Metabolism of L-[*sulfane*-^{34}S]Thiocystine by *Escherichia coli*†

Robert H. White

ABSTRACT: The metabolism of L-thiocystine [bis(2-amino-2-carboxyethyl) trisulfide] by *Escherichia coli* was studied by using L-[*sulfane*-^{35}S]thiocystine. This compound was found to serve as a source of sulfur for *E. coli* grown on a defined medium free of other sulfur sources and to incorporate its labeled sulfur into cysteine as well as the other sulfur-containing cellular components. For determination of the extent of the synthesis of new cysteine in these cells, cells were grown with [3,3-$^2H_2$]serine and L-[*sulfane*-^{34}S]thiocystine, and the extent of incorporation of both deuterium and ^{34}S into the cellular cysteine was measured by gas chromatography–mass spectrometry. The results show that approximately 50% of the cysteine which is incorporated into cellular macromolecules is derived from the thiocystine without cleavage of the carbon–sulfur bond, the remaining portion being newly biosynthesized from serine and ^{34}S-enriched H_2S. These results suggest that the first step in the metabolism of thiocystine by *E. coli* involves the β elimination of pyruvate. This type of reaction is characteristic of the cleavage reactions catalyzed by β-cystathionase.

Thiocystine was first isolated from the acid hydrolysates of cystine-rich proteins by Fletcher & Robson (1963). This material has also been isolated along with the trisulfide of glutathione from *Rhodopseudomonas spheroides* (Sandy et al., 1975). Thiocystine offers a unique biological source of sulfur in that it can simultaneously supply organic reduced sulfur, i.e., cysteine, and inorganic sulfane sulfur to a cell. Sulfane sulfur specifically refers to divalent sulfur present in a molecule with the same oxidation state as elemental sulfur (Roy & Trudinger, 1973). In the case of thiocystine, it is the central polysulfide sulfur that is bound only to other sulfur atoms (Figure 1).

The fact that these different sources of sulfur are supplied to cells simultaneously greatly reduces the possible differential utilization of one type of sulfur source over another as would be the case if they were supplied separately, i.e., cystine for the organic sulfur and thiosulfate for the sulfane sulfur (Roberts et al., 1955). It should, therefore, be possible using appropriately labeled thiocystine to specifically label each of these different sulfur pools. In this paper, using thiocystine labeled at the sulfane sulfur with ^{34}S, it will be shown that it is in fact possible to differentially label these two sulfur pools. In addition, data will be presented which show that approximately half of the cysteine incorporated into the cells from the thiocystine is incorporated without cleavage of the carbon–sulfur bond, the remainder being biosynthesized from serine and a H_2S pool greatly enriched in ^{34}S. These experiments open up the possibility of in vivo stable isotope experiments to determine the biosynthetic origins of the sulfur atoms in biotin, lipoic acid, and the thiazole ring of thiamin and the sulfur in iron–sulfur proteins.

Materials and Methods

Materials

Bacterial Strains. Escherichia coli strains were grown on a defined liquid medium at 37 °C as previously described (White & Rudolph, 1978). *E. coli* K12 strain CU 332 (formerly AT 2475) *serA ara* was supplied by Dr. H. E. Umbarger of Purdue University, and *E. coli* B strain WG 1143 *ser A12* was supplied by Dr. W. B. Dempsey, Verterans Administration Hospital, Dallas, TX.

Compound Synthesis. DL-[3,3-$^2H_2$]Serine was prepared by the condensation of formaldehyde (98 atom % $^2H_2$) with diethyl acetamidomalonate followed by acid hydrolysis of the resulting product as described by King (1947). L-[*sulfane*-^{34}S]Thiocystine was prepared by Eddie DeMoll, University of Texas at Austin, from L-cysteine and elemental sulfur as described by Fletcher & Robson (1963). The sulfur used in the synthesis was supplied by Monsanto Research Corp. and had the following isotopic abundances: ^{32}S, 5.44 atom %; ^{33}S, 2.02 atom %; ^{34}S, 90.83 atom %; ^{35}S, 1.71 atom %.

