[58] Overview: Inorganic Sulfur and Sulfate Activation

By JEROME A. SCHIFF and TEKCHAND SAIDHA

In nature sulfur exists in a wide range of oxidation states. The oxidized and reduced forms of sulfur can be interconverted by various organisms (Fig. 1). The metabolism of the most highly oxidized naturally occurring form of sulfur, sulfate, begins with the entrance of sulfate into the cell, which is accomplished through active transport mediated by a carrier-enzyme system.[1] The uptake of sulfate is followed by activation and by transfer and reduction reactions in appropriate organisms.

Reactions of Sulfate Activation and Their Distribution

Sulfate must be converted to an activated form before it can be utilized metabolically. There are two forms of activated sulfate, adenosine 5′-phosphosulfate (APS) and 3′-phosphoadenylylsulfate (adenosine 3′-phosphate 5′-phosphosulfate, PAPS). They are formed, as described in this volume [59], in two sequential enzyme-catalyzed reactions involving ATP-sulfurylase (sulfate adenylyltransferase, EC 2.7.7.4) and APS kinase (adenylylsulfate kinase, EC 2.7.1.25) (Fig. 2). The first step in the biosynthesis of PAPS, leading to the formation of APS, is catalyzed by the enzyme ATP-sulfurylase. This reaction is greatly favored energetically in the reverse direction as the free energy of hydrolysis of the sulfate group in APS is considerably higher than the free energy of the phosphate linkage of ATP. Therefore, the forward reaction proceeds to a reasonable extent only when the products of the reaction, APS and PP_i, are removed. PP_i is cleaved by the ubiquitous inorganic pyrophosphatase, and APS is removed by phosphorylation by APS-kinase and ATP in the second step or by utilization in other reactions. The resulting overall standard free energy change (Fig. 2)[2,3] helps explain accumulation of PAPS.

APS-kinase has a very high affinity for APS, giving the highest reaction rates at the lowest measurable concentration (5 μM). This characteristic of the enzyme helps to drive the first reaction in the forward direction by eliminating APS from the equilibrium. The three reactions together

[1] J. A. Schiff, *Encycl. Plant Physiol., New Ser.* **24,** 401 (1983).

[2] A. B. Roy and P. A. Trudinger, "The Biochemistry of Inorganic Compounds of Sulfur," p. 91. Cambridge Univ. Press, London and New York, 1970.

[3] T. W. Goodwin and E. I. Mercer, eds., "Introduction to Plant Biochemistry," p. 273. Pergamon, Oxford, 1982.

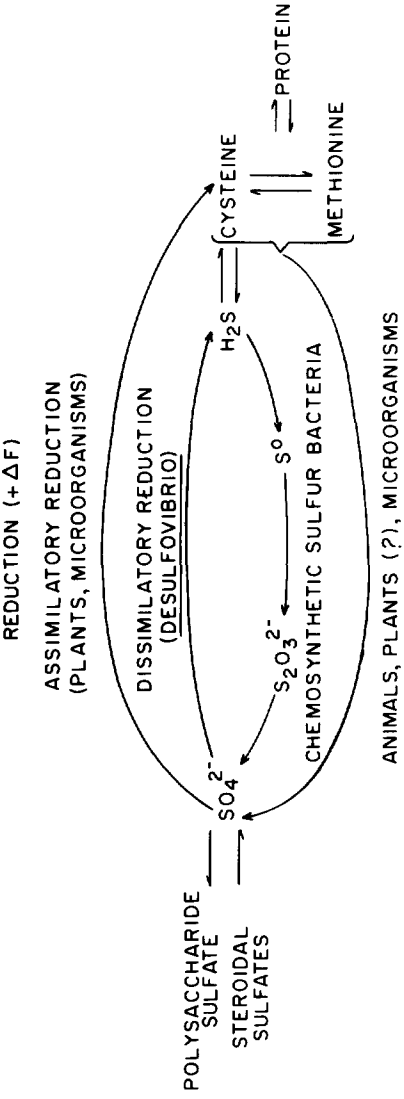


FIG. 1. Reactions of sulfur in the biosphere.

