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METABOLIC REGULATION OF ADENOSINE TRIPHOSPHATE SULFURYLASE IN YEAST

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ABSTRACT

DE VITO, PETER C. (Princeton University, Princeton, N.J.), AND JACQUES DREYFUSS. Metabolic regulation of adenosine triphosphate sulfurylase in yeast. J. Bacteriol. **88**:1341-1348. 1964. —The metabolic regulation of adenosine triphosphate sulfurylase (ATP-sulfurylase) from baker's yeast was studied. The enzyme was strongly inhibited by low concentrations of adenosine-5′-phosphosulfate, 3′-phosphoadenosine-5′-phosphosulfate, and sulfide. Sulfide ion was a competitive inhibitor of ATP-sulfurylase. Cysteine, methionine, sulfite, and thiosulfate were not inhibitors of the enzyme. ATP-sulfurylase was repressed when yeast was grown in the presence of methionine, and derepressed when yeast was grown in the presence of cysteine. In contrast to these results, the enzyme sulfite reductase was repressed in cysteine-grown cells. Thus, the sulfate-reducing pathway in yeast appears to be regulated at its first step both by feedback inhibition (by sulfide) and by repression (by methionine). Other known controls in the cysteine biosynthetic pathway are discussed.

The generally recognized metabolic intermediates for the assimilation of sulfate into cysteine in yeast and in bacteria are adenosine-5′-phosphosulfate (APS), 3′-phosphoadenosine-5′-phosphosulfate (PAPS), sulfite, and sulfide (Schlossmann and Lynen, 1957; Wilson, Asahi, and Bandurski, 1961; Dreyfuss and Monty, 1963*a*). According to Leinweber and Monty (1963), thiosulfate is metabolized in *Salmonella typhimurium* by a reductive dismutation to sulfite and sulfide.

Robbins and Lipmann (1958*a*) succeeded in separating two distinct enzymes in yeast, both of which were involved in the synthesis of PAPS, "active sulfate." One of the enzymes, designated as adenosine triphosphate sulfurylase (ATP-sulfurylase), catalyzes the formation of APS according to the following reaction:

$$SO_4^{=} + \text{adenosine triphosphate (ATP)} \xrightarrow[Mg^{++}]{\text{ATP-Sulfurylase}} \text{APS} + \text{pyrophosphate}$$

The second enzymatic activity, designated as APS-kinase, catalyzes the synthesis of PAPS from APS by the following reaction:

$$\text{APS} + \text{ATP} \xrightarrow[Mg^{++}]{\text{APS-kinase}} \text{PAPS} + \text{adenosine diphosphate}$$

Various aspects of the metabolic regulation of cysteine biosynthesis from sulfate were studied in *S. typhimurium* (Dreyfuss and Monty, 1963*b*), in *Escherichia coli* (Mager, 1960; Pasternak, 1962; Kemp et al., 1963), in *Bacillus subtilis* (Pasternak, 1962), and in yeast (Wilson et al., 1961). However, until recently (Pasternak et al., 1964), the regulation of the first enzyme in the sulfate-reducing pathway had not been studied in these organisms. The experiments described herein demonstrate that ATP-sulfurylase is regulated both by feedback inhibition and by enzyme repression.

MATERIALS AND METHODS

Chemicals. Glucose-6-phosphate (barium salt) was obtained from Sigma Chemical Co., St. Louis, Mo.; it was converted to the sodium salt before use. Glucose-6-phosphate dehydrogenase and nicotinamide adenine dinucleotide phosphate (NADP) were purchased from C. F. Boehringer and Soehne, Mannheim, Germany. ATP (disodium salt) was supplied by Pabst Laboratories, Milwaukee, Wis. Inorganic pyrophosphatase was obtained from Worthington Biochemical Corp., Freehold, N.J. Elon (*p*-methylaminophenol sulfate) was purchased from Eastman Kodak Co., Rochester, N.Y.

Synthesis of APS and PAPS. APS was prepared by the method of Baddiley, Buchanan, and

Letters (1957) with use of the chromatographic elution procedure of Wilson et al. (1961); it was recovered as the ammonium salt. APS was judged to be better than 95% pure by the examination of electrophoretic patterns after the method of Hilz and Lipmann (1955).