Characterization of L-[*sulfane*-^{34}S]*Thiocystine.* Cold cyanolysis of the labeled thiocystine by the method of Fletcher & Robson (1963) showed 0.85 mol of labile S per 274 g of the L-[*sulfane*-^{34}S]thiocystine. Reduction of a small amount of the sample with $NaBH_4$ in 0.5 M $NaHCO_3$ led to the isolation of cysteine which was characterized by gas chromatography–mass spectrometry (GC–MS) of its *S*-methyl-*N*-trifluoroacetyl *n*-butyl ester derivative (White, 1981). Analysis of the *m/e* 61 ion in the mass spectra of this material showed no excess ^{34}S over the natural abundance, indicating that the enriched ^{34}S was contained only in the sulfane sulfur. Decomposition of a sample of the material by dilute cyanide at pH 7.4 as described by Abdolrasulnia & Wood (1980) led to the precipitation of elemental sulfur. The resulting sulfur was reduced to H_2S by reduction with $NaBH_4$ in ethanol and converted into dibenzyl sulfide by reaction with benzyl chloride. GC–MS analysis of this material showed it to have a $^{34}S/(^{32}S + {}^{34}S)$ ratio of 0.8. The reduction in this ratio from the expected value of 0.943 calculated from the known isotopic abundances of the sulfur used in the synthesis of the thiocystine can be explained by the possible incorporation of a small amount of the cysteine into the precipitated sulfur during the cyanide decomposition. Attempts to obtain mass spectral data on the intact thiocystine by using a number of different derivatives were unsuccessful.

Methods

Measurement of Isotope Distribution. Analyses of the isotopic distributions of ^2H and/or ^{34}S in the bound serine, cysteine, and methionine were performed by GC–MS of the *N*-trifluoroacetyl *n*-butyl ester derivative of methionine, the *N,O*-trifluoroacetyl *n*-butyl ester derivative of serine, and the *S*-methyl-*N*-trifluoroacetyl *n*-butyl ester derivative of cysteine.

† From the Department of Biochemistry and Nutrition, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061. *Received September 21, 1981; revised manuscript received May 10, 1982.*

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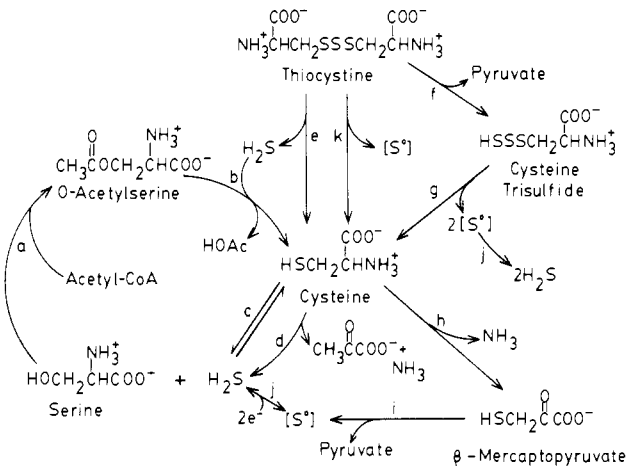

FIGURE 1: Possible routes for the metabolism of thiocystine in *E. coli*: (a) serine transacetylase (Kredich & Tomkins, 1966); (b and c) cysteine synthase [*O*-acetyl-L-serine acetate-lyase (adding H_2S)] (Brueggeman et al., 1962; Wiebers & Garner, 1967); (d) cysteine desulfhydrase (Fromageot, 1951; Ohkishi et al., 1981); (e) enzymatic or nonenzymatic reductive cleavage by intracellular thiols, i.e., glutathione (Flavin, 1962); (f) β-cystathionase (Flavin & Slaughter, 1964; Delavier-Klutchko & Flavin, 1965b); (g) decomposition into sulfane sulfur and cysteine (Flavin, 1962); (h) transamination (Rudman & Meister, 1953); (i) mercaptopyruvate sulfurtransferase (Meister et al., 1954; Vachek & Wood, 1972); (j) reduction of sulfane sulfur [S^0] to sulfide catalyzed by sulfane reductase. The reaction could proceed with or without the involvement of rhodanese (Westley, 1981).

