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CYSTEINE DESULFHYDRASE ACTIVITIES OF *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

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SUMMARY

1 Both wild type and a cysteine-auxotroph (cysE⁻) mutant of *Salmonella typhimurium* LT2, as well as strains B and W of *Escherichia coli*, presented an inducible cysteine desulfhydrase (L-cysteine hydrogen sulfide-lyase (deaminating), EC 4 4 1 1) activity free of catabolite repression The enzymatic activity was rapidly induced in the presence of either glucose or glycerol

2 The cysteine desulfhydrase activity of *S typhimurium* showed a pH optimum of 8 5 and, in intact cells, both cysteine and cystine were used as substrates, the presence of glucose did not enhance the activity Toluene-treated cells lost the ability to desulfhydrate cystine, while the activity towards cysteine was increased In contrast, the cysteine desulfhydrase activity of *E coli* showed a pH optimum of 7 2 and in intact cells the presence of glucose increased the activity Toluene-treated cells lost all activity for the desulfhydration of cysteine

3 The enzymatic activity of *S typhimurium* was not inhibited *in vitro* by NH_4^+ unless they were added along with pyruvate Pyruvate alone was slightly inhibitory, while H_2S had a strong inhibitory effect

4 The cysteine desulfhydrase activity of *S typhimurium* apparently is different from the cystathionase activity as both were induced and repressed independently

INTRODUCTION

During the characterization of a cysteine-requiring mutant of *Salmonella typhimurium*, it was found that its growth in glucose minimal liquid medium was limited by the rapid desulfhydration of the required amino acid, a finding very similar to those reported for cysteine auxotrophs of *Escherichia coli*[1,2] This result and the fact that no information about the cysteine desulfhydrase (L-cysteine hydrogen

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sulfide-lyase (deaminating), EC 4 4 1 1) activity of *S typhimurium* was available prompted us to study some of the characteristics of this activity, as well as some aspects related to its inducibility, and to compare them with those reported for the same enzymatic activity of *E coli*[1–4,13]

MATERIALS AND METHODS

Terminology

The use given in this paper to the term cysteine desulfhydrase activity (L-cysteine hydrogen sulfide-lyase (deaminating), EC 4 4 1 1) refers to the classical pyridoxal phosphate cysteine desulfhydrase yielding H_2S and the unstable α-amino acrylic acid which spontaneously hydrolyzes to NH_3 and pyruvic acid (*cf* ref 4)

Bacterial strains

Salmonella typhimurium LT2 was kindly provided by the late Dr M Demerec Mutant strain SM103-2 was obtained from the LT2 parental strain as a spontaneous cysteine auxotroph resistant to streptomycin (20 μg/ml) *Escherichia coli* strains B and W were kindly provided by Dr Gene M Brown All strains were kept on nutrient glucose agar slants, but for mutant SM103-2 the medium also contained 40 μg/ml of L-cystine

Chemicals

All chemicals used were reagent grade Pyridoxal phosphate and amino acids were products of Sigma Chemical Co , except *O*-acetyl-L-serine that was purchased from Cyclo Chemical Corporation All amino acids used were the L isomers, except DL-lanthionine and DL-cystathionine Bacto-tryptone and Bacto-Casamino acids were products of Difco Laboratories

Culture conditions

The minimal medium used was that of DAVIS AND MINGIOLI[5], citrate omitted The different carbon sources were used at the concentrations indicated in the text Cysteine, cystine, *O*-acetylserine and lanthionine were sterilized by filtration and added to the corresponding media at the final concentrations indicated in the text

Experiments of induction and repression of enzymatic activities

The bacteria were grown overnight (12 h) at 37° in the media and the culture conditions indicated for each experiment Fresh media were inoculated with a sample of the cells grown overnight (approx 5–10 $\pm$ of the final volume) and incubated under the appropriate conditions until the absorbance of the culture at 660 nm reached a value of 0 1 (read in a Colemen Jr spectrophotometer, model GA) At that time the cells were harvested aseptically by centrifugation in the cold, washed twice with sterile minimal medium and resuspended in approx 5 ml of the sterile minimal medium Aliquots of this suspension were used to inocculate the induction media

When the experiment was designed to study the induction of the cysteine desulfhydrase activity in a defined time, the inoculated media were incubated for that time, and at the end of it the cells were harvested by centrifugation in the cold, washed and used as such or for preparation of toluenized cells or cell-free extracts