PAPS was synthesized by the method of Wilson et al. (1961); it had an indicated purity of 40% as determined by measuring the optical density at 260 mμ of the eluted ultraviolet absorbing spots after electrophoresis. The contaminant was 3′-phosphoadenosine-5′-phosphate (PAP).

Growth of yeast. National brand bakers' yeast was purified by a single colony isolation. Yeast cultures were grown at 30 C on a gyrotory shaker in the minimal medium of Maw (1963). Any desired sulfur source at a final concentration of 2×10^{-4} M was added to this minimal medium.

Assay procedure for ATP-sulfurylase. The activity of ATP-sulfurylase was measured according to the method of Wilson and Bandurski (1958) as described by Robbins (1962). This method is based on the fact that group VI anions such as molybdate will substitute for sulfate, thereby increasing the rate of formation of pyrophosphate from ATP. The reaction mixture contained, in a final volume of 1.0 ml, 1 μg of inorganic pyrophosphatase, 0.6 mg of yeast protein (40 to 50% ammonium sulfate fraction); and the following (in μmoles): tris(hydroxymethyl)aminomethane (tris) buffer (pH 8.0), 100; ATP, 10; magnesium chloride, 10; and sodium molybdate, 5. After incubating for 30 min at 37 C, 1.0 ml of ice-cold 10% trichloroacetic acid was added and the mixture was centrifuged. A 1.0-ml sample was immediately assayed for inorganic phosphate by the method of Fiske and SubbaRow (1925). In place of the Fiske and SubbaRow (1925) color-developing reagent, 0.5 ml of 1% Elon in 3% sodium bisulfite was used. The optical density of the developed samples was measured with a Klett-Summerson photoelectric colorimeter (red filter). By working rapidly, nonenzymatic hydrolysis of ATP in acid solutions was avoided. A tube without molybdate was used as a control to check for inorganic phosphate produced by enzyme reactions other than that catalyzed by ATP-sulfurylase. Controls without enzyme but with molybdate and a zero time sample were used to check for the nonenzymatic hydrolysis of ATP. The latter two controls produced negligible phosphate. Specific activity is defined as micromoles of phosphate formed per hour per milligram of protein.

Special precautions were taken to prevent sulfide from escaping from the reaction mixture during the incubation period. An ice-cold sodium sulfide solution was prepared, from which the desired amount of sulfide was removed and rapidly transferred to the ice-cold reaction mixture containing all the components except the partially purified ATP-sulfurylase. After addition of the enzyme, the tubes were immediately closed with Parafilm-wrapped rubber stoppers.

When analyzing for phosphate in the presence of high concentrations of sulfide, i.e., 10^{-3} to 10^{-2} M, a dark-brown precipitate formed which subsequently redissolved when the mixture was diluted to 10 ml. The formation of this precipitate did not interfere with the recovery of phosphate, even when sulfide was present at a concentration of 10^{-2} M.

Protein was determined by the method of Lowry et al. (1951) with bovine plasma albumin as a standard.

Assay procedure for sulfite reductase. As a measure of sulfite reductase activity, the disappearance of sulfite (present as HSO_3^-) was followed. The reaction mixture contained, in a final volume of 2.0 ml, 1 to 4 mg of protein, 0.2 units of glucose-6-phosphate dehydrogenase, and the following (in micromoles): sodium phosphate buffer (pH 7.7), 120; glucose-6-phosphate, 9.3; NADP, 0.5; and sodium bisulfite, 0.3. Incubation was carried out for 30 min at 37 C. The amount of sulfite reduced was analyzed by the method of Grant (1947), as previously described (Dreyfuss and Monty, 1963*a*). The color was allowed to develop for exactly 15 min, and then the optical density was determined at 580 mμ. The optical density values were converted to millimicromoles of sulfite reduced by use of a standard curve prepared with an iodometrically standardized solution of sodium bisulfite.

Sulfite reductase activity as assayed by this procedure is proportional to time for at least 40 min, and proportional to protein concentration up to at least 4 mg.

Partial purification of ATP-sulfurylase. At the outset of this study, it was intended to repeat the purification of yeast ATP-sulfurylase as described by Robbins and Lipmann (1958*a*). Repeated attempts were made to duplicate this purification procedure without any success.

