STUDIES ON THERMOPHILIC SULFATE-REDUCING BACTERIA

III. ADENOSINE TRIPHOSPHATE-SULFURYLASE OF *CLOSTRIDIUM NIGRIFICANS* AND *DESULFOVIBRIO DESULFURICANS*[1]

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ABSTRACT

AKAGI, J. M. (University of Kansas, Lawrence) AND L. LEON CAMPBELL. Studies on thermophilic sulfate-reducing bacteria. III. Adenosine triphosphate-sulfurylase of *Clostridium nigrificans* and *Desulfovibrio desulfuricans*. J. Bacteriol. **84**:1194–1201. 1962.—Adenosine triphosphate (ATP)-sulfurylase, which catalyzes the formation of adenosine-5′-phosphosulfate (APS) from ATP and $SO_4^=$, has been purified from crude extracts of *Clostridium nigrificans* and *Desulfovibrio desulfuricans* by $(NH_4)_2SO_4$ fractionation and triethylaminoethyl column chromatography. The enzyme from both sources operates over a broad pH range from 6.0 to 9.5. Below pH 6.0, activity decreases sharply, with no detectable activity at pH 5.0. Of the nucleotides tested (ATP and the triphosphates of deoxyadenosine, uridine, inosine, and guanosine), only ATP was acted upon by the enzyme from either source. The enzyme requires Mg^{++} for activity. Incubation of the enzyme from both organisms with ATP and $S^{35}O_4^=$ in the presence of helium resulted in the formation of an S^{35}-labeled nucleotide whose electrophoretic mobility was identical to that of chemically prepared APS. When incubated with ATP and the group VI anions (CrO_4, MoO_4, WO_4), the enzyme from both organisms formed an unstable intermediate, resulting in the accumulation of pyrophosphate. Thermal stability studies revealed that the ATP-sulfurylase of *C. nigrificans* was stable at higher temperatures than the enzyme obtained from *D. desulfuricans*. Exposure of the enzyme from *C. nigrificans* to 65 C for 2 hr gave virtually no decrease in activity. In contrast, the enzyme from *D. desulfuricans* was completely inactivated after 30 min at 55 C, after 3 min at 60 C, or after 1 min at 65 C.

The sulfur-containing nucleotides adenosine-5′-phosphosulfate (APS) and 3′-phospho-adenosine-5′-phosphosulfate (PAPS) have been established as the activated forms of sulfate in biological systems (Robbins and Lipmann, 1956*a*, *b*, 1957). The initial reaction leading to these intermediates involves the participation of the enzyme adenosine triphosphate (ATP)-sulfurylase. This enzyme catalyzes the nucleophilic displacement of the pyrophosphate moiety in ATP by the sulfate anion, resulting in the formation of APS. In yeast and in certain mammalian cells (Wilson and Bandurski, 1956, 1958; Lipmann, 1958; Robbins and Lipmann, 1958*a*), APS is further phosphorylated in the 3′-position of the ribose moiety forming PAPS. This latter compound can then participate in various sulfate-transfer reactions, resulting in the assimilatory metabolism of sulfate. In contrast, in the sulfate-reducing bacteria, *Clostridium nigrificans* and *Desulfovibrio desulfuricans*, the APS formed (Peck, 1959, 1962*a*; Ishimoto, 1959; Ishimoto and Fujimoto, 1959) is reduced to adenosine monophosphate (AMP) and sulfite through the mediation of APS-reductase (Peck, 1961*a*, *b*, 1962*a*, *b*; Ishimoto and Fujimoto, 1961). Hydrogen sulfide appears as the end product of the subsequent series of reactions. Thus, the dissimilatory process of sulfate reduction occurring in these organisms involves APS and not PAPS as the active intermediate compound (Peck, 1962*b*).

As part of a comparative program on the mechanism of sulfate reduction by *C. nigrificans*

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and *D. desulfuricans* (Campbell, Frank, and Hall, 1957; Akagi and Campbell, 1961; Baker, Papiska, and Campbell, 1962), the ATP-sulfurylase of these two organisms was purified and studied. A preliminary report of this work has appeared (Akagi and Campbell, 1962).

