to 9. Solid line, relative optical density at 280 mμ. Triangles, pH gradient. Circles, glutamine-binding activity in mμmoles bound per ml.

TABLE I

COMPARISON OF *E. coli* STRAIN 7 AND THE GLUTAMINE TRANSPORT MUTANT WITH RESPECT TO BINDING PROTEINS RELEASED BY OSMOTIC SHOCK[a]

Strain	Leucine binding		Glutamine binding	
	Specific activity (units/mg)	Total units	Specific activity (units/mg)	Total units
Parent	0.92	23	6.7	15
Mutant	0.66	16	16.7	50

[a] Osmotic shock was carried out on 6.5 g (wet weight) of cells, corresponding to 780 mg protein. This caused the release of 25 mg protein in the shock fluid, both for the parent (Strain 7) and the glutamine-transport mutant. Binding of glutamine was measured after DEAE-cellulose chromatography to remove glutaminase. Specific activity values are low because the Bio-Gel P-10 step was not used, and this separates certain inert proteins. Binding of leucine was measured in shock fluid. Two other runs gave similar results for glutamine and showed equal binding of leucine for the two strains. Specific activity is expressed as mμmoles bound per mg protein. One unit is 1 mμmole substrate bound.

ular weight of 29,000. Equilibrium sedimentation in a Spinco Model E ultracentrifuge gave a molecular weight of 23,000, assuming a value of $\bar{v}$ of 0.73. The binding of ^{14}C-glutamine was specific; it was not inhibited by L-asparagine nor by any natural amino acid. The binding was freely reversible with a dissociation constant of 1.5×10^{-7} M. The bound glutamine was not altered in the binding reaction, as judged by its chromatographic behavior. The extent of binding was not altered by variation in ionic strength or by changes in pH from 3 to 9. The binding protein was stable to heating at 100° for 15 min. It also maintained its activity after storage for 6 months at 3° or −90°.

The properties of the binding protein can be correlated with the behavior of the L-glutamine transport system of *E. coli* Strain 7. The protein specifically binds glutamine very tightly and the transport system is also specific and shows a high affinity, K_m being 0.5×10^{-7} M. Osmotic

shock results in a 99% decrease in the initial rate of transport, from 7 to 0.07 mμmoles per mg protein per minute. (In six experiments, the value for shock cells varied from 0.06 to 0.16). This is correlated with the appearance of glutamine-binding protein in the osmotic shock fluid. As a control, no glycine-binding protein was found, and glycine transport is not reduced by osmotic shock. We have recently isolated a mutant of Strain 7, using *N*-methyl-*N'*-nitro-*N*-nitroso-guanidine as mutagen, which is able to grow on glutamine as sole carbon source. This mutant showed a 4-fold higher initial rate of transport with no change in K_m, and released 3.3-fold more binding protein by osmotic shock (Table I). The experiment was carried out three times with similar results and each time the binding protein from both Strain 7 and the mutant was fractionated under standardized conditions. This tends to rule out the involvement of inhibitors or activators.

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