Therefore, it was decided to attempt a partial purification of the enzyme by an alternative method. One pound of fresh National bakers' yeast was disrupted in a French pressure cell in 300 ml of 0.1 M tris buffer (pH 7.5). The viscous exudate was diluted to 800 ml with 0.1 M tris buffer (pH 7.5). Whole cells and debris were removed by centrifugation. To the crude extract, sufficient solid ammonium sulfate was added to bring the final concentration to 40% saturation. The solution was centrifuged and the precipitate was discarded. Again, sufficient solid ammonium sulfate was added to bring the final concentration of the supernatant fluid to 50% saturation. The solution was centrifuged and the precipitate was dissolved in a minimal amount of 0.02 M tris buffer (pH 7.5), and was dialyzed overnight against 0.01 M tris-10^{-3} M ethylenediaminetetraacetic acid (pH 7.5). All steps were carried out at 0 to 4 C. This enzyme fraction was completely stable when stored at −15 C, even after thawing and freezing several times. The crude extract is not stable when stored under identical conditions.

Table 1 summarizes the specific activities of the various ATP-sulfurylase fractions recovered during the partial purification of the enzyme. A twofold purification was achieved. The 40 to 50% ammonium sulfate fraction was used in all experiments. The activity of this fraction was sufficient to give a difference of about 300 Klett units between the complete reaction mixture and the minus-molybdate control.

Isotopic competition experiments. Yeast cells were grown on minimal medium (10^{-4} M sodium sulfate as the sulfur source) to population limit, and were resuspended in fresh medium for 2 hr. The cells were then harvested by centrifugation and washed twice with sulfur-free minimal medium. Cell concentration was expressed on a wet weight basis. The packed yeast pellet was diluted with 4 volumes of minimal medium.

The reaction mixture contained 1.0 ml of minimal medium containing 10^{-4} M sodium sulfate and 10^6 counts per min per ml of $S^{35}O_4^=$. Sufficient yeast was added to give a final concentration of 0.5%. Various concentrations of inhibitor dissolved in minimal medium were added in a volume of 0.05 ml. As a control, minimal medium without inhibitor was added to one tube. The reaction mixture was incubated for 10 min at 30 C. After incubation, 1.0 ml of 25% trichloroacetic acid was added to stop the reaction. The precipitated protein was then recovered by filtering the mixture through a 0.45-μ Millipore filter. The filter was then washed with 5 ml of minimal medium containing 10^{-3} M sulfate, and then with 5 ml of water. The filter was dried and was placed in approximately 10 ml of scintillation fluid. The composition of the scintillation fluid was (per liter of toluene) 100 mg of 1,4-bis-2′-(5′-phenyloxazolyl)-benzene, and 4.0 g of 2,5-diphenyloxazole. The fluid-containing vials were counted in a Packard Tri-Carb liquid scintillation counting system (model 314-EX).

TABLE 1. *Partial purification of adenosine triphosphate-sulfurylase*

Fraction	Specific activity*
Crude extract	1.7
$(NH_4)_2SO_4$ (0 to 40%)	2.1
$(NH_4)_2SO_4$ (40 to 50%)	3.3

* Expressed as micromoles of phosphate formed per hour per milligram of protein.

RESULTS

Verification of ATP-sulfurylase assay. The assay for ATP-sulfurylase is linear with time for about 45 min, and is proportional to the amount of protein contained in the incubation mixture to 0.7 mg. A typical substrate saturation curve for molybdate is obtained (Fig. 1). The Michaelis constant for molybdate, as determined graphically by the method of Lineweaver and Burk (1934), is 3.6×10^{-4} M.

Inhibition of ATP-sulfurylase in vitro. A number of sulfur-containing compounds were

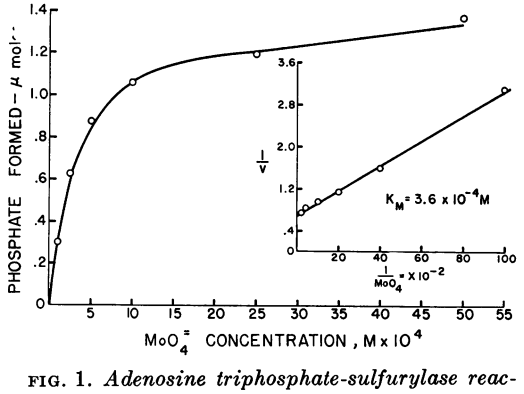

FIG. 1. *Adenosine triphosphate-sulfurylase reaction as a function of molybdate concentration.*

tested as potential inhibitors of ATP-sulfurylase in vitro. Of the compounds tested, L-cysteine, L-methionine, sulfite, and thiosulfate were not inhibitors of the enzyme, even when present at concentrations as high as 10^{-2} M. Positive inhibitory results were obtained with sulfate, APS, PAPS, and sulfide (Fig. 2). It is not surprising that sulfate inhibits the reaction, because it is the true substrate for ATP-sulfurylase and should compete directly with molybdate.