The preparation of these derivatives from the bound amino acids released by the 6 N acid hydrolysis of dialyzed cells previously alkylated with methyl iodide has been described by White (1981). The label in these bound amino acids is expected to reflect the average isotopic distribution of the free amino acids which were present in the cells over the entire growth of the bacteria. This is a valid conclusion for bacterial cells due to the lack of compartmentalization in the cells and the lack of protein turnover in growing cells (Hogness et al., 1955; Koch & Levy, 1955; Rotman & Spiegelman, 1954).

The observed isotopic distributions or abundances in the derivatives were calculated from the experimentally observed isotopic ion intensities which were corrected for the natural abundances of ^{13}C, ^{18}O, and ^{15}N by subtracting the experimentally observed isotopic ion intensities which were determined from unenriched samples. These ion intensity measurements can be taken from any ion in the mass spectra of these derivatives which contains all of the isotopically labeled atoms of interest. Due to interferences from other ions in the GC–MS runs, many of these ions were not suitable for accurate intensity measurements. Accurate measurements of the isotopic abundances could, however, be obtained from the m/z 61 ($CH_3SCH_2^+$) ion for the cysteine and methionine derivatives (White, 1981) and from the $M^+ - C_4H_7$ ion at m/z 298 for the serine derivative (Leimer et al., 1977). All isotopic abundances reported in this paper were obtained from isotopic intensity measurements of these ions.

Mathematical Description of the Cysteine Isotopic Cluster. The intensities of the observed isotopic cluster generated by the m/z 61 ion in the mass spectrum of the cysteine derivative from cells grown with [3,3-$^2H_2$]serine and L-[*sulfane*-^{34}S]-thiocystine can be viewed as the superposition of two separate isotopic patterns. The first of these patterns is generated from the cysteine present in the sample which was derived directly from the fed thiocystine without cleavage of the carbon–sulfur bond. It will have ion intensities at m/z 61 and m/z 63, reflecting the natural abundance of ^{34}S. The ratio of these peak intensities, i.e., the intensity of m/z 63 to m/z 61, can be represented by b/a where a is the mole fraction of the molecules containing ^{32}S and b is the mole fraction of the molecules containing ^{34}S.

A second pattern having major intensities at m/z 61, 63, and 65 is the result of the cysteine present in the sample which has been resynthesized in the cell from the deuterated serine and hydrogen sulfide enriched in ^{34}S. In this pattern, the m/z 61 ion intensity will originate from the cysteine with no deuterium or ^{34}S, the m/z 63 ion from cysteine with either two deuteriums or a ^{34}S, and the m/z 65 ion from cysteine containing both two deuteriums and a ^{34}S. The m/z 61 ion in both of these patterns will also have ion intensities at m/z 62, 64, and 66 due to the presence of ^{13}C, ^{33}S, ^{35}S, and $^2H_1$. Since these ion intensities are relatively small, they are not considered in these calculations. The ratios of these peak intensities can be calculated from the following expression: $(x + y)(m + n) = xm + (ym + xn) + yn$ where xm = the relative intensity of m/z 61, $ym + xn$ = the relative intensity of m/z 63, and yn = the relative intensity of m/z 65 and where x represents the mole fraction of the molecules containing ^{32}S, y the mole fraction of the molecules containing ^{34}S, m the mole fraction of the molecules containing no deuterium, and n the mole fraction of the molecules containing two deuterium atoms.

Equating the sum of contributions of the m/z 61, 63, and 65 ions from both of these patterns to the observed normalized intensities of the m/z 61, 63, and 65 ions leaves us with the following three equations:

$$\text{intensity of } m/z\ 61 = Aa + Bxm$$

$$\text{intensity of } m/z\ 63 = Ab + B(ym + xn)$$

$$\text{intensity of } m/z\ 65 = Byn$$

where A is the mole fraction of the total cysteine molecules responsible for the first pattern, i.e., those incorporated with no cleavage of the carbon–sulfur bond, and B is the mole fraction of the total cysteine responsible for producing the second pattern, i.e., those cysteine molecules newly biosynthesized in the cell.

