EVIDENCE FOR A TWO-STEP ELECTRON FLOW FROM TPNH TO SULFITE AND HYDROXYLAMINE IN EXTRACTS OF SALMONELLA TYPHIMURIUM*

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Recent work by Mager (1960), by Lazzarini and Atkinson (1961), and by Kemp, et al. (1963) has demonstrated that the sulfite reductase of Escherichia coli is capable of catalyzing the oxidation of triphosphopyridine nucleotide (TPNH) in the presence of such varied electron acceptors as sulfite, hydroxylamine and nitrite. No separation of these activities was observed during purifications of 100 to 200 fold, leading to the conclusion that the various reductions were catalyzed by the same protein. Kemp, et al. (1963) noted in addition the presence of a TPNH-cytochrome c reductase activity in all sulfite-reductase preparations. These latter workers also described a mutant of E. coli which lacked the ability to reduce sulfite, but retained a TPNH-cytochrome c reductase activity which was repressed during growth on cysteine.

In contrast to the above studies, Wainwright (1962) has reported the resolution of a sulfite reductase from yeast into six components. Dreyfuss and Monty (1963a), employing cysteine-requiring mutants of Salmonella typhimurium, demonstrated that the synthesis of the TPNH-sulfite reductase in that bacterium was dependent upon the integrity of six discrete cistrons (cys Ba, Bb, Bc, G, I and J), suggesting in a different manner a multi-component nature of sulfite-reductase. The present communication describes a further subdivision of the Salmonella mutants, based on their ability to utilize various electron acceptors for the oxidation of TPNH.

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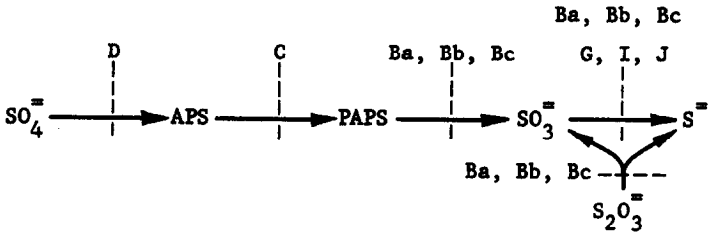
Presented in Scheme I is a summary of the pathway and the metabolic failures for the various classes of mutants as deduced by Dreyfuss and Monty (1963a), and by Leinweber and Monty (1963):

Scheme 1



Not depicted in this scheme are the cys A mutants, which lack a permease for sulfate and thiosulfate (Dreyfuss and Monty, 1963a; Dreyfuss, 1964), and the cys E mutants, which apparently lack the ability to incorporate the sulfide sulfur atom into cysteine (Dreyfuss and Monty, 1963a).

Representative mutants were grown on a minimal medium which contained L-djenkolic acid as the sole sulfur source to obtain a maximal degree of derepression (see Dreyfuss and Monty, 1963b). The cultures were harvested during the logarithmic phase of growth and washed with sulfur-free medium. Extracts were prepared by sonic disruption of the cell suspensions in 0.05 M sodium phosphate buffer, pH 7.7, followed by a thirty minute centrifugation at 30,000 times g. Protein was measured by the biuret method (Layne, 1957) with bovine serum albumin as a reference standard. Enzyme assays were carried out in a 1.0 ml. volume which contained 30 μmoles of pyrophosphate (pH 8.0), 0.2 μmole of TPNH, an appropriate amount of bacterial extract, and one of the following acceptors: 0.5 μmole of sodium bisulfite; 0.23 μmole of flavine adenine dinucleotide (FAD); 0.023 μmole of oxidized horse heart cytochrome c; or 10 μmoles of hydroxylamine hydrochloride. The rate of reduction of cytochrome c was observed at 550 mμ. With each of the other electron acceptors, the rate of TPNH oxidation was measured at 340 mμ, and corrected for any endogenous activity. All data was expressed as millimicromoles of TPNH oxidized per minute per milligram of bacterial protein present, with the assumption that the reduction of one mole of cytochrome c was accompanied by the oxidation of 0.5 mole of TPNH.