APS is a potent inhibitor of ATP-sulfurylase. Robbins (1962) pointed out that the inhibition by APS is difficult to measure because the rate of the reaction increases with time. This situation was found not to be the case in the partially purified yeast preparation. APS inhibition of ATP-sulfurylase does not vary with time, at least over a period of 30 min (Table 2).

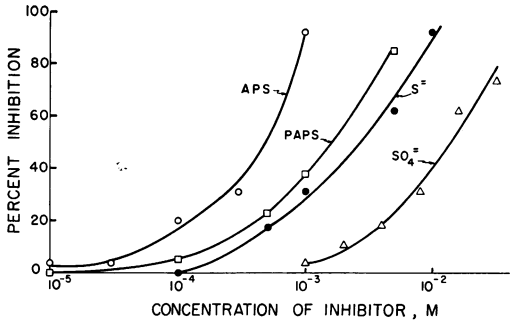

FIG. 2. *Inhibition of adenosine triphosphate-sulfurylase in vitro by various sulfur-containing compounds. Inhibitor was dissolved in 1 M tris buffer (pH 8.0), and was added to the other components so that the final volume of the reaction mixture was 1.0 ml. The inhibition curve for 3'-phosphoadenosine-5'-phosphosulfate (PAPS) is uncorrected for the degree of purity of the inhibitor. APS = adenosine-5'-phosphosulfate.*

TABLE 2. *Inhibition of adenosine triphosphate-sulfurylase by adenosine-5'-phosphosulfate (APS) and 3'-phosphoadenosine-5'-phosphosulfate (PAPS) as a function of time**

Length of incubation	Inhibitor	Inhibition
min		%
10	APS	90
20	APS	89
30	APS	92
10	PAPS	33
20	PAPS	55
30	PAPS	60

* APS and PAPS were present at final concentrations of 10^{-3} M.

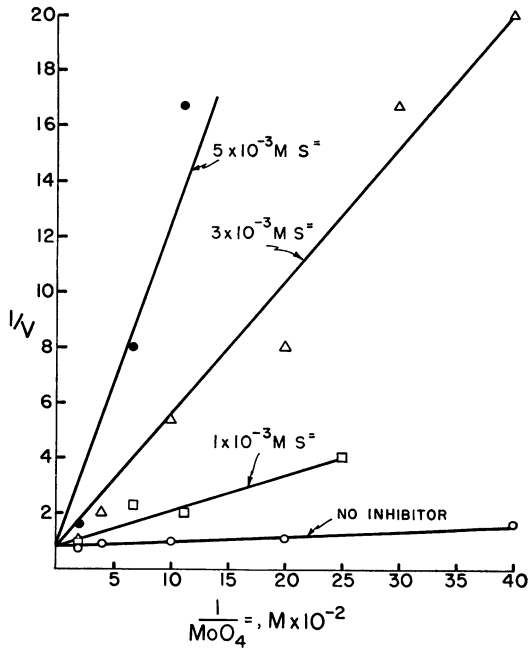

FIG. 3. *Competitive inhibition of adenosine triphosphate-sulfurylase by sulfide. The final molybdate concentration was 1.5 × 10^{-4} M.*

Another potent inhibitor of ATP-sulfurylase is PAPS. The degree of enzyme inhibition in this case did increase somewhat with time (Table 2). (It is not known whether the inorganic pyrophosphatase present in the reaction mixture was contaminated with a 3'-phosphatase.) Since the purity of the PAPS used in these experiments was only 40% with the hydrolysis product, PAP, present to the extent of 60%, it was important to determine which of the two components was the actual inhibitor. Accordingly, the compounds were separated from each other by paper electrophoresis, and were tested individually as inhibitors of ATP-sulfurylase. It was found that PAPS at a concentration of 6 × 10^{-4} M gave 70% inhibition, whereas PAP at a concentration of 10^{-3} M gave only 18% inhibition.

