EVIDENCE FOR CONJUGATES BETWEEN POLYAMINES AND GLUTATHIONE IN E. COLI

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Glutathione and the polyamine spermidine ($NH_2(CH_2)_3NH(CH_2)_4NH_2$) are prominent constituents of the cold-trichloroacetic acid-soluble fraction of E. coli grown in minimal glucose-salts medium (Roberts et al., 1955; Tabor et al., 1958). In the course of work on the metabolism of polyamines in E. coli B, a compound has been recovered which has been tentatively identified as a conjugate between glutathione and spermidine. Cells were grown in minimal medium at neutral pH, harvested, and treated with trichloroacetic acid; the extracts were then chromatographed in a Dowex-50-HCl gradient system, all as previously described (Tabor et al., 1958; Dubin and Rosenthal, submitted for publication). The unknown compound (I) was detected as a DNFB (2,4-dinitrofluorobenzene)-reacting peak eluted just after spermidine (200-250 ml.). It was qualitatively identified by the following: (1) the spectrum of its DNFB derivative was characteristic of a compound containing both primary and secondary amino groups (Dubin, submitted for publication);(2) it gave a positive nitroprusside test only after treatment with cyanide (Toennies and Kolb, 1951);(3) when obtained from cells grown in the presence of

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spermidine-C^{14}[2] it contained radioactivity which moved as a single ninhydrin-positive spot on paper chromatography in solvent I of Table I;(4) after exhaustive hydrolysis (12 hours at 110° C. in 6N HCl in a sealed tube) this spot disappeared, and all the radioactivity traveled with spermidine, the specific activity of which was the same as the free spermidine recovered from the same cells. Three other ninhydrin positive compounds were also released, none of which contained radioactivity. These cochromatographed with cysteic and glutamic acids and glycine in a Dowex-50-HCl system (Tabor et al., 1958), and in four solvents (those in Table I plus n-propanol, 3: concentrated HCl, 1: H_2O, 1, Schleicher and Schuell #598).

Table I

Products of Hydrolysis of Compound I and Glutathione

Compound Hydrolyzed	Compound Released (μmoles)				
	Glycine	Glutamic Acid	Cysteic Acid	Cystine	Spermidine
Compound I	0.23	0.22	0.11	<0.01	0.10
Glutathione	0.20	0.18	<.02	0.08	--

An aliquot of compound I, and 0.1 μmoles of oxidized glutathione were hydrolyzed for six hours in 0.2 ml. of 6N HCl at 110° C. in a sealed tube. Aliquots of hydrolysate were then chromatographed in three solvents: I: n-butanol, 4: glacial acetic acid, 1: H_2O, 2, Schleicher and Schuell #598; II: methanol, 20: H_2O, 5: pyridine 1, Schleicher and Schuell #507; and III: n-propanol, 85: H_2O, 15: diethylamine, 3, Schleicher and Schuell #507. Assays were performed with ninhydrin (Kay et al., 1956) using as standards amino acids and spermidine subjected to the same hydrolysis conditions. (No one or two of these systems separates each of the components from the others; when two compounds ran together, however, the total ninhydrin value was in agreement with the stoichiometry as given.) Values are expressed in terms of the total hydrolysates; those for compound I are normalized to 0.10 μmoles of spermidine.

Paper chromatography was employed to estimate the stoichiometry after less extensive hydrolysis (Table I). If the compound is in fact a conjugate of spermidine and glutathione, the data suggest it contains

[2] Kindly supplied by Drs. E. L. Jackson and S. M. Rosenthal (unpublished synthesis).

one mole of (oxidized) glutathione per mole of spermidine. The low yield of sulfur-containing amino acids, and the presence of cysteic acid rather than cystine is unexplained. Oxidized glutathione treated in parallel fashion yielded approximately equivalent quantities of glutamic acid, glycine, and cystine (Table I). Trace impurities (perhaps metals eluted from the column) may have catalyzed further reactions of cystine (in part oxidation to cysteic acid) during hydrolysis. On the other hand, the nature of the linkage to spermidine may have influenced the course of hydrolysis; although peptide linkage appears most likely, the possibility of an S-N bond exists; it is not known whether a compound with this structure would react with nitroprusside as disulfides do.

Table II

The Levels of Compounds I and II in *E. coli* B as Affected by Polyamine in the Growth Medium

Medium	Compound (μmole/gm. wet weight)	
	I	II
Minimal (experiment 1)	0.5	*
(experiment 2)	0.4	-
Minimal plus spermidine		
6×10^{-6} M	0.6	*
5×10^{-4} M	0.8	*
Minimal plus spermine		
2.5×10^{-4} M	*	0.3

The compounds were quantitated by assaying the polyamine released after hydrolysis of the material recovered from the appropriate Dowex fractions, and assuming one mole of polyamine per mole of unknown. Assays were performed by paper chromatography as described for Table I.

*none detected (<.03 μmole/gm. present).

An analogous compound (II) has been recovered from cells grown in the presence of spermine ($NH_2(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$), a polyamine which can replace the intracellular spermidine (Dubin and Rosenthal, submitted for publication). Compound II was eluted from Dowex-50 after spermine (270-310 ml.), and released spermine rather than spermidine on hydrolysis; in other respects it behaved like compound I in the qualitative identification procedures described. Table II summarizes the effects of growth in the presence of spermidine or spermine on the levels of compounds I and II in the cells.

Further work is in progress on more definite identification of these compounds, and elucidation of their function.

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