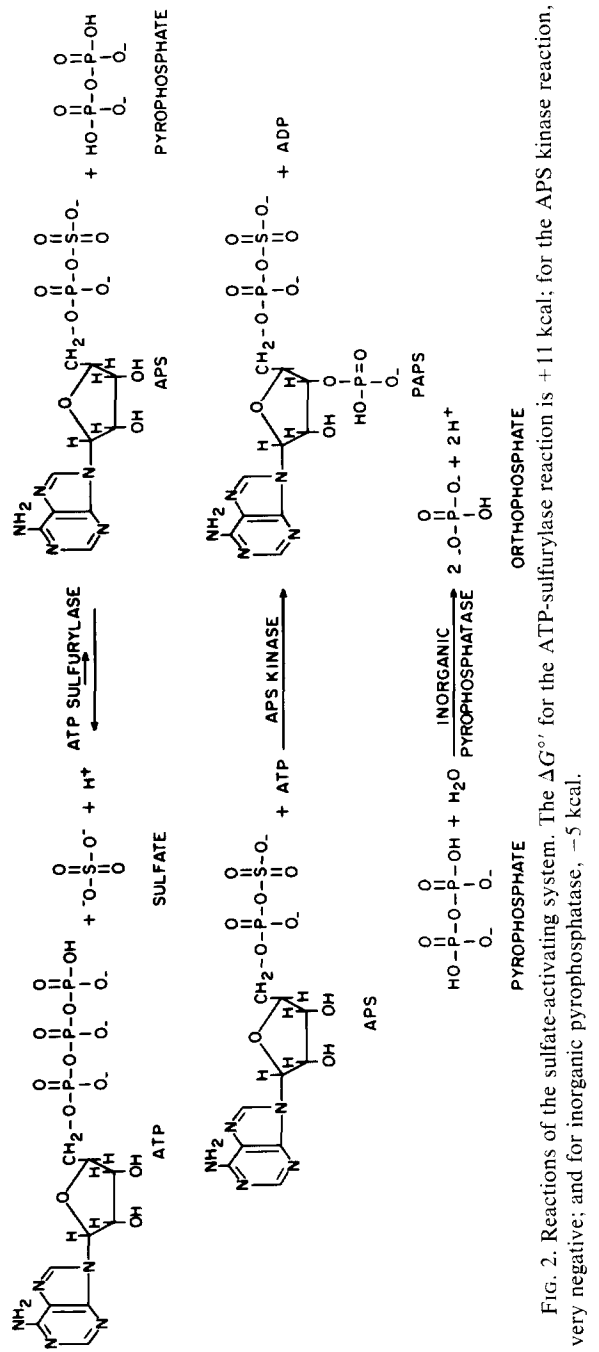


FIG. 2. Reactions of the sulfate-activating system. The $\Delta G^{o\prime}$ for the ATP-sulfurylase reaction is +11 kcal; for the APS kinase reaction, very negative; and for inorganic pyrophosphatase, −5 kcal.

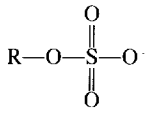
comprise the sulfate activating system. A widely distributed 3′(2′),5′-diphosphonucleoside 3′(2′)-phosphohydrolase (DPNPase) catalyzes the conversion of PAPS to APS. This enzyme may provide control of cellular levels of APS and PAPS which are required for different metabolic functions.[1]

The activating enzyme system that synthesizes PAPS catalyzes the following overall reaction: 2 ATP + SO_4^{2-} → ADP + PAPS + PP_i, and is distributed widely in nature.[4] It is present in most mammalian tissues including those of adrenal glands, brain, cartilage, cornea, heart, ovaries, pancreas, placenta, retina, skin, spleen, and a variety of tumors. It has also been found in chick embryo, hen oviduct, and frog liver. Among invertebrates it occurs in snails, some sea urchins, and the clam *Spisula solidissima*. It is also present in higher plants, yeast, *Fusarium solani*, *Euglena gracilis*, and a number of other algae. The anaerobic sulfate-reducing bacterium, *Desulfovibrio*, appears to have only ATP-sulfurylase and to lack APS kinase.