If the experiment was designed for the study of the kinetics of the induction of the cysteine desulfhydrase activity, the inoculated media were incubated under appropriate conditions and, at fixed times, samples of them (10–20 ml) were withdrawn Chloramphenicol was immediately added to a final concentration of 50 μg/ml, the samples were strongly shaken and placed in an ice bath The cells present in each sample were collected by centrifugation in the cold, washed once with 0 1 M Tris buffer (pH 8 5) resuspended in the same buffer, and the absorbance of the suspension at 660 nm was determined in a Beckman DB spectrophotometer These absorbance values were used for the determination of the units of absorbance volume for calculation of the specific activity of the enzyme preparations

Toluenization of the cells

To each ml of the bacterial suspension (with an absorbance between 2 and 10, read at 660 nm in the Beckman DB spectrophotometer), 0 01 ml of toluene was added The sample was transferred to an erlenmeyer flask of a volume at least 10 times greater than that of the sample, and the mixture was shaken for 30 min at 37° in a rotatory shaker set at 240 rev /min The toluenized preparations were kept for no longer than 8 h in an ice bath until they were used

Preparation of cell-free extracts

The bacterial cells were induced under the different experimental conditions described in the text and were harvested, washed and resuspended in buffer as in the case of those used for toluenization They were disrupted by sonication for 3 min in an MSE ultrasonic power unit set at 1 2 A Intact cells and debris were separated by centrifugation of the sonicate for 30 min, at 4°, at 30 000 × *g* The clear supernatant was dialyzed in the cold (4°) for 12–16 h against 200 times its volume of 0 1 M Tris buffer (pH 8 5) The dialyzed extracts were kept at 4° and used as such

Determination of the cysteine desulfhydrase and cystathionase activities

The cysteine desulfhydrase and cystathionase activities of the LT2 and SM103-2 strains were determined by the colorimetric measurement of the 2,4-dinitrophenylhydrazone of the enzymatically formed keto acid by a slight modification of the method of WIJESUNDERA AND WOODS[6] The incubation mixtures contained, in a final vol of 2 ml 100 μmoles of potassium phosphate buffer (pH 8 5), 10 μmoles of cysteine hydrochloride or 20 μmoles of cystathionine, 10 μmoles of pyridoxal phosphate, and either cell-free extract (0 3 to 0 5 mg of protein) or toluene-treated cells (0 2 to 0 5 mg, dry weight) The samples were incubated 30 min at 37°, and the enzymatic reaction was stopped by the addition of 0 5 ml of 25% (w/v) trichloroacetic acid The controls had the same composition, but the trichloroacetic acid was added at zero time The precipitated protein or cells were separated by centrifugation, and 0 1–0 5-ml aliquots were taken from the clear supernatant These samples were diluted to a final vol of 2 ml with twice distilled water, and then 1 ml of a 0 0125% (w/v) solution of 2,4-dinitrophenylhydrazine in 2 M HCl was added to each sample After a 10-min incubation at 37°, 1 ml of 2 5 M NaOH was added After development of the color for 15 min at room temperature, the absorbance of the samples was read at 450 mμ in a Beckman DB spectrophotometer

The cysteine desulfhydrase activities of the *S typhimurium* LT2 and *E coli* B

and W strains were determined by the measurement of either the keto acid or the H_2S produced The intact or toluene-treated cells or the cell-free extracts were incubated in 13 mm × 100 mm tubes for 3 min at 37° with either 120 μmoles of Tris buffer (pH 8 5), for the *S thyphimurium* preparations) or 100 μmoles of potassium phosphate buffer (pH 7 2, for the *E coli* preparations) Then 1 61 μmoles of cysteine hydrochloride were added, the tubes were hermetically sealed with parafilm-covered rubber stoppers and the samples were incubated for 2 min at 37° The final volume was 1 7 ml When the activity was measured by the production of keto acid, the reaction was stopped by the addition of 0 2 ml of a 25% (w/v) solution of trichloroacetic acid, and the formation and determination of the corresponding phenylhydrazone was carried out as before If the enzymatic activity was determined by the measurement of the H_2S evolved, the reaction was stopped by the addition of 0 1 ml of a 12% (w/v) solution of NaOH, and the product was transformed into methylene blue following the modification of SIEGEL[7] to the method of FOGO AND POPOWSKY[8]