MATERIALS AND METHODS

Organisms. C. nigrificans, strain 8351, was grown at 55 C in the PYG medium of Postgate (1951) as described by Campbell et al. (1957). The source and maintenance of the culture have been described (Campbell et al., 1957). *D. desulfuricans*, strain 8303, was obtained from J. R. Postgate. It was grown at 30 C in a medium containing NH_4Cl, 1.0 g; KH_2PO_4, 0.5 g; Na_2SO_4, 2.6 g; $MgSO_4 \cdot 7H_2O$, 2.0 g; $CaCl_2 \cdot 2H_2O$, 0.06 g; yeast extract (Difco), 1.0 g; and sodium lactate, 6.0 g/liter of distilled water. After sterilization, the medium was adjusted to pH 7.2 with sterile 1 N NaOH. Just prior to inoculation, $Fe(NH_4)_2 \cdot (SO_4)_2 \cdot 6H_2O$ was added from a filter-sterilized stock solution to give a final concentration of 10 μg/ml. Solid media were prepared by the addition of agar (20 g/liter).

Since these organisms are obligate anaerobes, they were grown in the presence of a potassium carbonate-pyrogallol seal.

Preparation of cell-free extracts. The methods employed for large-scale cultivation of both organisms were similar to those described previously (Campbell et al., 1957; Akagi and Campbell, 1961). Cells from 100-liter cultures were harvested with a Sharples centrifuge or a Servall continuous-flow centrifuge. Cell-free extracts were obtained by passing a 50% cell suspension in 0.1 M tris(hydroxymethyl)aminomethane(tris)hydrochloride buffer of appropriate pH value through a French pressure cell (20,000 lb/in^2). Cell debris was removed by centrifugation at 27,000 $\times$ *g* for 20 min.

Enzyme assays. ATP-sulfurylase was assayed by the molybdate method described by Bandurski, Wilson, and Squires (1956). The reaction mixture contained ATP, 5 μmoles; $MgCl_2$, 5 μmoles; Na_2MoO_4, 10 μmoles; tris buffer (pH 7.2, *C. nigrificans*; pH 8.0, *D. sulfuricans*), 50 μmoles; purified yeast inorganic pyrophosphatase, 50 μg; and enzyme in a total volume of 0.75 ml. Incubation time was 10 min at 30 C. (The temperature of assay for the sulfurylase of *C. nigrificans* was lowered to 30 C for two main reasons. One was that the presence of a very potent adenosine triphosphatase in the crude extracts at 55 C quickly reduced the availability of exogenous ATP. This hindered the assay for ATP-sulfurylase by the molybdate method. By lowering the assay temperature to 30 C, this complication did not arise, and the activity of the enzyme was readily detected. A second reason was that with as little as 2 μg of the purified sulfurylase the reaction was so rapid at 55 C that it was virtually complete at the end of 10 sec. Since this was found to increase the chances of error, in several of the experiments the temperature was kept at 30 C.) The reaction was stopped by the addition of 1.0 ml of 5 N H_2SO_4, and the necessary reagents were added for the determination of inorganic phosphate according to the method of Fiske and SubbaRow (1925). One unit of enzyme is defined as that amount which produces 1 μmole of inorganic phosphate per hr under the conditions specified. Specific activity is expressed as the number of units per mg of protein. Inorganic pyrophosphatase activity was followed by determining the liberation of inorganic phosphate from sodium pyrophosphate after 10 min of incubation at 30 C. The reaction mixture contained $MgCl_2$, 5 μmoles; $Na_4P_2O_7$, 5 μmoles; tris buffer (pH 7.5), 50 μmoles; and enzyme in a final volume of 0.6 ml. Adenosine diphosphate (ADP)-sulfurylase was assayed by the method of Robbins and Lipmann (1958*a*), and adenylate kinase by the method of Colowick (1955).