The values for a and b are easily determined by measuring the atom percent of ^{34}S in an unenriched cysteine sample. These values were found to be 95.8% and 4.2%, respectively. The observed b value is very close to the established natural abundance of ^{34}S of 4.18 atom % ^{34}S. The values of m and n can be determined from the mass spectrum of the serine isolated from the cells as described above. With these values and the intensities of the peaks in the cysteine isotopic cluster, the values of A, B, x, and y can be obtained from the above equations. These values not only give the percentage of cysteine which was newly biosynthesized by the cells but also give the ratio of $^{34}S/(^{32}S + {}^{34}S)$ in the H_2S used in the biosynthesis. (This ratio will be ~2% larger than the true atom percent ^{34}S since the amount of ^{33}S and ^{35}S in the sample has not been considered.)

Results and Discussion

Early work by Roberts et al. (1955) showed that *E. coli* B grown on a defined medium with either sulfate, cysteine, or cystine as the sole source of sulfur at a total sulfur concentration of 0.3 mM gave 90% of the growth rate and yield of cells as that observed for cells grown under non-sulfur-limiting conditions. I have confirmed this result and have also found this to be true for cells grown on the medium used in this work with thiocystine as the sole source of sulfur at a concentration of 0.104 mM. Since the total sulfur in this medium is only

Table I: Incorporation of ^{34}S from L-[*sulfane*-^{34}S]Thiocystine into Cysteine and Methionine by *E. coli*

strain	yield of cells (mg/mL of medium)	concn of [^{34}S]thiocystine fed (mM)	atom % ^{34}S Cys	atom % ^{34}S Met
E. coli B	9.2	0.021	25.5	25.3
E. coli B	13.0	0.104	27.9	27.5

0.3 mM, this would indicate that most of the sulfur atoms of the thiocystine were utilized. That the sulfane sulfur was being incorporated is evident by the increase in the atom percent ^{34}S found in the cysteine and methionine isolated from cells which were grown with [^{34}S]sulfane-labeled thiocystine (Table I). Considering that the carbon-bonded sulfur of the fed thiocystine was 4.18 atom % ^{34}S and that the sulfane sulfur was 90.83 atom % ^{34}S, we would expect, assuming complete utilization of all the sulfur atoms, an incorporation of 33.4% ^{34}S into the cysteine and methionine. Table I shows an average value of 26.5% ^{34}S, indicating that >75% of the sulfane sulfur is biosynthetically incorporated back into cysteine.

There are several possible mechanisms whereby the sulfane sulfur could be incorporated into cysteine. One possible mechanism would involve the reductive cleavage of the L-[*sulfane*-^{34}S]thiocystine to unlabeled cysteine and $H_2{}^{34}S$ followed by the incorporation of the $H_2{}^{34}S$ back into cysteine via cysteine synthetase as shown by eq e and b in Figure 1 (Brueggeman et al., 1962; Wiebers & Garner, 1967). The required reductive steps could be carried out either enzymatically or nonenzymatically by exchange reactions with glutathione or other cellular thiols (Flavin, 1962). The resulting glutathione disulfide would then be reduced back to glutathione by glutathione reductase (Williams et al., 1967). This same basic chemistry could also occur by the reaction depicted in eq k and j in Figure 1 where the sulfane sulfur either is transferred directly or is transferred by way of rhodanese (Abdolrasulnia & Wood, 1979) to a sulfane reductase whereupon it is reduced to hydrogen sulfide (Westley, 1981).

An alternative possibility would be a β elimination of one of the two cysteine residues of the thiocystine with the formation of pyruvate, ammonia, and cysteine trisulfide as described by Flavin & Slaughter (1964) for β-cystathionase-type reactions. The persulfide sulfur of the resulting cysteine trisulfide could then be reduced to H_2S and incorporated back into newly biosynthesized cysteine. These reactions are shown in eq f, g, j, and b in Figure 1. Both of these pathways operating either together or independently could generate the ^{34}S incorporations observed. Another possibility is that after the cysteine is produced, there is a scrambling of the cysteine sulfur with the H_2S pool. This could occur due to the reversibility of the cysteine synthetase (pathway c, Figure 1) or by one or more of three possible cyclic pathways involving reactions h–j, c, or b; c and d; and/or a–c as shown in Figure 1. All of these reactions have been demonstrated to occur in *E. coli* as referenced in Figure 1.