Sulfide ion proved to be an excellent inhibitor of ATP-sulfurylase. An analysis of the kinetics of inhibition by the method of Lineweaver and Burk (1934) indicated that sulfide was a competitive inhibitor of the enzyme (Fig. 3). The de-

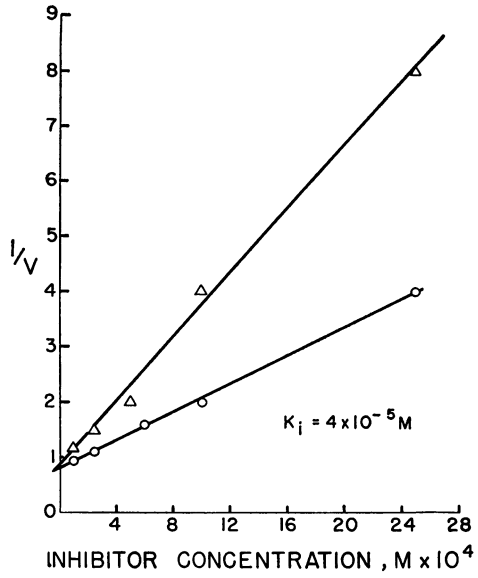

FIG. 4. *Graphical determination of inhibitor constant for sulfide. Symbols:* ○, *9* × *10⁻⁴ M molybdate;* △, *4* × *10⁻⁴ M molybdate.*

termination of the inhibitor constant for sulfide by the graphical method of Dixon (1953) gave a K_i of 4×10^{-5} M (Fig. 4). The inhibition of ATP-sulfurylase by sulfide was found to be noncompetitive with ATP in similar studies.

Inhibition of $S^{35}O_4^=$ incorporation in vivo. To ascertain whether the inhibition of ATP-sulfurylase by sulfide in vitro had regulatory significance to the intact yeast cell, the incorporation of $S^{35}O_4^=$ into protein was measured in the presence of various potential inhibitors as well as in their absence. The results (Fig. 5) indicate that sulfide and sulfite are good inhibitors of $S^{35}O_4^=$ incorporation at concentrations greater than 10^{-4} M. The K_i for sulfide in vivo (i.e., the sulfide concentration at which $S^{35}O_4^=$ incorporation is inhibited by 50%) is about 10^{-4} M. This value is in reasonable agreement with the K_i determined for sulfide in vitro of 4×10^{-5} M. Methionine is also an effective inhibitor at low concentrations, but the inhibition levels off at a maximum of about 75%. Thiosulfate also inhibits $S^{35}O_4^=$ incorporation, but is not as effective as sulfide, sulfite, or methionine. The end product of the sulfate-reducing pathway, cysteine, is not an effective inhibitor of $S^{35}O_4^=$ incorporation into protein.

Repression of ATP-sulfurylase. Yeast was grown in the presence of various sulfur-containing compounds. The ATP-sulfurylase activity was then determined in a crude extract of the disrupted cells (Table 3). It is interesting to note that, relative to sulfate-grown yeast, cysteine-grown cells are derepressed three- to fourfold, whereas methionine-grown cells are repressed three- to fourfold. The ATP-sulfurylase activity of cysteine plus methionine-grown cells is not significantly different from that of yeast grown solely in the presence of methionine.

The growth rates of yeast in the presence of sulfate, cysteine, methionine, and cysteine plus methionine may, in part, explain the results obtained above (Table 4). The cells most derepressed for ATP-sulfurylase, namely, those grown in the presence of cysteine, had the longest generation time. Similarly, sulfate-grown cells had a longer generation time than did methionine-grown cells, and possessed greater ATP-sulfurylase activity. Finally, cysteine plus methionine-grown cells grew at the same rate as did methionine-grown cells, and possessed very similar ATP-sulfurylase activities.