The specific activities are expressed as functions of the mg of dry weight, or mg of protein (determined by the method of LAYNE[9]) or units of absorbance volume (determined at 660 nm) present in the different assays Under the conditions employed, 1 absorbance unit was equivalent to 0 434 mg bacterial dry weight per ml

RESULTS

Characterization of mutant SM103-2

In solid media mutant SM103-2 grew only in the presence of cysteine, cystine, *O*-acetylserine or lanthionine, the growth, however, was rather poor When the mutant was grown in minimal glucose liquid medium supplemented with either cysteine or cystine, the growth stopped after some time unless new amounts of the corresponding amino acid were added periodically At the same time a strong odor of hydrogen sulfide was apparent practically from the beginning of the experiment It appeared as if the mutant limited its growth by destruction of the required amino acid Lanthionine, however, allowed a continuous though slow growth of the mutant (Fig 1)

The fact that the mutant grew only when either cysteine, cystine or *O*-acetylserine were provided, while serine was inactive, indicated its lack of L-serine transacetylase activity This was confirmed to be the case when the activities of L-serine transacetylase and *O*-acetyl-L-serine sulfhydrase were determined[10] in cell-free extracts of both parental and mutant strains In the mutant strain the first activity was missing, while the second was slightly enhanced Mutant SM103-2 was, therefore, classified as a *cysE*⁻ mutant[10]

Some characteristics of the cysteine desulfhydrase reaction of S typhimurium

pH optimum For intact or toluene-treated cells, as well as for cell-free extracts, the pH optimum for the desulfhydrase reaction was found to be around 8 5

Kinetics For any of the three systems mentioned before, the desulfhydration of the amino acid was linear for at least 6 min The activity also showed linearity with respect to enzyme concentration

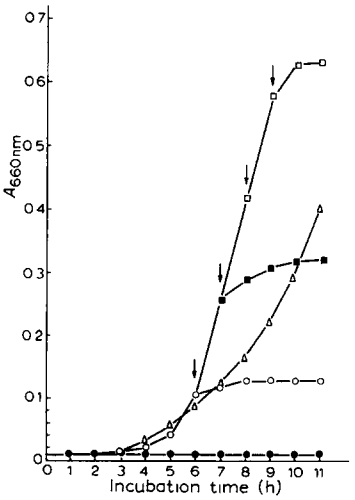
Stoichiometry As shown in Table I, a 1 1 stoichiometry in the production of H_2S and keto acid from the desulfhydration of cysteine could not be demonstrated, twice as much H_2S as keto acid was always determined No lactate dehydrogenase

TABLE I

STOICHIOMETRY OF THE CYSTEINE DESULFHYDRASE ACTIVITY OF CELL-FREE EXTRACTS OF *S typhimurium* LT2

Cells were grown in glucose (0.2%, w/v) liquid medium, aerobically at 37° and induced under these conditions for 2 h in the presence of 40 μg/ml of cystine

Amount of protein (μg)	*Product (μmoles)*		*Ratio H_2S/ keto acid*
	H_2S	*Keto acid*	
0	0.0	0.0	
10	12.5	6.3	1.98
20	19.2	9.5	2.02
30	28.5	13.1	2.17
40	39.5	17.6	2.24
50	47.5	21.7	2.18



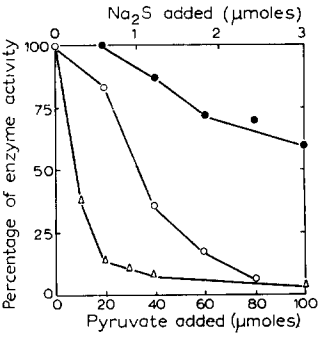


Fig. 1. Growth of mutant SM103-2 in the presence of cystine or lanthionine. Cells were grown overnight aerobically, at 37°, in glucose (0.4%, w/v)–Casaminoacids (0.1%, w/v)–cystine (40 μg/ml). Cells were collected and washed with sterile saline solution. Inoculated media were incubated aerobically at 37°. ●, minimal glucose; ○, minimal glucose *plus* cystine (40 μg/ml); ■ and □, minimal glucose *plus* cystine (40 μg/ml) at the times indicated by the arrows new additions of cystine (40 μg/ml) were made, in the first case only one addition; △, minimal glucose *plus* lanthionine (270 μg/ml).