Special reagents. Nucleotide triphosphates were obtained from the Sigma Chemical Co., St. Louis, Mo. $Na_2S^{35}O_4$ and sodium radiophosphate were obtained from the Abbott Laboratories, North Chicago, Ill. Synthetic APS was prepared by the method of Baddiley, Buchanan, and Letters (1957) and isolated by paper electrophoresis. Electrophoresis was performed on Whatman 3 MM paper in 0.03 M citrate buffer (pH 5.9), with a Spinco model R apparatus operating at 400 v for 3 hr at 4 C. Nucleotides were detected with a shortwave ultraviolet lamp. APS was eluted from the paper with water, a sample was hydrolyzed with 0.1 N HCl, and the amount of APS formed was determined by treatment with 5′-adenylic acid deaminase. The 5′-adenylic acid deaminase was prepared by the method of Nikiforuk and Colowick (1955). Purified yeast inorganic pyrophosphatase was prepared by the procedure of Heppel and Hilmoe (1951). Protein was measured

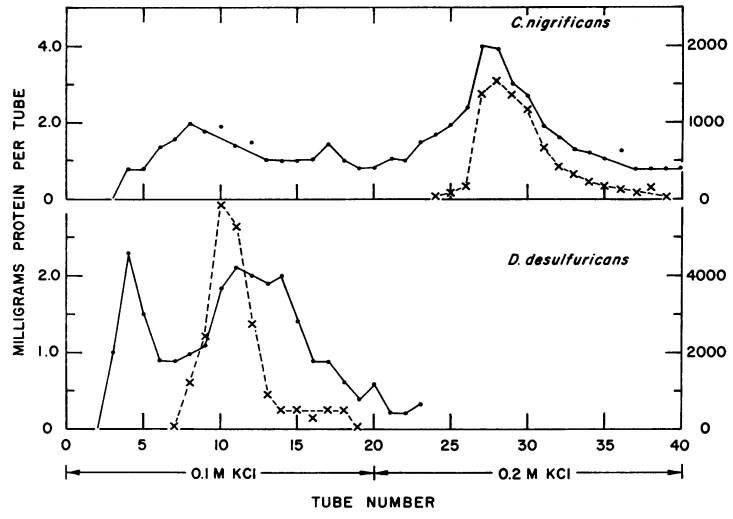

FIG. 1. *Chromatographic purification of ATP-sulfurylase of Clostridium nigrificans and Desulfovibrio desulfuricans on TEAE.*

by the method of Lowry et al. (1951). Inorganic pyrophosphate labeled with P^{32} was prepared by pyrolysis of sodium radiophosphate with non-radioactive K_2HPO_4.

Triethylaminoethyl cellulose (TEAE) with an exchange capacity of 0.75 ± 0.1 meq/g was obtained from Bio-Rad Laboratories. The resin (100 g) was suspended in 4 liters of 0.1 N NaOH and stirred for 1 hr. After a settling time of 1 hr, the alkali was decanted and the procedure repeated once. Distilled water (4 liters) was added and the resin was stirred for 20 min and allowed to settle for 30 min. This procedure was repeated twice. The resin was then adjusted to pH 8.0 by the addition of 6 N HCl and washed twice with distilled water with 30-min settling periods; 2 liters of 0.5 M KCl-0.05 M tris buffer (pH 8.0) were added to the resin and the slurry was stirred for 4 hr. The resin was then washed five times with 4 liters of distilled water, with 30-min settling periods, and stored in water at 4 C until used.

Purification of ATP-sulfurylase from D. desulfuricans. The cell-free extract was adjusted to a protein concentration of 10 to 15 mg per ml and centrifuged at 105,000 × *g* for 2 hr. The supernatant fluid was carefully decanted from the black pellet. Very little, if any, sulfurylase activity was detected in the pellet fraction. Solid $(NH_4)_2SO_4$ was slowly added with gentle stirring to the supernatant liquid to a saturation of 50%. After standing for 1 hr at 0 C, the mixture was centrifuged at 27,000 × *g* for 30 min. The precipitate was discarded, and additional $(NH_4)_2SO_4$ was slowly added with stirring until 60% saturation was obtained. After 30 min at 0 C, the mixture was centrifuged as above. The precipitate was dissolved in a minimal amount of 0.1 M tris buffer (pH 8.0) and dialyzed for 3 hr against 200 volumes of 0.025 M tris-0.001 M ethylenediaminetetraacetate (EDTA) buffer (pH 8.0). The dialyzed enzyme was applied to a TEAE column (1.8 × 20 cm for 60 mg of protein), and eluted with a 0.1 M KCl-0.05 M tris buffer, pH 8.0 (Fig. 1). The tubes (8 to 15) containing sulfurylase were pooled and 1 mg of Cγ alumina gel was added per mg of protein. The gel-enzyme mixture was dialyzed for 3 hr at 0 C against 50 volumes of 0.025 M tris-0.001 M EDTA buffer (pH 7.0). All of the sulfurylase adsorbed to the gel and was eluted completely by successive treatments with small portions of 0.001 M ATP in 0.1 M tris buffer (pH 7.0). Table 1 presents the purification scheme of the sulfurylase of *D. desulfuricans*.