Repression of sulfite reductase. In view of the results obtained for the repressive regulation of ATP-sulfurylase, it was of interest to assay the activity of a second enzyme in the sulfate-reducing pathway. Sulfite reductase was the enzyme

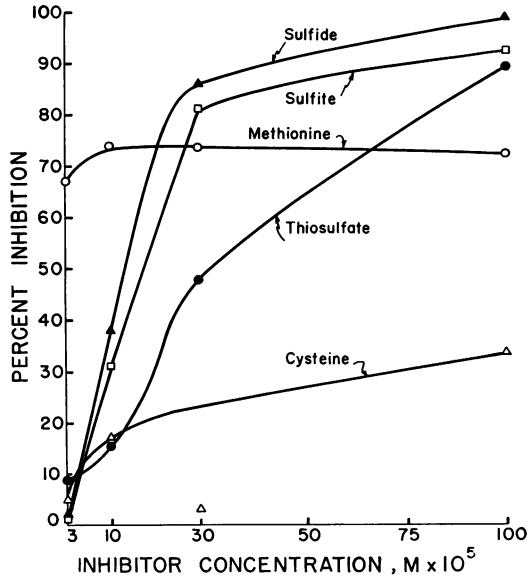

FIG. 5. *Inhibition of $S^{35}O_4^=$ incorporation in vivo by various sulfur-containing compounds. The reaction mixture was incubated for 10 min at 30 C.*

TABLE 3. *Adenosine triphosphate-sulfurylase and sulfite reductase activities of yeast grown on various sulfur sources**

Sulfur source for cell growth	ATP-sulfurylase activity†		Sulfite reductase activity‡	
	Expt 1	Expt 2	Expt 1	Expt 2
Sulfate	2.3	1.3	56.3	34.8
L-Cysteine	8.0	5.7	1.0	1.1
L-Methionine	0.5	0.3	28.8	26.0
L-Cysteine + L-methionine	0.2	0.4	31.7	21.9

* Yeast were grown on minimal medium containing the desired sulfur source at a final concentration of 2×10^{-4} M. The cells were harvested in the exponential phase of growth, washed once with minimal medium, and disrupted in a French pressure cell in 0.1 M tris buffer (pH 7.5). Whole cells and debris were removed by centrifugation. The enzyme assays are described in Materials and Methods.

† Results expressed as micromoles of phosphate formed per hour per milligram of protein.

‡ Results expressed as millimicromoles of sulfite reduced per 30 min per milligram of protein.

TABLE 4. *Generation time of yeast growing on various sulfur sources**

Sulfur source	Generation time
	min
None	300
Sulfate	90
L-Cysteine	125
L-Methionine	70
L-Cysteine + L-methionine	70

* Final population density attained by yeast when growing solely in the presence of L-cysteine was about one-half that attained by yeast growing in the presence of sulfate, L-methionine, or L-cysteine plus L-methionine. All sulfur sources were present at final concentrations of 2×10^{-4} M.

chosen for study because it has been well studied in other organisms (see Discussion).

The sulfite reductase activity of yeast grown in the presence of sulfate, cysteine, methionine, or cysteine plus methionine is indicated in Table 3. In this case, cysteine-grown cells exhibit severely repressed levels of sulfite reductase. Sulfate-grown cells show the highest levels of enzyme activity. Methionine- and cysteine plus methionine-grown cells have levels of sulfite reductase which are intermediate between those possessed by cysteine- and sulfate-grown cells.

DISCUSSION

The inhibition of ATP-sulfurylase in vitro by four sulfur-containing compounds was observed. Sulfate, the normal substrate for the enzyme, inhibits the reaction by competing with molybdate. Sulfide is a competitive inhibitor of ATP-sulfurylase. That APS and PAPS inhibit an early reaction in the sulfate-reducing pathway in yeast was previously reported by Wilson et al. (1961), who showed that sulfite formation from APS or PAPS is severely inhibited by concentrations of these substrates in excess of 5×10^{-4} M. It should be emphasized that cysteine, methionine, sulfite, and thiosulfate are not inhibitors of ATP-sulfurylase in vitro.

ATP-sulfurylase apparently has a high degree of specificity for both its substrate and various inhibitors. The affinity of the enzyme for sulfate or molybdate must be very specific, because thiosulfate, a structural analogue of both sulfate and molybdate, does not inhibit ATP-sulfurylase, nor does sulfite. Likewise, sulfide is an effective inhibitor of the enzyme, whereas cysteine, with its reactive sulfhydryl group, is not an inhibitor. It appears likely that the substrate-binding site is very specific with respect to the molecular dimensions of the substrate and inhibitor.