Fig. 2. Inhibition of cysteine desulfhydrase activity of cell-free extracts of *S typhimurium* LT2 by its reaction products. Cell-free extracts were prepared from cells that had been induced aerobically for 2 h at 37° in glucose (0.4%, w/v)–cystine (40 μg/ml) liquid medium. In the experiments in which pyruvate or pyruvate *plus* NH_4^+ were used, the enzymatic activity was followed by the production of H_2S. In experiments in which Na_2S was used as inhibitor, the activity was measured by the production of keto acid. Each sample contained 25 μg of protein. ●, pyruvate alone, at the indicated concentrations; ○, pyruvate at the indicated concentrations, *plus* 100 μmoles of $(NH_4)_2SO_4$; △, Na_2S at the indicated concentrations.

activity could be demonstrated in the cell-free extracts to account for the lower amount of keto acid found

Inhibition by reaction products Of the three products of the desulfhydration of cysteine (NH_3, pyruvate and H_2S), NH_3, as NH_4^+, did not inhibit the reaction up to a 100 mM concentration The effects of the reaction products upon the activity *in vitro* of the desulfhydrase are presented in Fig 2 The addition of increasing amounts of sodium pyruvate produced increased inhibition, when the concentration of the inhibitor was approx 60 times higher than that of the substrate (100 *vs* 1 6 μmoles) there was a 40% inhibition The simultaneous addition of NH_4^+ and pyruvate had, however, a strong inhibitory effect It should be pointed out that this strong inhibition was apparent only if the inhibitors were added to the incubation mixture before the substrate, if they were added after the cysteine there was practically no inhibition

S^{2-}, on the other hand, had a very marked inhibitory effect At a concentration equal to that of the substrate (1 6 μmoles), the inhibition was practically complete If the inhibitor was eliminated by bubbling nitrogen through the incubation mixture, practically all the activity was restored, indicating that the inhibition by S^{2-} was not due to enzyme denaturation

Serine and alanine, at a 60 mM concentration, caused 80% and 50% inhibition of the activity, respectively, while threonine, methionine and *S*-ethylcysteine at the same concentration produced no inhibition

Differences between the desulfhydrase activities of S typhimurium and E coli

When the cysteine desulfhydrase activities of *S typhymurium* LT2 and *E coli* B and W were compared, some differences were found (Table II) Intact cells of *S typhimurium* desulfhydrated both cysteine and cystine Toluene-treated cells lost the ability to attack cystine while the desulfhydration of cysteine was enhanced In the case of *E coli*, all the desulfhydrase activity was lost after the cells were treated with toluene Using intact cells, the addition of glucose to the incubation media did not affect the desulfhydrase activity of *S typhimurium* (with either cysteine or cystine

TABLE II

CYSTEINE DESULFHYDRASE ACTIVITY IN INTACT AND TOLUENE-TREATED CELLS OF *S typhymurium* AND *E coli*

Cells were grown aerobically at 37° in glucose (0 4%, w/v) minimal medium and induced under these conditions for 2 h in the presence of cystine (60 μg/ml) When indicated, 100 μmoles of glucose were added to the incubation mixtures Specific activity is expressed as nmoles of H_2S produced per min per unit of absorbance volume

Strain	*Cells*	*Specific activity in the presence of*			
		Cysteine	*Cysteine plus glucose*	*Cystine*	*Cystine plus glucose*
S typhimurium LT2	Intact	39 1	45 2	13 1	15 2
S typhimurium LT2	Toluenized	142 1	146 1	0 0	0 0
E coli B	Intact	9 3	27 3	—	—
E coli B	Toluenized	0 0	0 0	—	—
E coli W	Intact	2 0	12 8	—	—
E coli W	Toluenized	0 0	0 0	—	—

as substrates) while in the case of the *E coli* strains the activity was markedly increased, as had been found by ANDERSON AND JOHANSSON[4]

The pH optimum for activity was also different, as stated before for the *S typhimurium* activity, it was 8 5 while for that of the *E coli* strains it was 7 2