The contribution of each of these processes can be determined by measuring that portion of the cysteine which is derived directly from the thiocystine without breakage of the C–S bond. This can be done by measuring the abundances of ^{34}S and ^{2}H in the cysteine and serine produced by cells which have been grown with [3,3-$^{2}H_2$]serine and L-[*sulfane*-^{34}S]thiocystine and applying the mathematical methods discussed under Methods. However, before this procedure can be carried out, conditions must be established in which a fairly large portion of the cellular serine would be derived from the fed labeled serine. This proved to be rather difficult due to the continued synthesis of unlabeled serine by wild-type cells when grown in the presence of labeled serine. Growth of cells on amino acid mixtures containing 11.4 mM [3,3-$^{2}H_2$]serine and no glucose produced cells in which only ~10% of the cellular serine contained deuterium (experiment 1, Table II). The inclusion of glucose in the medium reduced this incorporation to only 2%.

E. coli K12 and *E. coli* B serine-requiring mutants were used in order to increase the extent of the serine labeled. As can be seen from the data in Table II, higher levels of [3,3-$^{2}H_2$]serine incorporation were now possible. It was, however, still impossible to completely replace the cellular serine with the labeled serine in any of the mutants tested. The reason for this inability to obtain complete labeling of the intercellular serine with two deuterium atoms results in part from the exchange of the C-3 deuterium atoms of the serine catalyzed by the following set of enzymes functioning under reversible conditions: serine hydroxymethyltransferase (EC 2.1.2.1); N^5,N^{10}-methylenetetrahydrofolate dehydrogenase (EC 1.5.1.5); N^5,N^{10}-methylenetetrahydrofolate reductase (EC 1.1.1.68). A discussion of this exchange will appear in a forthcoming paper by this author.

Using these mutant strains made it possible to make the required measurements and to determine the percentage of the cysteine newly biosynthesized as well as the atom percent ^{34}S in the H_2S used in this biosynthesis. These values are shown in Table III. They were determined from the calculated distributions of deuterium presented in Table II (data

Table II: Incorporation of [3,3-$^{2}H_2$]Serine into *E. coli* Cells[a]

expt no.	strain/mutant	DL-[3,3-$^{2}H_2$]serine fed (mM)	thiocystine fed (mM)	distribution of deuterium in cellular serine; no. of ^{2}H 0	1	2
1	*E. coli* B	11.4	0.104	(a) 100	8.4	11.7
				(b) 83.3	7.0	9.7
2	*E. coli* B/WG 1143 *ser A*	76.0	0.243	(a) 100	1.3	16.7
				(b) 83.1	3.0	13.9
3	*E. coli* K12/CU 332 *ser A*	76.0	0.024	(a) 100	18.9	100
				(b) 48.2	5.1	46.6
4	*E. coli* K12/CU 332 *ser A*	76.0	0.243	(a) 100	29.2	346.3
				(b) 21.9	3.4	74.7

[a] Cells were grown in the presence of labeled serine and thiocystine at the concentrations indicated as described in the text with the exception of experiment 1 which had no glucose. At the end of log-phase growth, the bound serine was released from the cells by acid hydrolysis and converted into the *N,O*-trifluoroacetyl *n*-butyl ester derivative for GC-MS analysis (White, 1981). Deuterium distributions are first reported (a) as the observed ion intensities of the $M^+ - C_4H_7$ ion at *m/z* 298, 299, and 300 in the mass spectrum of the serine derivative and then (b) as the calculated normalized percentages expected for the *m/z* 61 ion of the cysteine derivative having the same deuterium distribution as the serine.

