EVIDENCE FOR A SULFATE TRANSPORT SYSTEM IN *ESCHERICHIA COLI* K-12

S.E. SPRINGER and R.E. HUBER
Department of Chemistry, University of Calgary, Calgary, Alberta, T2N IN4, Canada

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1. Introduction

Although the sulfate transport system of *Salmonella typhimurium* has been well studied [1–5] very little work has been done on the sulfate transport system of *Escherichia coli.* Pardee [6] has suggested that there is a barrier which prevents sulfate from diffusing into the *E. coli* cell and an indirect form of evidence has been provided by Ellis [7]. He found that although the synthesis of the enzymes that reduce inorganic sulfate to sulfide in *E. coli* is under repressive control by the end product of sulfate reduction, L-cysteine, this repressive control is not nearly rapid enough to explain the sudden cessation of uptake of sulfate in the presence of L-cysteine. He has theorized that L-cysteine may be a specific allosteric inhibitor of the sulfate uptake system.

The results of the study reported in this paper suggest that there is an active uptake mechanism in *E. coli* for sulfate.

2. Materials and methods

2.1. *Culture conditions*

E. coli K-12 was grown and maintained in Davis minimal salt media [8] (sulfate salts were replaced by chloride salts and 5×10^{-3} M Na_2SO_4 was added separately) at 28° and constant shaking. Lactose (0.8%) was the energy source.

2.2. *Measurement of the initial velocity of sulfate uptake*

Cells were harvested during exponential growth and washed once with minimal medium without sulfate. The cells were then taken up in the minimal media lacking sulfate and incubated at 28° for 2 hr to deplete the cells of intracellular sulfate.

Initial rates of sulfate uptake were measured at several different concentrations of sulfate with no selenate present. Solutions contained from 1.5×10^{-5} M to 6.0×10^{-4} M [^{35}S]sulfate in 20 ml of minimal media (specific activities of the [^{35}S]sulfate were 10 μCi/375 μmoles for the saturation studies and 100 μCi/375 μmoles for the other studies). For the selenate inhibition study the solution contained, in addition, 5×10^{-3} M selenate. Radioactive carrier free [^{35}S]sulfate 50 μl), 1 ml of cold sulfate, and 1 ml of selenate (in the inhibition study) were added at zero time to 18 or 19 ml of the sulfur-depleted bacteria (2.5 mg dry weight) to obtain a final volume of 20 ml with the above concentrations of sulfate and selenate. Cultures were swirled vigorously and 1 ml samples were withdrawn for filtration with a Millipore Suction Filtration Apparatus (25 mm diameter filters, Millipore (Canada) Ltd., Montreal, 0.8 μ pore size). The filters were first washed with 10 ml of 4° non-radioactive media and the sample was then applied. When the sample had drained into the filter it was quickly washed with 100 ml of 4°, non-radioactive media. (The concentrations of non-radioactive sulfate in the wash solution corresponded to the concentrations of sulfate in the experimental cultures.) Four samples were taken within the first 60 sec and the times of sampling were recorded. Initial velocity of sulfate uptake was determined by a plot of counts *vs.* time.

The initial velocities at various temperatures were studied in the same manner and the effect of energy depletion was carried out after incubating the cells for 2 hr in medium which had neither lactose nor sulfate.

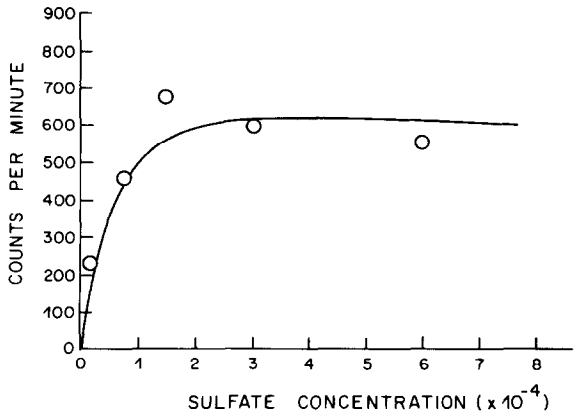

Fig. 1. Plot of cpm of [^{35}S]sulfate taken up at various initial concentrations of [^{35}S]sulfate (specific activity 10 μCi/375 μmoles).