Another difference found was that in *S typhimurium* the cysteine desulfhydrase and the cystathionase activities appear to be different entities ROWBURY AND WOODS[11], working with *E coli*, found that the two afore-mentioned activities were repressed and derepressed simultaneously From this observation they concluded that probably the same protein was responsible for the two activities When this possibility was studied in the LT2 and SM103-2 strains of *S typhimurium*, it was found that the two activities behaved differently As can be seen from the data presented in Table III, when the cells were grown in the presence of cystine, the cysteine desulfhydrase activity increased 15-fold while the cystathionase activity was not affected, as compared with the activities present in the LT2 strain grown in the absence of the inducer Also, when the cells were grown in the presence of cystine and methionine the level of the cysteine desulfhydrase activity remained stable while that of the cystathionase decreased markedly

Induction and repression of the cysteine desulfhydrase activity of S typhimurium LT2 and E coli B

Fig 3 shows the kinetics of induction of the cysteine desulfhydrase activity in cells of S *typhimurium* LT2 and *E coli* B growing in glucose In both cases the activity can be demonstrated almost immediately after the addition of the inducer

TABLE III

CYSTEINE DESULFHYDRASE AND CYSTATHIONASE ACTIVITIES OF CELL-FREE EXTRACTS OF LT2 AND SM103-2 STRAINS OF *S typhimurium*

Expt A Cells were grown aerobically for 12 h at 37 in the indicated media (glucose (0 4%, w/v) cystine (40 μg/ml)) Specific activities μmoles of keto acid produced per 30 min per mg of protein Expt B Cells were grown aerobically for 6 h at 37 in the indicated media (glucose and cystine concentrations as in Expt A, methionine, 150 μg/ml) When indicated, 150 μg of methionine were added to the incubation mixtures Specific activities given as in Expt A

Expt	*Extract of strain*	*Cells grown in*	*Specific activity*		
			Cysteine desulfhydrase		*Cystathionase**
A	LT2	Glucose	0 10		1 24
	LT2	Glucose–cystine	1 57		1 31
	SM103-2	Glucose–cystine	1 56		1 22
			Cysteine desulfhydrase in the presence of		*Cystathionase**
			Cysteine	*Cysteine plus methionine*	
B	SM103-2	Glucose–cystine	2 49	2 55	0 403
	SM103-2	Glucose–cystine–methionine	2 52	2 55	0 083

* The specific activities are given considering that the L-isomer was the only substrate used in the reaction

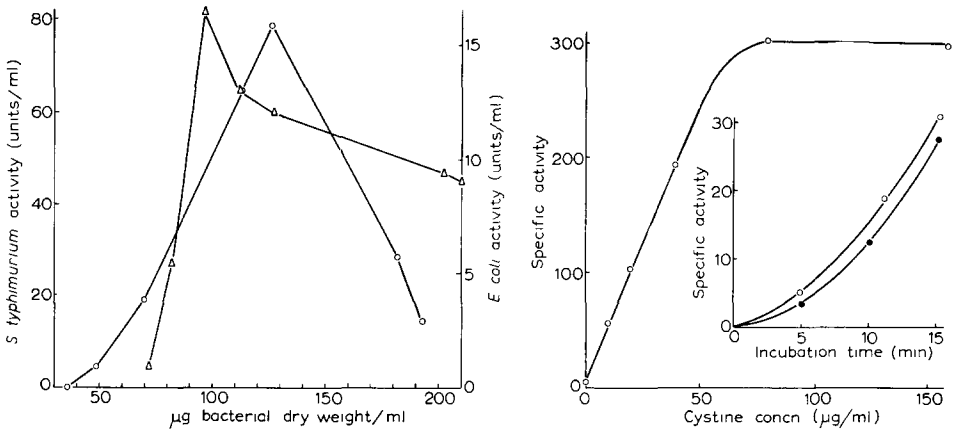


Fig 3 Kinetics of induction of cysteine desulfhydrase activity in *S typhimurium* LT2 and *E coli* B in glucose minimal medium Cells were grown aerobically in glucose (0 2 %, w/v) minimal liquid medium and induced under these conditions in the presence of cystine (40 μg/ml) Enzyme units nmoles of hydrogen sulfide produced per min ○, kinetics of induction of the *S typhimurium* activity, △, kinetics of induction of the *E coli* activity

Fig 4 Effect of inducer concentration in the induction of the cysteine desulfhydrase activity of *S typhimurium* LT2 The experimental conditions were as those stated in Fig 3, except for the indicated amounts of cystine used the time of induction was 2 h Specific activity is expressed as in Table II Inset Kinetics of induction of cysteine desulfhydrase activity of *S typhimurium* LT2 in glucose media with either cysteine or cystine as inducers Experiment was carried out as the one reported in Fig 3, except that the concentrations of inducer were 66 μg/ml for cysteine and 33 μg/ml for cystine ○, cysteine as inducer, ●, cystine as inducer