Some insight can be gained into the mechanism of the ATP-sulfurylase reaction based on the observation that sulfide inhibition is competitive with molybdate but noncompetitive with ATP. A distinct site must exist on the enzyme which binds sulfate, one of the substrates of the reaction. In addition, ATP either must be bound at a site separate from the sulfate-binding site or must react with sulfate in an unbound form. The present data do not allow one to make a distinction between these two possibilities.

Of the sulfur-containing compounds tested as potential inhibitors of $S^{35}O_4^{=}$ incorporation in vivo, sulfide is the only inhibitor of ATP-sulfurylase in vitro. It is worth noting that sulfide does not inhibit $S^{35}O_4^{=}$ incorporation in vivo by virtue of its being toxic to the intact cell, because sulfide is a good sulfur source for yeast at a concentration of 10^{-4} M (Schultz and McManus, 1950). It is possible that, in vivo, sulfite.

methionine, and thiosulfate inhibit enzymes involved in cysteine biosynthesis beyond the reaction catalyzed by ATP-sulfurylase. In fact, sulfide might act as an inhibitor of more than one enzyme-catalyzed reaction. Leinweber and Monty (1963) showed that sulfide is a potent inhibitor of thiosulfate metabolism in vivo in *S. typhimurium*. The end product of the sulfate-reducing pathway, cysteine, does not appear to be an inhibitor of any enzyme-catalyzed reaction in the pathway.

Although it would appear that elaborate feedback mechanisms exist for the regulation of cysteine biosynthesis in yeast, these isotopic competition experiments in vivo should be interpreted with caution. The results obtained can be equally well explained by assuming that pools of substrates exist for each of the sulfur-containing intermediates, and that exogenous sulfur sources exert their inhibitory effect by a simple isotopic dilution. This point can only be clarified by making direct measurements of the pool sizes of the various biosynthetic intermediates.

In addition to being regulated by feedback inhibition, ATP-sulfurylase is regulated by the phenomenon of enzyme repression. The regulation of enzyme synthesis in yeast was previously described (MacQuillan and Halvorson, 1962*a*, *b*). The level of ATP-sulfurylase can be changed about 20-fold, depending on the sulfur source used for cell growth. Relative to the activity of ATP-sulfurylase in sulfate-grown cells, cysteine-grown cells are derepressed, whereas methionine-grown cells are repressed. By contrast, the activity of sulfite reductase is repressed to very low levels in cysteine-grown cells, but not in sulfate- or methionine-grown cells.

Considerable information is available concerning the repressive regulation of enzymes involved in cysteine biosynthesis. Sulfite reductase was shown to be repressed in cysteine-grown cells of *E. coli* (Mager, 1960; Kemp et al., 1963; Pasternak et al., 1964) and of *S. typhimurium* (Dreyfuss and Monty, 1963*a*). Our results are in agreement with these findings. In *S. typhimurium*, it was further shown that the enzymes concerned with the reduction of PAPS and thiosulfate are repressed in cysteine-grown cells (Dreyfuss and Monty, 1963*b*), as well as the component parts of a sulfate-transporting system (Dreyfuss, 1964). Pasternak et al. (1964) indicated that, in *E. coli*, ATP-sulfurylase and APS-kinase are completely repressed in cystine-grown cells. In yeast, it appears as though ATP-sulfurylase is repressed in methionine-grown cells but not in cysteine-grown cells. This difference in the nature of the repressing metabolite for ATP-sulfurylase may reflect a difference in the biochemical evolution of yeast and of the Enterobacteriaceae.

The control of cysteine biosynthesis in yeast, therefore, would be exerted on two levels. The most immediate control would be product inhibition by APS and PAPS. In addition, ATP-sulfurylase would be effectively feedback-inhibited by the sulfide ion. With these effective feedback controls, it is unlikely that much cysteine would be formed. However, if for some physiological reason the feedback controls should fail to function, methionine would repress the synthesis of ATP-sulfurylase, and cysteine would repress the formation of sulfite reductase.

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LITERATURE CITED

BADDILEY, J., J. G. BUCHANAN, AND R. LETTERS. 1957. Synthesis of adenosine-5′-sulphatophosphate. A degradation product of an intermediate in the enzymic synthesis of sulphuric esters. J. Chem. Soc., p. 1067–1071.

DIXON, M. 1953. The determination of enzyme inhibitor constants. Biochem. J. **55**:170–171.

