Dihydrostreptomycin Inhibition of Cysteine Desulfhydrase in Cell Free Extracts of *Escherichia coli.* (22105)

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The inhibitory effect of streptomycin on hydrogen sulfide production from organic and inorganic sulfur compounds by non-proliferating *Brucellae* and *Enterobacteriaceae* was reported by Olitzki(1,2). It was shown that preincubation of bacteria with streptomycin inactivates enzymes responsible for H_2S formation from various organic and inorganic sulfur compounds. These experiments were carried out using whole bacteria. The present paper examines the action of streptomycin on cysteine desulfhydrase in cell free extracts of *E. coli.*

Materials and methods. The organism used was *E. coli B/r strain.* Cysteine was determined by the method of Binkley and Nakamura(3). Streptomycin was assayed by the method of Sulitzeanu and Olitzki(4). This method is based on growth intensity of a streptomycin dependent strain of *Vibrio* and the inhibition by streptomycin of a sensitive one. Sulfide was determined by the method of Delwiche(5). Preliminary experiments having shown that cysteine desulfhydrase of *E. coli B/r* is an adaptive enzyme, the bacteria were grown on the surface of "Difco" nutrient agar in Roux bottles, to which cysteine at a final concentration of 0.1% was added aseptically. After 18 hours incubation at 37°, the cells were harvested in distilled water, centrifuged, twice washed in phosphate buffer (M/15, pH 7.2), disrupted by sonic vibration in a Raytheon Magnetostriction Oscillator for 15-20 minutes and centrifuged at 10000 rpm for 10 minutes. The supernatant solution was used in all experiments. Protein content of the extracts was determined by the biuret method in a Coleman Jr. spectrophotometer at 540 mμ, using a standard curve prepared for this purpose. Preliminary experiments on dihydrostreptomycin inhibition of H_2S production by cell free extracts of *E. coli* had shown a marked correlation between the degree of inhibition and the time of preincubation of streptomycin with the extracts before the addition of substrate. Preincubation of extracts with streptomycin for 15, 30, 60 and 90 minutes resulted in about 5, 13, 30 and 50% inhibition respectively. Preincubation of extracts with streptomycin for 2 and more hours caused almost complete inhibition of H_2S production. In all experiments reported in this paper the extracts were therefore preincubated at 37° for 2 hours with streptomycin and cysteine was then added. In order to ascertain that the loss of hydrogen sulfide production was not due to enzyme inactivation merely by incubation, in each experiment controls without streptomycin were subjected to the same incubation. All experiments were carried out in small test tubes closed by rubber stopper to prevent loss of hydrogen sulfide. For sulfide determination the reaction was stopped by the addition of sodium hydroxide. For cysteine determination the reaction was stopped by trichloracetic acid. In some experiments the extracts after preincubation with streptomycin were dialysed against distilled water. The dialysis was carried out in a cold room at 5°, with stirring for 18 and 35 hours before the substrate was added, the water being changed every 8 hours.

Results. Effect of streptomycin concentration on H_2S production from cysteine. To determine the minimal concentration of streptomycin required for almost complete inhibition of hydrogen sulfide production, 1 ml of extracts, equivalent to 7 mg protein, was preincubated for 2 hours with amounts of streptomycin ranging from 40 to 0.3 μg. After preincubation 10 μmoles cysteine was added. The pH of the reaction mixture was adjusted by phosphate buffer to 7.2 and sulfide was determined after 2 hours incubation at 37°. Samples without streptomycin were run as controls. The results (Table I)

TABLE I. Effect of Streptomycin Concentration on H_2S Production from Cysteine.

Amt of streptomycin (preincubated μg/7 mg protein)	Sulfide produced (μmoles)	% inhibition
40	.19	92
20	.19	92
10	.31	87
5	.70	71
2.5	1.4	42
1.25	1.58	35
.6	1.72	29
.3	1.82	25
0	2.43	0

Conditions as described in text.

show that the lowest concentration of streptomycin required for more than 90% inhibition of hydrogen sulfide production from cysteine was 20 μg per 7 mg protein.

Effect of cysteine concentration on H_2S production in presence and absence of streptomycin. Increasing amounts of cysteine were added to the extracts with and without preincubation with streptomycin in order to determine whether or not the inhibition of hydrogen sulfide production by streptomycin is of a competitive type. The results are summarized in Table II. Increasing the cysteine concentration until the system was saturated with the substrate caused a corresponding increase in hydrogen sulfide production by extracts not preincubated with streptomycin. The failure to produce hydrogen sulfide by preincubated extracts was not overcome by increase of cysteine concentration, indicating that the inhibition of hydrogen sulfide production from cysteine by streptomycin is not of the competitive type.

TABLE II. Effect of Cysteine Concentration on H_2S Production by Extracts of *E. coli* with and without Streptomycin.

Cysteine (μmoles)	Sulfide produced (μmoles) Without streptomycin	With streptomycin
10	4.10	.30
20	7.18	.30
40	8.12	.30
80	5.93	.26

Each vessel contained: Phosphate buffer (pH 7.2) 100 μmoles, cysteine as indicated, extract equivalent to 7 mg protein, streptomycin (when added) 20 μg. Sulfide was determined after 3 hr incubation at 37°.

