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*CELL-FREE ACTIVITY OF A SULFATE BINDING SITE INVOLVED IN ACTIVE TRANSPORT**

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Communicated by Colin S. Pittendrigh, November 17, 1965

Salmonella typhimurium possesses a repressible system for active transport of sulfate.[1] Certain mutants are unable to grow on sulfate or thiosulfate, owing to a defect in this transport system. These mutants retain a repressible ability to bind firmly a small amount of sulfate,[2] which appears to be involved in the active transport process.[2, 3] The firmness of binding, the apparent absence of an energy requirement, and the simplicity of the chemistry of sulfate suggested that this binding might be observed in cell-free preparations.[2] In this communication a method of measuring binding in cell-free systems is described. Binding of sulfate in such a system is reported.

Methods and Materials.—The mutant strain of *S. typhimurium* used in these studies, Cys CD-519, is a deletion mutant which possesses an active transport mechanism, but lacks the first two enzymes in the sulfate reduction pathway.[4]

The bacteria were grown on E medium plus 2 gm/liter glucose. Repressed and derepressed bacteria were grown with 2×10^{-4} *M* L-cysteine or 1.5×10^{-4} *M* djenkolic acid as sulfur sources, respectively.[1] Techniques of growth, sources of chemicals, counting methods, etc., were described in previous communications.[1-3]

Results.—Binding activity was measured by ability of preparations to release sulfate into solution from Dowex 1-×8. The resin (0.34 gm dry weight) in 1 ml H_2O was mixed with 0.05 ml of 4×10^{-5} *M* sulfate containing about 4.5×10^5 cpm S^{35}, and was incubated at 37° for 10 min to achieve equilibrium. Then 0.4 ml of the cell preparation in E medium salts + glucose + 30 μg/ml chloramphenicol

was added, incubated for 5 or 10 min, mixed again, and resin was allowed to settle for 5 min. Then 0.08 ml of the supernatant fluid was counted for 5 min in a Packard liquid scintillation spectrometer. The background of 15–20 cpm was subtracted from all counts.

The medium contained 3,300 cpm/ml, about 1 per cent of the quantity bound to the resin. When repressed bacteria were added, no further sulfate was brought into solution (Table 1). (Quenching caused an apparent loss.) Derepressed bacteria brought about three times more counts into solution, depending on the number of cells added. These results were highly reproducible. Centrifugation of cells from the reaction mixture caused the extra counts to disappear, showing that they were combined with the bacteria.

TABLE 1

BINDING BY INTACT CELLS AND CELL FRACTIONS

Treatment	Change in Cpm/Ml	
	Repressed	Derepressed
Intact cells	−900	12,000
Intact cells (cent. after resin)	−500	−400
Broken cells	400	3,400
Broken (cent. after resin)	−500	3,500
Extract	1,000	3,700
Extract (2 min incubation)	1,400	3,800
Supernatant, 1 hr cent. 100,000 × *g*	900	2,100
Precipitate, 1 hr cent. 100,000 × *g*	−500	600
Extract, 5 min at 100°	300	600
Extract, $BaCl_2$ added to supt.*	50	40
Extract, resin added to supt.*	200	800

Overnight cultures of bacteria were concentrated approximately 100 × in E salts (protein = 45 mg/ml). Portions were disrupted at 0° with a Branson sonifier for 0.5–1.0 min at setting 7, to provide clarified broken cell preparations. These were centrifuged at 10,000 × *g* for 10 min to prepare "extracts." Parts of these extracts were treated and assayed for binding ability as described in the text. The numbers in the table are cpm/ml minus 3,300 cpm/ml found in the supernatant when only E salts were added to the resin and sulfate.

* Cpm/ml minus background (20 cpm).

Binding ability of cell fractions was next tested. Sonically broken, repressed cells did not release sulfate from the resin, but broken, derepressed cells were about one third as active as intact cells. Centrifugation of these preparations (which should remove any remaining intact cells), either before or after mixing with resin, did not decrease this activity. The activity of these extracts is attributed to binding, as opposed to both binding and transport by intact cells.

Similar experiments were performed with transport-negative mutants.[3] These cells and their extracts both were similar in activity to the extracts of CD-519. Wild-type *S. typhimurium* LT-2 also had a repressible binding activity.

Preliminary experiments indicated that the active fraction was not readily sedimented in a Spinco angle-head rotor at 100,000 × *g* in 1 hr, but was partly sedimented at 150,000 × *g* in 5 hr. It passed through G 25 Sephadex with the protein peak. It was labile to 100° for 5 min, but was stable to toluene or storage for 1 day at 0°. These data suggest that activity is associated with a macromolecular entity.

The ability of extracts to release sulfate from the resin could be attributed to binding or to metabolic conversion of sulfate into forms which no longer have a strong affinity for the resin. Several tests were made to distinguish these alternatives. Mutant CD-519 is incapable of activating sulfate and possesses no known pathway of sulfate metabolism. After 2 or 10 min incubation, similar counts were

released from the resin, arguing against gradual metabolism of sulfate. Properties of the radioactivity in the supernatant appeared to be the same as those of sulfate. A portion of the supernatant was treated with $BaCl_2$ plus carrier Na_2SO_4 to precipitate sulfate, and centrifuged. Virtually all counts were precipitated. Other 0.2-ml aliquots were mixed with 0.3-ml batches of fresh resin. The radioactivity was adsorbed to the resin; binding was shown to be reversible, and metabolism of sulfate to a nonbinding product was eliminated by this experiment.

Discussion and Summary.—A method of measuring binding to subcellular entities is described. It was applied to binding of sulfate to cells and cell-free extracts of *S. typhimurium*. Binding by extracts of derepressed cells but not by extracts of repressed cells was observed. Chemical changes of sulfate into nonbinding compounds does not seem to be involved.

This binding is thought to be a step in active transport of sulfate.[2, 3] To the best of our knowledge, this communication is the first report of activity of such a binding site in extracts. Data on preliminary separation of a radioactively labeled and presumably inactive binding site for β-galactoside transport have been presented recently.[5] These results open the avenue to characterization of such binding material and a study of active transport at the subcellular level.

Note added in proof: Binding activity was released into solution by osmotically shocked bacteria. It could be purified by DEAE and hydroxylapatite so as to move as a single band on gel electrophoresis. Both the number of binding sites per mg protein and behavior of the material on Sephadex columns suggest that the binding activity is associated with a protein of molecular weight about 70,000.

* This work was supported by U.S. Public Health Service grant AI-04409.

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*HYDRODYNAMIC CHANGES ACCOMPANYING THE THERMAL DENATURATION OF TRANSFER RIBONUCLEIC ACID**

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Communicated by Walter Kauzmann, November 15, 1965

The secondary structure of sRNA,[1] as well as other types of RNA, has been interpreted as resulting from the folding back on itself of a single polynucleotide chain at one or several points so as to bring together shorter or longer nucleotide sequences that have the capacity to form helical arrays of Watson-Crick-type complementary base pairs.[2, 3] It has been postulated, moreover, that noncomplementary bases may occur in these sequences as imperfections looped out of the helical regions, and they also serve to separate helical regions.[3, 4] While these proposals define secondary structure and suggest possibilities for tertiary structure,