Purification of ATP-sulfurylase from C. nigrificans. Essentially the same fractionation procedure was employed for purifying the sulfurylase from the thermophile as was used for *D. desulfuricans*. Some differences were noted, however, during the fractionation: (i) the enzyme precipitated at an $(NH_4)_2SO_4$ concentration between 0 to 50% saturation, and (ii) the enzyme from *C. nigrificans* was eluted from the TEAE column with 0.2 M

TABLE 1. *Purification of ATP-sulfurylase from Desulfovibrio desulfuricans*

Fraction	Volume	Units	Specific activity	Recovery
	ml		*units/mg*	%
Crude extract	42	55,440	94	100
Dialyzed crude extract	43	56,760	98	100
105,000 × *g* supernatant liquid	43	56,760	155	100
$(NH_4)_2SO_4$ ppt	6.5	41,340	980	75
TEAE eluate	103	19,470	1,070	35
Gel eluate	10	8,160	4,080	14.7

TABLE 2. *Purification of ATP-sulfurylase from Clostridium nigrificans*

Fraction	Volume	Units	Specific activity	Recovery
	ml		*units/mg*	%
Crude extract	50	27,440	22.4	100
105,000 × *g* supernatant liquid	72	23,904	41.5	87
$(NH_4)_2SO_4$ ppt	11	10,580	87.5	38.5
TEAE eluate	42	5,508	306	20
Gel eluate	5	288	500	1.1

KCl-0.05 M tris buffer, pH 8.0 (Fig. 1). The purification scheme for the sulfurylase of *C. nigrificans* is presented in Table 2.

The sulfurylase of both *C. nigrificans* and *D. desulfuricans* showed a tendency to inactivate fairly rapidly during the fractionation procedure, and it was necessary to accomplish the steps leading to the application onto the TEAE column within a 12-hr period. The eluates from the alumina gel, however, were stable for several months when stored at −20 C under an atmosphere of hydrogen. The purified ATP-sulfurylase of both organisms was free from adenosine triphosphatase, adenylate kinase, ADP-sulfurylase and inorganic pyrophosphatase activities.

RESULTS

Nucleotide specificity. Incubation with ATP and triphosphates of guanosine, inosine, cytidine, uridine, and deoxyadenosine demonstrated that ATP was the only nucleotide acted upon by the sulfurylase of both organisms (Table 3).

Activity with different group VI anions. Table 4 shows that with molybdate, chromate, and tungstate the enzyme forms an unstable anhydride, resulting in the accumulation of pyrophosphate. With sulfate and selenate, very little inorganic phosphate was detected. This indicates that stable intermediates were formed which were converted back to ATP and the respective anions.

TABLE 3. *Nucleotide specificity of ATP-sulfurylase**

Nucleotide	Inorganic phosphate formed	
	D. desulfuricans	*C. nigrificans*
	μmoles	*μmoles*
ATP	0.51	0.61
deATP	0.05	0.08
CTP	0.00	0.00
GTP	0.00	0.05
ITP	0.00	0.00
UTP	0.00	0.00

* Each tube contained 50 μmoles of tris buffer (pH 7.2 for *C. nigrificans*; pH 8.0 for *D. desulfuricans*), 5 μmoles of $MgCl_2$, 0.5 μmole of nucleotide, 10 μmoles of Na_2MoO_4, 25 μg of purified yeast inorganic pyrophosphatase, and 4 μg of enzyme, in a total volume of 0.75 ml. Incubation was for 30 min at 30 C.

TABLE 4. *Effect of group VI anions on ATP-sulfurylase**

Group VI anion	Inorganic phosphate formed	
	D. desulfuricans	*C. nigrificans*
	μmoles	*μmoles*
SO_4	0.04	0.03
SeO_4	0.00	0.02
MoO_4	1.31	1.12
CrO_4	0.98	0.61
WO_4	0.48	0.40

* Each tube contained 25 μmoles of tris buffer (pH 7.2 for *C. nigrificans*; pH 8.0 for *D. desulfuricans*), 5 μmoles of $MgCl_2$, 2.5 μmoles of ATP, 2 μmoles of anion, 25 μg of purified yeast inorganic pyrophosphatase, and 20 μg of enzyme in a total volume of 0.75 ml. Incubation was for 10 min at 30 C.