To exclude the possibility that the loss of hydrogen sulfide production was due to some interaction between dihydrostreptomycin and cysteine the following experiments were carried out:

1. To extracts with and without streptomycin, cysteine was added and after 3 hours of incubation at 37° aliquots were tested for the presence of cysteine. The results showed that in the presence of streptomycin all added cysteine remained free and could be recovered. The following experiments with dialysed extracts served the same purpose.

2. Extracts without and in the presence of streptomycin (20 μg/7 mg protein) were dialysed as described above. After dialysis, cysteine was added and hydrogen sulfide determined after various periods of incubation. Dialysis for 35 hours caused about 40% loss of activity of cysteine desulfhydrase, in extracts without streptomycin. An almost complete loss of hydrogen sulfide production by preincubated extracts was observed when more than 75% of the original amount of streptomycin was removed by dialysis as shown in Table III. It was observed that after 18 hours of dialysis of an aqueous solution of streptomycin containing 125 μg in a volume of 5 ml, 5.5 μg/1 ml remained and after 35 hours of dialysis no streptomycin was left. When extracts con-

TABLE III. Effect of Dialysis of Extracts of *E. coli* on H_2S Production from Cysteine, with and without Preincubation with Streptomycin.

Previous treatment of extracts: Preincubation with streptomycin	Previous treatment of extracts: Dialysis (hr)	Incubation with cysteine (hr)	Sulfide produced (μmoles)
Not preincubated		1	1.25
" "		2	2.12
Preincubated		1	.12
"		2	.15
Not preincubated	35	1	.63
" "	"	2	1.20
" "	"	3	1.79
Preincubated	"	1	.07
"	"	2	.10
"	"	3	.10

Each vessel contained: Extract equivalent to 7 mg protein, cysteine 10 μmoles, phosphate buffer (pH 7.2) 100 μmoles, streptomycin (when present) 20 μg. Final vol, 3 ml.

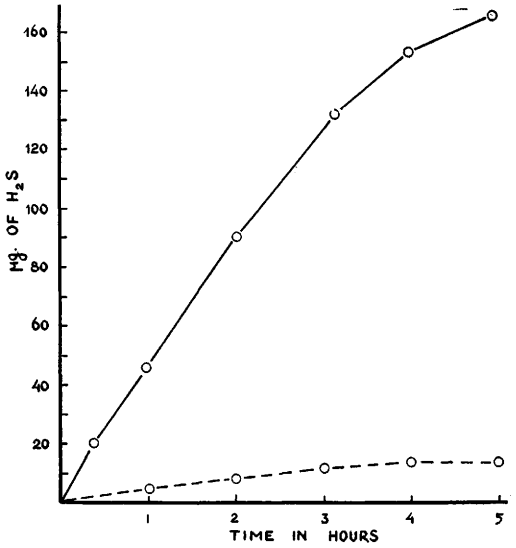

FIG. 1. The kinetics of H_2S production from cysteine by extracts of *E. coli* without and after preincubation with streptomycin. Each vessel contained: Extracts equivalent to 7 mg protein, cysteine 10 μmoles, phosphate buffer (pH 7.2) 100 μmoles. Final vol, 3 ml. Continuous line (———) H_2S production by extracts without streptomycin. Dotted line (- - - -) *Idem*, with streptomycin (20 μg).

taining the same concentration of streptomycin (25 μg/1 ml extract equivalent to 10 mg protein) in the same total volume were dialysed under the same conditions, the amount of streptomycin left was 19.8 and 7.1 μg/1 ml respectively. These experiments showed that in the presence of extracts of *E. coli*, diffusion of streptomycin was retarded. However after 35 hours of dialysis the concentration of streptomycin was too low to give appreciable inhibition of hydrogen sulfide production from cysteine by non dialysed extracts. In spite of this there was almost complete inhibition of hydrogen sulfide production by dialysed extracts, indicating the irreversibility of cysteine desulfhydrase inhibition by streptomycin.

Discussion. It seems evident that low concentrations of streptomycin (20 μg/7 mg protein) caused almost complete and irreversible inactivation of cysteine desulfhydrase of *E. coli*. The inactivation became apparent after 2 hours preincubation of streptomycin with extracts. A shorter preincubation time caused a corresponding smaller effect. Similar observations were made by following authors: Geiger(6) observed that in *E. coli* streptomycin inhibited aspartate oxidation but only after 1-2 hours had elapsed. Umbreit and Tonhazy(7) had shown that streptomycin inhibition of oxygen uptake by rat kidney mitochondria in presence of fumarate and pyruvate was evident only when streptomycin has been in contact with mitochondria for a period of 2 hours in the cold before the substrates were added. Paine and Clark(8) had shown that the inhibitory effect of streptomycin on oxygen uptake by resting cells of *E. coli* became apparent after 1-2 hours incubation.

Summary. 1) The effect of streptomycin on the cysteine desulfhydrase in cell free extracts of *E. coli* is reported. 2) 2.5 μg of streptomycin/1 mg of crude extract protein caused almost complete inhibition of enzyme activity. 3) Inhibition is of a non-competitive type, since it is not reversed by increasing concentrations of substrate. 4) Inhibition is irreversible, since the removal of streptomycin by prolonged dialysis did not cause a restoration of enzyme activity.

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