In *S typhimurium* the activity increases steadily (for about 2 h) until the bacterial mass has approximately tripled and then diminishes rather sharply A similar pattern was found when glycerol was used as carbon source although the activity was approximately one-third of that found in glucose When the cells were induced under anaerobic conditions, using glucose as carbon source, similar results were obtained, but the activity of the desulfhydrase was markedly lower, approximately one-fifth of that obtained in aerobiosis

In the case of *E coli* the increase in the cysteine desulfhydrase activity during induction in glucose-minimal medium was faster than in the case of *S typhimurium*, the maximum activity was reached before the initial bacterial mass increased by one-half (between 30 and 45 min after the addition of the inducer), the activity declined gradually as if it were diluted among the increasing bacterial population When the induction was made in glycerol minimal medium, the kinetics of induction of the activity followed very closely that obtained with glucose

When the induction of the activity in *S typhimurium* was determined after a fixed time of induction (2 h) with variable concentrations of inducer, it was found that the desulfhydrase activity increased linearly with the concentration of the inducer until it reached a plateau A further increase in the inducer concentration did not increase the activity (Fig 4) The same results were obtained either with glucose or glycerol as carbon sources When short periods of induction were used, it was found that the kinetics of induction were the same either with cysteine or cystine (Inset, Fig 4)

TABLE IV

REPRESSION BY TRYPTONE OF THE CYSTEINE DESULFHYDRASE ACTIVITY OF *S typhimurium* LT2

Cells were grown in glycerol (0.2%, w/v) minimal liquid medium and induced in the indicated media (glucose and glycerol, 0.2%, w/v; tryptone, 1%, w/v; cystine, 80 μg/ml), aerobically at 37°. At intervals aliquots were withdrawn and toluene-treated cells were prepared. Specific activities are expressed as in Table II.

Induction medium	*Specific activity at*	
	1 h	*2 h*
Glucose + cystine	237	411
Glucose + tryptone	12	—
Glucose + tryptone + cystine	172	131
Glycerol + cystine	276	—
Glycerol + tryptone	9	—
Glycerol + tryptone + cystine	161	—
Tryptone + cystine	158	122

With tryptone as carbon and energy source, the desulfhydrase activity of *S typhimurium* was repressed, even when the concentration of the inducer was increased. This repression was not affected by the addition of either glucose or glycerol (Table IV). The results (not shown) obtained in studying the induction of the cysteine desulfhydrase activity in tryptone medium for *E coli* B were similar to those obtained by ANDERSON AND JOHANSSON[4] for the *E coli* strain Crookes: the activity was greater in the absence than in the presence of glucose.

Finally, NH_4^+, pyruvate and glucose had a stimulatory effect on the induction of the cysteine desulfhydrase activity of *S typhimurium* up to concentrations of 30, 20 and 40 mM, respectively (Table V).

TABLE V

EFFECT OF VARIABLE CONCENTRATIONS OF GLUCOSE, PYRUVATE AND NH_4^+ ON THE INDUCTION OF CYSTEINE DESULFHYDRASE ACTIVITY OF *S typhimurium* LT2

Expt. A: Cells were grown in glucose (0.2%, w/v) liquid medium, aerobically at 37°. Induction was carried out for 2 h in minimal medium with 100 μg/ml of cystine and the indicated concentrations of glucose. Expt. B: Cells were grown in pyruvate (0.22%, w/v) minimal liquid medium, aerobically at 37°. Induction was carried out for 2 h in minimal medium with 100 μg/ml of cystine and the indicated amounts of pyruvate. Expt. C: Cells were grown as in Expt. A. Induction was carried out for 2 h in minimal medium without NH_4^+ with glucose (0.2%, w/v) and cystine (100 μg/ml), and the indicated amounts of $(NH_4)_2SO_4$. In all cases the preparation of toluene-treated cells and the determination of enzyme activities were carried out as indicated in MATERIALS AND METHODS. Specific activities are given as in the experiment of Table II.