Table III: Calculated Values

expt no.	strain/mutant	concn of [^{34}S]thiocystine fed (mM)	$^{34}S/(^{32}S + ^{34}S)$ ratio in newly biosynthesized cysteine	% of total cellular cysteine biosynthesized	calcd atom % ^{34}S in cellular Cys and Met
2	*E. coli* B/ WG 1143 *ser A*	0.243	52.3	59.2	32.7
3	*E. coli* K12/ CU 332 *ser A*	0.024	57.9	43.0	26.6
4	*E. coli* K12/ CU 332 *ser A*	0.243	48.0	46.0	24.4

Table IV: Isotopic Distributions for the *m/z* Ions in the Mass Spectrum of the *S*-Methylcysteine Derivative Prepared from Cells Grown with L-[*sulfane*-^{34}S]Thiocystine and [3,3-$^{2}H_2$]Serine[a]

		concn of [^{34}S]thio-cystine	obsd *m/z*					calcd *m/z*				
expt no.	strain/mutant	(mM)	61	62	63	64	65	61	62	63	64	65
1	*E. coli* B	0.104	63.9	4.3	25.1	2.6	4.2					
2	*E. coli* B/ WG 1143 *ser A*	0.243	58.4	3.0	32.1	2.0	4.4	59.6	2.2	31.4	0.9	4.3
3	*E. coli* K12/ CU 332 *ser A*	0.024	58.0	3.6	23.6	3.1	11.6	61.2	2.9	22.9	1.3	11.6
4	*E. coli* K12/ CU 332 *ser A*	0.243	53.8	3.0	24.7	2.3	16.2	55.0	2.7	25.0	0.8	16.5

[a] The results are reported as the percent of the total molecules having the indicated masses. The calculated data are from the results reported in Tables II and III by using the methods discussed in the text. The thiocystine had 90 atom % ^{34}S in the persulfide sulfur.

b) and the observed distribution of deuterium for the cysteine in Table IV. These values, when substituted back into the original equations, gave the calculated values shown in Table IV.

The data show that roughly 50% of the cysteine incorporated into the cellular proteins by cells grown with thiocystine as their sole sulfur source is not derived directly from the thiocystine but from cysteine newly biosynthesized by the cells. Had the thiocystine been metabolized only by a reductive cleavage as shown in reaction e, Figure 1, we would expect from 0 to 33% of the cysteine to be newly biosynthesized, with the exact percentage depending upon the efficiency of utilization of the sulfane sulfur. Alternatively, metabolism of the thiocystine by a β elimination (reaction f, Figure 1) followed by reduction of the two persulfide sulfurs to hydrogen sulfide and reincorporation of the hydrogen sulfide into cysteine by reactions g, j, and b in Figure 1 would require that less than 66% of the cysteine be newly biosynthesized by the cells with the remaining percentage arising intact from the thiocystine. Again, the exact percentage would depend on the efficiency at which the persulfide sulfur which results from the β cleavage was biosynthetically incorporated back into new cysteine. Only this second model is consistent with the observed data.

The metabolism of thiocystine by a β elimination is consistent with the observed wide variety of "cysteine"-containing substrates cleaved by β-cystathionase from *E. coli* (Delavier-Klutchko & Flavin, 1965a). That the enzyme is present in the cells studied is evident as it is required for the biosynthesis of methionine which the cells are clearly producing (Delavier-Klutchko & Flavin, 1965b).

The extent of labeling of the H_2S pool is also consistent with a β-cystathionase cleavage reaction. We would expect to produce two persulfide sulfurs for each molecule cleaved; one would contain the natural abundance of ^{34}S (4.2%) and the other, 90.83 atom % ^{34}S. An equal mixture of these sulfur atoms would give an inorganic sulfur pool containing approximately 50 atom % ^{34}S, which is in agreement with the calculated data presented in Table III.

