SULFITE METABOLISM IN *E. COLI**

by

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Numerous microorganisms are capable of growth with inorganic sulfate as the sole source of sulfur, but the pathway by which this sulfur is converted to cysteine has, in no case, been clearly elucidated. Several possibilites are apparent, including: (a) reduction to sulfite with subsequent fixation in β-sulfinyl pyruvate followed by transamination and reduction to cysteine; (b) reduction to sulfide followed by reversal of the cysteine desulfhydrase reaction[1,2]; (c) reduction to sulfide, addition to a 2-carbon compound which may be converted to β-mercaptoethylamine followed by CO_2 fixation. The present report describes preliminary studies designed to test the first of these possibilities which is, essentially, reversal of the "desulfinase" series of reactions[3,4].

EXPERIMENTAL

E. coli, N.T.C.C. 6522, were grown on a liquid medium, pH 7.2 containing per liter, 2.0 g glucose, 4.0 g NaCl, 2.0 g anhydrous KH_2PO_4, 2.0 g $(NH_4)_2HPO_4$ and 0.2 g anhydrous $MgSO_4$. When a sulfur-free medium was desired, $MgCl_2$ was substituted for $MgSO_4$. Growth was measured as optical density at 500 mμ.

$^{35}SO_3^=$ was prepared by careful combustion of elemental ^{35}S in oxygen, trapping the $^{35}SO_2$ in *N* NaOH containing 0.01 % versene to prevent autoxidation. Cysteine sulfinic acid (CSA) was synthesized essentially according to LAVINE[5].

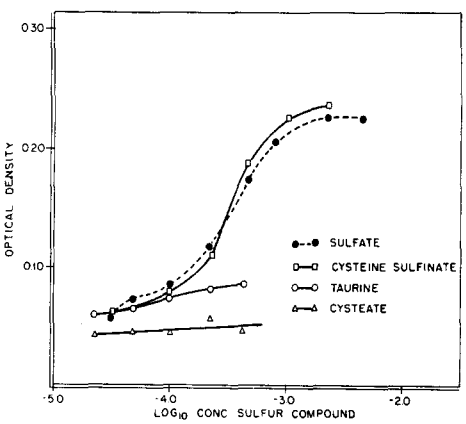

Fig. 1. Availability of organic sulfur compounds for growth of *E. coli* in a sulfur deficient medium.

If the pathway, cysteine → cysteine sulfinate → β-sulfinyl pyruvate → pyruvate + SO_2 → $SO_3^=$, known to be operative in *Proteus vulgaris*[3], is reversible, both cysteine sulfinic acid and sulfite should be capable of replacing sulfate in the medium of an organism which can grow with sulfate as the sole source of sulfur. Fig. 1 shows that cysteine sulfinate was as effective as sulfate for the growth of *E. coli*, whereas taurine

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was considerably less effective and cysteic acid was essentially useless under these conditions. It was not established whether failure of the latter was due to lack of appropriate enzymes or to "impermeability".

Sulfite could not be compared with sulfate for aerobic growth because of autoxidation to sulfate under these conditions. However, anaerobically, sulfite was fully as effective as sulfate; at 0.005 *M*, both supported half-maximal growth. When $^{35}SO_3^{=}$ was included in such incubations, 32% of the radioactivity was found in the trichloracetic acid precipitable material, presumably indicating utilization of the $^{35}SO_3^{=}$ for synthesis of sulfur-containing amino acids.

These experiments demonstrated that both $SO_3^{=}$ and CSA are available to *E. coli* for growth but leave open the questions of whether $SO_3^{=}$ may be fixed to CSA or whether CSA is "desulfinated" and the $SO_3^{=}$ reduced to $S^{=}$ before incorporation into organic linkage. Attempts were made, therefore, to determine whether cell-free extracts of *E. coli* possess "desulfinase" activity and, if so, whether this activity is reversible. Washed cells were disintegrated ultrasonically in water and the material which sedimented at 3 000 r.p.m. discarded. Desulfinase activity was determined by incubating 1 ml of cell-free extract with 25 μ moles of CSA and 0.2 mg versene in a final volume of 2.5 ml of phosphate buffer for 2 hours at 30° under nitrogen. After deproteinization, the filtrate was examined by descending chromatography on paper with water-saturated phenol or *n*-butanol, water, acetic acid (4:1:1) as the solvent and the chromatogram developed with ninhydrin.

When the cell-free extract was dialyzed before incubation, there was no diminution in the intensity of the CSA spot. Addition of α-ketoglutarate (25 μ moles) to the incubation mixture resulted in the appearance of a strong glutamate spot while that due to CSA amost disappeared. If the pH of the reaction mixture was raised to 7.8, the glutamate spot was much less intense, and alanine appeared in its stead, whereas if the reaction was permitted to proceed at pH 5.6 or less, again the yield of glutamate was decreased but an equivalent amount of γ-aminobutyrate appeared. Thus over the pH range 4.5–8.0, CSA + α-ketoglutarate → β-sulfinyl pyruvate + glutamate. In the pH range 4.5 to 5.6 the glutamate was decarboxylated to γ-aminobutyrate[6], whereas from pH 7.2 to 8.0 the glutamate engaged in transamination with pyruvate, arising from the desulfination of β-sulfinyl pyruvate, to yield alanine[3]. The fate of CSA in extracts of *E. coli*, then appears to be identical with that observed in *Proteus*[3]. At no pH was there any indication of decarboxylation of CSA to hypotaurine (aminoethane sulfinic acid).