Activity as a function of enzyme concentration. Under the conditions of the standard assay, sulfurylase activity was proportional to the amount of enzyme present over the range studied (Fig. 2).

Effect of Mg^{++} ion on enzyme activity. Table 5 shows that the sulfurylase prepared from *C.*

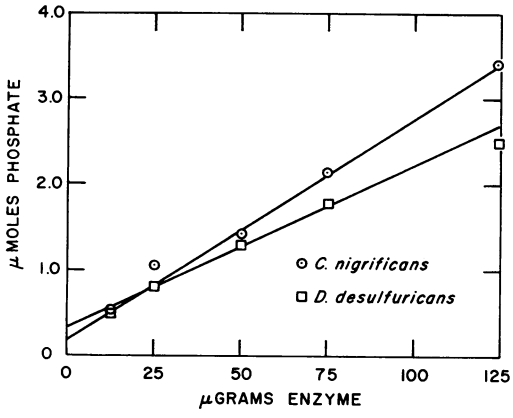

FIG. 2. *Effect of enzyme concentration on ATP-sulfurylase activity. Reaction mixtures contained 5 μmoles of ATP; 5 μmoles of $MgCl_2$; 10 μmoles of Na_2MoO_4; 50 μmoles of tris buffer (pH 7.2 for C. nigrificans; pH 8.0 for D. desulfuricans); 50 μg of purified yeast inorganic pyrophosphatase; and the indicated amount of enzyme in a total volume of 0.75 ml. Incubation was at 30 C for 10 min.*

TABLE 5. *Effect of Mg^{++} on ATP-sulfurylase activity**

Mg^{++}	Inorganic phosphate formed	
	D. desulfuricans	*C. nigrificans*
μmoles	*μmoles*	*μmoles*
0.00	0.00	0.00
0.05	0.10	0.77
0.15	0.23	1.50
0.25	0.33	2.30
0.50	0.68	3.30
1.25	1.16	4.6
2.5	2.40	4.6

* Each tube contained 50 μmoles of tris buffer (pH 7.2 for *C. nigrificans*; pH 8.0 for *D. desulfuricans*), 10 μmoles of Na_2MoO_4, 2.5 μmoles of ATP, $MgCl_2$ as indicated, and 20 μg of enzyme, in a final volume of 0.75 ml. After 20 min of incubation at 30 C, the tubes were placed in a boiling-water bath for 90 sec and quickly cooled. To each tube were added 5 μmoles of $MgCl_2$ and 50 μg of purified yeast inorganic pyrophosphatase, and the tubes were incubated for 10 min at 30 C.

nigrificans and *D. desulfuricans* requires Mg^{++} ion for activity.

Effect of pH on sulfurylase activity. The effect of pH on enzyme activity is shown in Fig. 3. The enzyme from *D. desulfuricans* showed a broad optimum from pH 8.0 to 9.5, while that from *C. nigrificans* exhibited maximal activity at pH 7.0 to 7.5.

Thermal stability studies. Because of differences in the temperature for growth of *C. nigrificans* and *D. desulfuricans*, the effect of temperature on enzyme stability was studied. Figure 4 shows that the sulfurylase purified from the thermophile (*C. nigrificans*) is quite stable to temperatures from 60 to 65 C. In contrast, the sulfurylase of *D. desulfuricans* was rapidly inactivated when tested under the same conditions. It was found, however, that the enzyme of *D. desulfuricans* could be protected against inactivation at 60 C by the presence of ATP. Under these conditions, the enzyme retained 60% of its activity after 15 min (Table 6). This is in contrast to the complete loss of activity in 3 min when exposed to 60 C in the absence of ATP (Fig. 4).