The magnitudes of the other reactions shown in Figure 1 are impossible to accurately assess from the data presented here, but since almost half of the cysteine found in the cell is incorporated without cleavage of the carbon–sulfur bond, they must not be of major importance, at least in terms of sulfur cycling into and out of cysteine. This lack of sulfur cycling has recently been demonstrated for the metabolism of cysteine by *E. coli* (R. White, unpublished experiments). When *E. coli* cells grown with labeled serine and concentrations of cystine of 0.05 and 0.45 mM were used, 16% and 5%, respectively, of the cellular cysteine incorporated into protein were found to be derived from serine. It was also shown that at the lower concentration of the fed cystine, about half of the cystine was metabolized by the β elimination of pyruvate, which means that not more than ~8% of the cysteine sulfur could be exchanged by pathways which separate and re-form the cysteine C–S bond (e.g., eq c, Figure 1).

The route proposed in this paper for the metabolism of thiocystine is different from that proposed by Szczepkowski & Wood (1967) and Abdolrasulnia & Wood (1979). In their in vitro work, they described the transfer of the sulfane sulfur of thiocystine to rhodanese without the cleavage of the cystine C–S bond as a first step in its metabolism. If this were operating in *E. coli*, we would see about 30% of the cysteine arising from serine and H_2S. In terms of the expected distribution of the sulfur label, this would be analogous to the reductive cleavage shown in eq e in Figure 1, which we have shown is not consistent with the data. The operation of the γ-cystathionase–rhodanese system for the metabolism of the thiocystine is also not possible in bacteria because of the absence of γ-cystathionase in bacteria (Delavier-Klutchko & Flavin, 1965b).

As a result of this work, it is now possible to differentially label the cysteine and H_2S sulfur pools in *E. coli*. This has already led to the demonstration that the cysteine sulfur is the most likely biosynthetic precursor for the lipoic acid sulfur (White, 1982). Similar experiments are under way, using these methods to determine the in vivo biosynthetic origins of the

sulfur atoms in biotin, thiamin, and iron–sulfur proteins.

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Viscosity Dependence of the Kinetics of the Diffusion-Controlled Reaction of Carbon Monoxide and Myoglobin†

Brian B. Hasinoff* and S. Bano Chishti

ABSTRACT: The kinetics of the reaction of CO with myoglobin have been studied by laser flash photolysis in glycerol–water as a function of solvent viscosity and temperature. At high viscosities and low temperatures the second-order rate constant is inversely proportional to the viscosity raised to approximately the 0.5 power. This result parallels the behavior of the oxygen diffusion coefficient in glycerol–water. It is concluded that the reaction kinetics in high viscosity glycerol–water are largely diffusion controlled. At higher temperatures, though, the effect of simultaneous chemical activation control of the reaction is observed. The diffusion-controlled rate constant is 1.4×10^{-3} of that predicted from simple von Smoluchowski theory based on diffusion coefficients and molecular radii. Several models with steric requirements for diffusion-controlled reactions are examined.

The effect of solvent viscosity on the binding of CO or O_2 to Mb[1] in glycerol–water has been the object of several studies (Strother et al., 1959; Fesenko et al., 1972; Austin et al., 1975; Hasinoff, 1977; Beece et al., 1980; McKinnie & Olson, 1981). Nonlinear Arrhenius plots of the second-order ligand recombination rate constant were interpreted in a previous study as due to simultaneous chemical activation and diffusion control of the reaction (Hasinoff, 1977). At temperatures below ~ -25 °C, an additional fast phase is seen exhibiting kinetics indicative of a caged geminate transient diffusion process (Hasinoff, 1981). In a recent study (Beece et al., 1980) the ligand combination rate constant was observed to vary inversely as the solvent viscosity raised to a fractional power.

Simple von Smoluchowski (1917) theory predicts that for a diffusion-controlled reaction

$$k_D = 4\pi RDN/1000 \quad (1)$$

where k_D is the diffusion-controlled rate constant in M^{-1} s^{-1}, R is the encounter distance in cm, D is the sum of the

† From the Department of Chemistry and Faculty of Medicine, Memorial University of Newfoundland, St. John's, Newfoundland, Canada A1B 3X7. *Received March 25, 1982.* This work was supported in part by Natural Sciences and Engineering Research Council (Canada) Grant A9430.

* Address correspondence to this author at the Department of Chemistry.

[1] Abbreviation: Mb, ferrous myoglobin.

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