DREYFUSS, J. 1964. Characterization of a sulfate- and thiosulfate-transporting system in *Salmonella typhimurium*. J. Biol. Chem. **239**: 2292–2297.

DREYFUSS, J., AND K. J. MONTY. 1963*a*. The biochemical characterization of cysteine-requiring mutants of *Salmonella typhimurium*. J. Biol. Chem. **238**:1019–1024.

DREYFUSS, J., AND K. J. MONTY. 1963*b*. Coincident repression of the reduction of 3′-phosphoadenosine-5′-phosphosulfate, sulfite, and thiosulfate in the cysteine pathway of *Salmonella typhimurium*. J. Biol. Chem. **238**:3781–3783.

FISKE, C. H., AND Y. SUBBAROW. 1925. The colorimetric determination of phosphorus. J. Biol. Chem. **66**:375–400.

GRANT, W. M. 1947. Colorimetric determination of sulfur dioxide. Anal. Chem. **19**:345–346.

HILZ, H., AND F. LIPMANN. 1955. The enzymatic activation of sulfate. Proc. Natl. Acad. Sci. U.S. **41**:880–890.

KEMP, J. D., D. E. ATKINSON, A. EHRET, AND R. A. LAZZARINI. 1963. Evidence for the identity of the nicotinamide adenine dinucleotide phosphate-specific sulfite and nitrite reductases of *Escherichia coli*. J. Biol. Chem. **238**: 3466–3471.

LEINWEBER, F.-J., AND K. J. MONTY. 1963. The metabolism of thiosulfate in *Salmonella typhimurium*. J. Biol. Chem. **238**:3775–3780.

LINEWEAVER, H., AND D. BURK. 1934. The determination of enzyme dissociation constants. J. Am. Chem. Soc. **56**:658–666.

LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, AND R. J. RANDALL. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.

MACQUILLAN, A. M., AND H. O. HALVORSON. 1962*a*. Metabolic control of β-glucosidase synthesis in yeast. J. Bacteriol. **84**:23–30.

MACQUILLAN, A. M., AND H. O. HALVORSON. 1962*b*. Physiological changes occurring in yeast undergoing glucose repression. J. Bacteriol. **84**:31–36.

MAGER, J. 1960. A nicotinamide adenine dinucleotide phosphate-linked sulfite reductase and its relation to hydroxylamine reductase in enterobacteriaceae. Biochim. Biophys. Acta **41**:553–555.

MAW, G. A. 1963. The uptake of some sulphur-containing amino acids by a brewer's yeast J. Gen. Microbiol. **31**:247–259.

PASTERNAK, C. A. 1962. Sulphate activation and its control in *Escherichia coli* and *Bacillus subtilis*. Biochem. J. **85**:44–49.

PASTERNAK, C. A., C. E. CRICHTON, R. J. ELLIS, M. C. JONES-MORTIMER, AND J. F. WHELDRAKE. 1964. The control of sulphate reduction in *Escherichia coli*. Intern. Congr. Biochem. 6th, New York, p. 730.

ROBBINS, P. W. 1962. Sulfate activating enzymes, p. 964–977. *In* S. P. Colowick and N. O. Kaplan [ed.], Methods in enzymology, vol. 5. Academic Press, Inc., New York.

ROBBINS, P. W., AND F. LIPMANN. 1958*a*. Separation of the two enzymatic phases in active sulfate synthesis. J. Biol. Chem. **233**:681–685.

ROBBINS, P. W., AND F. LIPMANN. 1958*b*. Enzymatic synthesis of adenosine-5′-phosphosulfate. J. Biol. Chem. **233**:686–690.

SCHLOSSMANN, K., AND F. LYNEN. 1957. Biosynthese des Cysteines aus Serin und Schwefelwasserstoff. Biochem. Z. **328**:591–594.

SCHULTZ, A. S., AND D. K. MCMANUS. 1950. Amino acids and inorganic sulfur as sulfur source for the growth of yeasts. Arch. Biochem. Biophys. **25**:401–409.

WILSON, L. G., T. ASAHI, AND R. S. BANDURSKI. 1961. Yeast sulfate-reducing system. I. Reduction of sulfate to sulfite. J. Biol. Chem. **236**:1822–1829.

WILSON, L. G., AND R. S. BANDURSKI. 1958. Enzymatic reactions involving sulfate, sulfite, selenate, and molybdate. J. Biol. Chem. **233**: 975–981.