Formation of APS and measurement of equi-

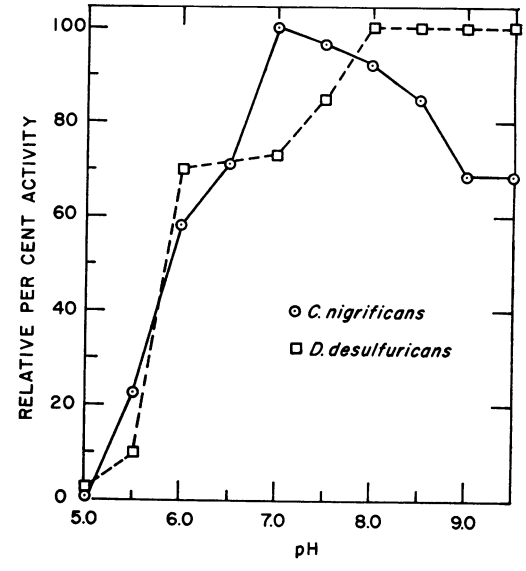

FIG. 3. *Effect of pH on ATP-sulfurylase activity. Reaction mixtures contained 5 μmoles of ATP; 5 μmoles of $MgCl_2$; 10 μmoles of Na_2MoO_4; 50 μmoles of tris-maleate buffer of the pH indicated; 10 μg of enzyme in a total volume of 0.75 ml. Incubation was at 30 C for 10 min. At the end of the reaction time, 1.0 ml of a 1 M tris-HCl buffer (pH 7.2) was added to each tube, and the tubes were immediately immersed in a boiling-water bath for 90 sec. After cooling, 50 μg of purified yeast inorganic pyrophosphatase were added to each tube. The tubes were incubated for 30 min at 30 C and analyzed for inorganic phosphate.*

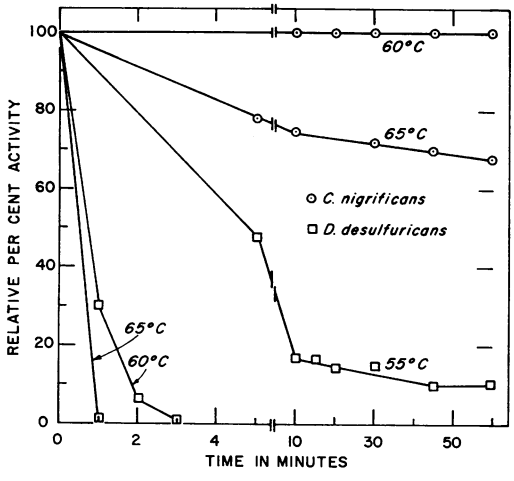

FIG. 4. *Thermal stability of ATP-sulfurylase of Clostridium nigrificans and Desulfovibrio desulfuricans. A series of tubes containing the purified enzyme (5 μg for C. nigrificans; 150 μg for D. desulfuricans) in 50 μmoles of tris buffer (pH 7.2 for C. nigrificans; pH 8.0 for D. desulfuricans) was incubated at the temperatures indicated in the absence of substrate. At suitable time intervals, tubes were removed and immersed in an ice bath. To each tube were added 5 μmoles of ATP; 5 μmoles of $MgCl_2$; 10 μmoles of Na_2MoO_4; and 50 μg of purified yeast inorganic pyrophosphatase. Incubation was at 30 C for 10 min. Zero-time controls were kept in an ice bath during the exposure time. All activities at the different temperatures and exposure times were compared to the zero-time controls.*

librium constant. The formation of S^{35}-APS by the sulfurylase of both organisms was established in the following manner. Reaction mixtures contained 5 μmoles of ATP, 5 μmoles of $MgCl_2$, 50 μmoles of tris buffer (pH 8.0 for *D. desulfuricans* and pH 7.2 for *C. nigrificans*), 5 μmoles of Na_2SO_4 ($Na_2S^{35}O_4$, corresponding to 1.06×10^5 count/min), 100 μg of purified yeast inorganic pyrophosphatase, and 20 μg of purified ATP-sulfurylase. After incubation for 1 hr at 30 C, the reaction was stopped by boiling for 90 sec. The reaction mixtures were subjected to paper electrophoresis as described in Materials and Methods. A radioactive nucleotide was detected which migrated identically with authentic chemically synthesized APS.