Since desulfination of β-sulfinyl pyruvate is an appreciably exergonic reaction, simple reversal of this process appeared unlikely. However, the possibility did exist of β-sulfinyl pyruvate synthesis from pyruvate and sulfite by an alternate pathway, in analogy to the malic enzyme[7] and oxalacetate carboxylase[8]. This was tested in the following manner. CSA (25 μ moles) was incubated with 25 μ moles α-ketoglutarate, 25 μ moles $^{35}SO_3^{=}$ containing $2 \cdot 10^6$ counts per minute, 0.01% versene, and 1 ml of cell-free *E. coli* extract in a total volume of 2.5 ml. The incubations were performed under nitrogen for 45 minutes, during which approximately half of the CSA disappeared as determined by paper chromatography. After deproteinization the filtrate was placed on a 1 × 20 cm column of Dowex-50 in the H^+-form and eluted with successively higher concentrations of HCl. CSA came off as a clean fraction in 1*N* HCl, permitting easy separation from glutamate, alanine, sulfite and sulfate. Carrier, non-

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radioactive sulfite and sulfate were added to the CSA which was rechromatographed on Dowex-50, isolated and counted in a gas flow counter.

The CSA obtained in this manner was not significantly radioactive. In separate runs, the reaction mixture was fortified with alanine, glutamate, phosphopyruvate, pyruvate, phosphopyruvate + ADP + hexokinase + glucose, ATP, oxalacetate carboxylase, Mg^{++}, Mn^{++}, K^{++} and various combinations of these. In no case was significant incorporation of ^{35}S into CSA observed.

DISCUSSION

The growth experiments amply demonstrated that sulfite and cysteine sulfinate can be used as efficiently as sulfate for protein synthesis by *E. coli*. Since extracts of this organism efficiently catalyze the series of reactions whereby cysteine sulfinate is converted to sulfite plus pyruvate, it remains entirely possible that the sulfur of CSA is converted to sulfite and reduced to the level of sulfide before it can be utilized for cysteine synthesis, perhaps by reversal of the cysteine desulfhydrase reaction. There is, at present, no evidence for the direct reduction of cysteine sulfinate back to cysteine in any system, nor, it should be remarked, is anything known of the enzymic reduction of sulfite to sulfide.

The attempt at reversal of the desulfinase reactions was designed with the thought that reversal might indicate that this is a pathway for direct sullfur fixation in bacterial and plant cells. In various experiments, ATP and phosphopyruvate were employed as energy donors to overcome the energy barrier to SO_2 fixation, but no reversal was observed. This was interpreted as an indication that direct SO_2 fixaton may not be an important operative metabolic pathway in *E. coli*. FROMAGEOT *et al.* have since observed a small but net fixation of $^{35}SO_3^=$ into CSA by acetone-dried rabbit kidney preparations incubated with $^{35}SO_3^=$, pyruvate and glutamate[9]. The mechanism of this SO_2 fixation and the biological role and distribution of the responsible enzyme system remain to be established.

SUMMARY

E. coli efficiently employ cysteine sulfinic acid and sulfite for growth in an otherwise sulfur-deficient medium, whereas taurine is used poorly and cysteic acid not at all. Sonic extracts of this organism were found to catalyze the desulfinase reactions wherein cysteine sulfinic acid plus α-ketoglutarate are converted to pyruvate, glutamate and SO_2. Attempts to demonstrate overall reversal of these reactions in such extracts, fortified with ATP, or phosphopyruvate, and incubated with CSA and $^{35}SO_3^=$, were unsuccessful.

RÉSUMÉ

E. coli utilise efficacement l'acide cystéine sulfinique et le sulfite pour sa croissance dans un milieu ne renfermant pas d'autre source de soufre; au contraire la taurine n'est utilisée que faiblement et l'acide cystéique pas du tout. Des extraits soniques de cet organisme catalysent les réactions désulfinasiques qui transforment l'acide cystéinesulfinique et l'α-cétoglutarate en pyruvate, glutamate et SO_2. Les auteurs n'ont pas réussi à rendre ces réactions réversibles en ajoutant à de tels extraits de l'ATP ou du phosphopyruvate et en les incubant avec du CSA et du $^{35}SO_3^=$

ZUSAMMENFASSUNG

In einem sonst schwefellosen Medium werden Cysteinsulfinsäure und Sulfit weitgehend, Taurin nur wenig und Cysteinsäure überhaupt nicht für das Wachstum von *E. coli* verbraucht. Es wurde

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festgestellt, dass aus diesem Organismus hergestellte Schallextrakte die Desulfinasereaktionen katalysieren, im Laufe deren Cysteinsulfinsäure und α-Ketoglutarat in Pyruvat, Glutamat und SO_2 umgewandelt werden. Es wurde erfolglos versucht, in solchen Extrakten mit Hilfe von ATP oder Phosphopyruvat, und bei Inkubierung mit CSS und $^{35}SO_3^=$, die vollständige Reversibilität dieser Reaktionen zu beweisen.

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