Expt. A		*Expt. B*		*Expt. C*	
Glucose (mM)	*Specific activity*	*Sodium pyruvate (mM)*	*Specific activity*	*$(NH_4)_2SO_4$ (mM)*	*Specific activity*
	46.1		28.8	—	192.0
1	211.7	1	74.0	$7.5 \cdot 10^{-2}$	198.4
5	219.0	5	124.5	$7.5 \cdot 10^{-1}$	198.1
10	235.4	10	139.2	7.5	201.6
20	293.3	20	139.9	15	205.7
40	290.7	40	115.8	75	165.3
60	242.6	60	93.1	150	128.8
100	197.8	100	115.8		

DISCUSSION

The results presented in this paper establish some similarities and differences between the cysteine desulfhydrase activities of *S typhimurium* and *E coli* In both organisms the enzymatic activity is not repressed by glucose and for the one of *S typhimurium* there is also no repression by either pyruvate or NH_4^+, end products of its activity While the induction of the activity in both organisms follows initially the same pattern, there is a clear difference in the permanence of the enzyme after the maximal activity has been reached In the case of the *S typhimurium* activity, there is a sharp decrease of it, probably due to rapid inactivation of the enzyme in the conditions used For that of *E coli* it appears as if the enzyme stops being synthesized and then is diluted along in the increasing population

There is also a difference in the induction and repression of the cysteine desulfhydrase and cystathionase activities While it has been reported[11] that in *E coli* both activities were repressed and derepressed simultaneously, in the *S typhimurium* strains used in the present work both activities were induced and repressed independently

There is also a difference in the stability of the enzyme towards toluene In the case of *E coli* the desulfhydrating activity is totally lost after toluenization of the cells while in *S typhimurium* the activity towards cysteine is enhanced after the treatment However, the toluenized cells of the latter organisms lost the ability to desulfhydrate cystine, indicating that the cystine reductase necessary for the formation of cysteine from cystine is lost by this treatment In intact cells, the presence of glucose increases the desulfhydration of cysteine by *E coli* cells while it has no effect in the activity of the *S typhimurium* cells, this could indicate that the mechanism of entrance of the amino acid into the cells is different in the two organisms

As expected, the cysteine desulfhydrase activity of *S typhimurium* was markedly inhibited *in vitro* by S^{2-} or by a mixture of pyruvate and NH_4^+ During its activity, however, no 1 1 stoichiometry in the production of H_2S and keto acid could be demonstrated, instead an excess of the first compound was always found Although no satisfactory explanation for this anomaly can be given (no disappearance of pyruvate due to remnant lactic dehydrogenase activity could be demonstrated), the possibility remains that a non-enzymatic reaction between the keto acid formed and the cysteine present could have taken place[12], although no test was done to prove it This situation is similar to that found by ANDERSON AND JOHANSSON[4] with the cysteine desulfhydrase of *E coli* in which, depending on the experimental conditions used, there was a greater production of H_2S (with regard to NH_4^+) than the 1 1 stoichiometry expected from the reaction

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REFERENCES

1 J O LAMPEN, R R ROEPKE AND M J JONES, *Arch Biochem*, 13 (1947) 55
2 R B ROBERTS, P H ABELSON, D B COWIE, E T BOLTON AND R J BRITTEN, *Carnegie Inst Wash Publ*, 607 (1955) 390

3 P DESNUELLE AND C FROMAGEOT, *Enzymologia*, 6 (1939) 80
4 D C ANDERSON AND K R JOHANSSON, *J Gen Microbiol*, 30 (1963) 485
5 B D DAVIS AND E S MINGIOLI, *J Bacteriol*, 60 (1950) 17
6 S WIJESUNDERA AND D D WOODS, *J Gen Microbiol*, 29 (1962) 353
7 L M SIEGEL, *Anal Biochem*, 11 (1965) 126
8 J K FOGO AND M POPOWSKY, *Anal Chem*, 21 (1949) 732
9 E LAYNE, in S P COLOWICK AND N O KAPLAN, *Methods in Enzymology*, Vol 3, Academic Press, New York, 1957, p 447
10 N M KREDICH AND G M TOMKINS, *J Biol Chem*, 241 (1966) 4955
11 R ROWBURY AND D D WOODS, *J Gen Microbiol*, 35 (1964) 145
12 A DUGAICZYK, M T MALECKI AND J J EILER, *J Biol Chem*, 243 (1968) 2236
13 R B ROBERTS, P H ABELSON, D B COWIE, E T BOLTON AND R J BRITTEN, *Carnegie Inst Wash Publ*, 607 (1955) 383