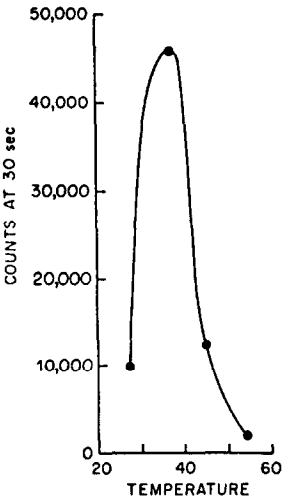

Fig. 2. Plot of cpm of [^{35}S]sulfate taken up at various temperatures (specific activity of [^{35}S]sulfate 100 μCi/375 μmoles).

(For these latter two experiments the sulfate concentration was 1.5×10^{-4} M.)

2.3. *Measurement of the initial velocity of glucose and methionine uptake*

In order to determine whether selenate inhibition was specific towards selenate or perhaps due to some general permeability effects of selenate on the *E. coli* K-12 membrane, the effect of selenate on the initial uptake rates of [U-^{14}C]glucose (1.25 μCi/μmole) and [^{14}C]methionine methyl (1.25 μCi/μmole) was followed. In the case of glucose, the cells were grown in glucose and then were washed and treated as for the sulfate studies except that the cells were depleted of glucose rather than of sulfate. The initial uptake rates of [U-^{14}C]glucose (10^{-4} M) in the presence and absence of 5×10^{-3} M selenate were followed as for the sulfate studies. In the case of methionine the same procedure was used except methionine (10^{-4} M) was substituted for glucose.

3. Results

3.1. *Concentration effects of sulfate on initial velocity*

The initial velocity of sulfate uptake showed the pattern of concentration dependence as shown in fig. 1. At low concentrations of sulfate the rate increased with sulfate and then the rate leveled off. The uptake of sulfate became saturated at about 1.0×10^{-4} M sulfate.

3.2. *Temperature effects*

Fig. 2 shows the effect of temperature on the initial uptake of sulfate by the bacteria. Sulfate uptake increased with temperature from 28–37° and thereafter declined rapidly at 54.5° there was virtually no sulfate uptake. This effect was not reversible.

3.3. *Uptake of sulfate in presence and absence of an energy source*

The deprivation of lactose caused a large reduction in the ability of the bacteria to take up sulfate. Approx. 60% less counts were taken up in the absence of lactose or other energy source (1400 cpm without *vs.* 3400 cpm with lactose).

3.4. *Uptake of sulfate in the presence of selenate*

Table 1 shows the effect of the absence and presence of selenate on sulfate, glucose and methionine transport It is obvious that selenate inhibits sulfate uptake but does not inhibit glucose or methionine uptake.

Table 1
The uptake of sulfate, glucose and methionine in the presence of 5×10^{-3} M selenate.

Control (no selenate) (cpm)	Selenate (5×10^{-3} M) (cpm)
1.5×10^{-4} M $^{35}SO_4$ (100 μCi/375 μmoles)	
5650	490
10^{-4} M [U-^{14}C]glucose (1.25 μCi/μmole)	
1410	1400
10^{-4} M [^{14}C]methionine (methyl) (1.25 μCi/μmole)	
2640	2890

4. Discussion

The fact that the sulfate uptake is saturable, temperature sensitive, energy requiring and specifically inhibited by its structural analog, selenate, is very suggestive that in *E. coli* K-12, at least, sulfate uptake is an active process. The evidence presented correlates very well with the evidence for active uptake of sulfate in other micro-organisms [1, 9] and is the first report of a sulfate transport system in *E. coli.*

Acknowledgement

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