An estimation of the equilibrium constant for the two sulfurylases was obtained from the experiment described in Table 7. After incubation of the reaction mixture with pyrophosphatase,

TABLE 6. *Effect of ATP on thermal stability of ATP-sulfurylase of Desulfovibrio desulfuricans**

Heating time	Inorganic phosphate formed	
	Without ATP	With ATP (5 μmoles)
min	*μmoles*	*μmoles*
0	0.51	0.53
3	0.00	0.36
5	0.00	0.33
10	0.00	0.28
15	0.00	0.28

* The enzyme (20 μg) was heated at 60 C in the presence and absence of ATP in 0.5 ml of 0.1 M tris buffer (pH 8.0) for the times indicated. Activity was then determined by the standard sulfurylase assay described in Materials and Methods.

500 mg of acid-washed charcoal (Norite A) were added to each tube, followed by 5 ml of cold distilled water. The tubes were stirred for 60 min to adsorb the nucleotide(s) to the charcoal. The charcoal was then washed five times with 5 ml of cold distilled water. The wash water was pooled and samples counted for radioactivity. The nucleotides were eluted from the charcoal with an alcohol-ammonia solution (50% ethanol-2% NH_4OH). Samples of the eluate were counted for radioactivity. To verify that the radioactivity counted in the alcohol-ammonia eluate was due to ATP^{32}, paper electrophoresis of a portion of the eluate was performed. The only nucleotide detected corresponded to ATP and contained all of the radioactivity. The over-all recovery of radioactivity was 93% in both cases. The apparent sulfurylase equilibrium constants calculated from the data were 1.8×10^{-8} for *C. nigrificans* and 6.2×10^{-9} for *D. desulfuricans*. While these are only approximate values, they are in essential agreement with those obtained (4×10^{-8} and 1×10^{-8}) for yeast ATP-sulfurylase by Wilson and Bandurski (1958) and Robbins and Lipmann (1958*b*).

DISCUSSION

The data presented show that the ATP-sulfurylase of *C. nigrificans* is similar to that of *D. desulfuricans* and that both enzymes are similar to yeast ATP-sulfurylase (Wilson and Bandurski, 1958; Robbins and Lipmann, 1958*b*) with respect to their catalytic activity. This is evidenced by (i) their identical behavior with regard to ATP

TABLE 7. *Estimation of sulfurylase equilibrium constant by P^{32}-pyrophosphate incorporation into ATP**

Sulfurylase	P^{32}-PP remaining at equilibrium		ATP^{32} found at equilibrium		K†
	Amount	Activity	Amount	Activity	
	μmoles	*count/min*	*μmoles*	*count/min*	
D. desulfuricans	5.8×10^{-4}	3.24×10^{3}	5.28	15.59×10^{5}	6.2×10^{-9}
C. nigrificans	9.7×10^{-4}	5.4×10^{3}	5.28	15.52×10^{5}	1.8×10^{-8}

* Reaction mixtures contained 5 μmoles of ATP, 10 μmoles of Na_2SO_4, 5 μmoles of $MgCl_2$, 50 μmoles of tris buffer (pH 8.0 for *D. desulfuricans*; pH 7.2 for *C. nigrificans*), 0.3 μmole of APS, 0.3 μmole of P^{32}-PP (inorganic pyrophosphate, corresponding to 16.74×10^5 count/min) and 2 μg of enzyme. After incubation for 2 hr at 30 C, the reaction was stopped by boiling for 90 sec. After cooling, purified yeast inorganic pyrophosphatase (100 μg) was added to each tube, and incubation was continued for 30 min at 30 C to hydrolyze any remaining PP to inorganic phosphate. Tubes were then analyzed as described in the text.

† In calculating K, the assumption was made that the system was in equilibrium after 2 hr of incubation and that the amount of APS utilized was equivalent to the amount of PP utilized.

specificity, (ii) the utilization of a number of group VI anions in place of sulfate, (iii) the requirement for Mg^{++} ion, and (iv) the close correlation of their equilibrium constants.

Some differences in structure probably exist, however, between the sulfurylases prepared from *C. nigrificans* and *D. desulfuricans*. They behave differently with respect to (i) precipitability with $(NH_4)_2SO_4$, (ii) elution properties from TEAE, (iii) pH and temperature optima, and (iv) thermal stability. The latter difference is not surprising since many enzymes prepared from thermophilic bacteria are considerably more heat-stable than the corresponding enzymes prepared from mesophilic organisms (see review of Koffler, 1957).

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