The data obtained from the application of these assays to appropriate mutants are presented in Table 1. Three phenotypic patterns are obvious from these data. Mutant extracts which can reduce sulfite also can reduce cytochrome c and hydroxylamine (types cys A, C, D and H), and

Table 1

The Rate of TPNH Oxidation in the Presence of Various Electron Acceptors as Catalyzed by Mutant Extracts

Culture Identity	Rate of TPNH Oxidation in the Presence of			
	Cyt. c	NH_2OH	HSO_3^-	FAD
	mμ moles TPNH/min./mg. protein			
Wild type (LT-2)	174	112	15.4	118
Cys Aabc-20 (deletion)	174	116	16.0	
Cys C-80	184	114	15.1	
Cys D-220	233	143	19.3	
Cys H-363	183	112	16.7	
Cys Ba-25	4.7	2.1	0	3.9
Cys Bb-403	2.2	0	0	0.8
Cys Bc-482	2.3	0	0	3.9
Cys J-266	3.0	1.2	0.3	8.2
Cys G-439	171	0	0	114
Cys I-68	135	0	0	96
Cys HI-36 (deletion)	81	0	0	

the three activities appear in the same ratio observable in extracts of wild-type cells. Mutant extracts which cannot reduce sulfite are subdivisible into two classes, i.e., those which have lost the ability to reduce all of the acceptors (types cys Ba, Bb, Bc and J), and those which have retained the ability to reduce cytochrome c (types cys G and I). The capacity for FAD reduction apparently accompanies the capacity to reduce cytochrome c.

The repressibility of the Salmonella sulfite-reductase was described by Dreyfuss and Monty (1963b). From the data of Table 2 a coordinate repression of the reductions of cytochrome c, hydroxylamine and sulfite is apparent over a wide range of absolute activities obtainable by varying the sulfur-source employed for growing the culture. Limited data not included in the table indicate that the coordinate repression extends to the ability of FAD to serve as an electron acceptor. Furthermore, the cytochrome c-reductase activity of the mutant cys I-68 is repressible in a fashion like that illustrated with the wild-type cultures. It is significant that all of the TPNH-cytochrome c reductase activity found in the 30,000 x g supernatants is repressible during growth on cysteine.

The data of Table 1 indicate that the passage of electrons from TPNH to sulfite or hydroxylamine proceeds in at least two steps, as

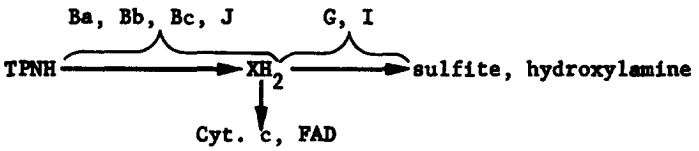
Table 2

Coordinate Repression of the TPNH-Oxidase Activities in Wild-Type Cells Grown on a Variety of Sulfur Sources

Sulfur Source for Growth	Rate of TPNH Oxidation in the Presence of: Cyt. c (A)	NH_2OH (B)	HSO_3^- (C)	Ratio A/B/C
	mμ moles TPNH/min./mg. protein			
Djenkolate	174	112	15.4	11.3/7.3/1
Sulfate	78	50	6.8	11.5/7.3/1
Cysteine Sulfinate	51	32	4.6	11.1/7.0/1
Sulfate + Methionine	30	19	2.7	11.1/7.0/1
Cysteine	3.2	0.1	0	

demonstrated by the flow diagram of Scheme 2. An intermediate carrier, depicted as "XH_2", is reduced by TPNH either directly or indirectly. The existence of "X" and the ability for TPNH to reduce it are dependent upon the integrity of the four cistrons cys Ba, Bb, Bc and J. "XH_2" can

Scheme 2



in turn serve as an electron donor for the reduction of cytochrome c and FAD. Alternatively, "XH_2" can serve indirectly as an electron donor for the reduction of either sulfite or hydroxylamine, this latter function being dependent upon the integrity of the cys G and I cistrons. The depiction of sulfite reduction as a two step process is, of course, the simplest picture which is compatible with the present data. In view of the dependence of each of the two steps upon more than one cistron, a strong possibility exists that both the reduction of "X" and the re-oxidation of "XH_2" by sulfite are themselves multi-step processes.

A unique explanation of the coordinate repression demonstrated by the data of Table 2 is not possible at present. The data might reflect the regulation of the synthesis of all components of the sulfite-reducing

system simultaneously, but could equally well arise from the regulated synthesis of a single key component such as the hypothetical component "X", or something which mediates the reduction of "X" by TPNH.

Finally, the demonstration of a phenotype difference between cys I and cys J mutants completes the demonstration that each of the five cistrons of the cys C region (C, D, H, I and J) is responsible for a separate function, and strengthens the earlier argument (Dreyfuss and Monty, 1963a; Mizobuchi, et al. 1962) that each of these cistrons is a separate structural gene for some component of the cysteine biosynthetic pathway.

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