Evidence relating to the cellular localization of the sulfate-activating system is scanty. The activating enzymes have been shown to be present in spinach chloroplasts[5] and on the outer surface of the mitochondrial inner membrane in *Euglena*.[6]

Sulfate Transfer and Reduction

Sulfate is used by living systems to form sulfate esters



of polysaccharides, phenols, steroids, and other organic compounds through transfer reactions in which PAPS is the sulfuryl group donor, catalyzed by sulfotransferases of various specificities.[4,7,8] APS or PAPS has also been suggested as a donor of the sulfonic acid group

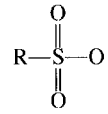
[4] R. H. De Meio, *in* "Metabolic Pathways" (D. M. Greenberg, ed.), 3rd ed., Vol. 7, p. 287. Academic Press, New York, 1975.

[5] J. D. Schwenn and A. Trebst, *in* "The Intact Chloroplast" (J. Barber, ed.), p. 315. Elsevier, Amsterdam, 1976.

[6] T. Saidha, A. I. Stern, D.-H. Lee, and J. A. Schiff, *Biochem. J.* **232,** 357 (1985).

[7] J. A. Schiff and R. C. Hodson, *Annu. Rev. Plant Physiol.* **24,** 381 (1973).

[8] W. B. Jakoby, R. D. Sekura, E. S. Lyon, C. J. Marcus, and J.-L. Wang, *in* "Enzymatic Basis of Detoxication" (W. B. Jakoby, ed.), Vol. 2, p. 199. Academic Press, New York, 1980.



of the plant sulfolipid component, 6-sulfo-6-deoxy-D-glucose (6-sulfoquinovose); the sulfonic acid group is at the redox level of sulfite.[9] Other sulfonic acids of biological interest are cysteic acid, which will serve as a sulfur source for *Neurospora,* and taurine, which is present in animal systems and can be formed from oxidation of cysteine or from sulfate in chick embryos. Homocysteic acid, another sulfonic acid, is formed by Sat_2^-, a mutant of *Chlorella pyrenoidosa* which is blocked late in the sulfate reduction pathway.

Dissimilatory sulfate reduction occurs in certain anaerobic bacteria such as *Desulfovibrio* which use sulfate in place of molecular oxygen (Fig. 1). In this process APS is the nucleotide sulfate donor, and reduction results in the accumulation of hydrogen sulfide and in ATP synthesis through oxidative phosphorylation.[10] Assimilatory sulfate reduction, in which sulfate is reduced to form sulfur at the thiol level in sulfur-containing amino acids, coenzymes, and other organic compounds, now appears to exhibit two distinct patterns.[1] (1) In oxygen-evolving photosynthesizers including some blue-green algae (cyanobacteria), eukaryotic algae, higher plants, and spinach chloroplasts, the nucleotide sulfate donor is APS acting with a highly specific APS sulfotransferase (adenylyl sulfate : thiol sulfotransferase) which will not use PAPS. This is the "APS pathway." (2) PAPS is the nucleoside phosphosulfate donor for reduction by a specific PAPS sulfotransferase (3′-phosphoadenylyl : thiol sulfotransferase) which will not use APS. This is the "PAPS pathway" that operates in organisms which lack oxygen-evolving photosynthesis (besides animals which do not reduce sulfate to the thiol level), such as yeast and *Escherichia coli,* in other bacteria, and in a few oxygen-evolving blue-green algae. Detailed reactions of the two assimilatory pathways have been presented.[1]

Control of sulfate activation takes place in some of the microorganisms that reduce sulfate.[4] In *E. coli* the formation of PAPS is repressed by cysteine, and in *Bacillus subtilis* by both cysteine and glutathione. The two steps of activation seem to be repressed simultaneously, and there is an inverse relationship between the specific activity of the activating sys-

[9] J. L. Harwood, *in* "The Biochemistry of Plants" (P. K. Stumpf, ed.), Vol. 4, p. 301. Academic Press, New York, 1980.

[10] H. Bothe and A. Trebst, eds., "Biology of Inorganic Nitrogen and Sulfur." Springer-Verlag, Berlin and New York, 1981.

tem and the intracellular concentration of cysteine. This type of control is not present in all microorganisms. In *Desulfovibrio,* for instance, ATP-sulfurylase is not repressed by either cysteine or sulfite.[4] This could be explained by the fact that in this microorganism APS is the only activated form of sulfate that is produced; also, sulfate reduction is required constitutively for respiration and coupled phosphorylation. The sulfate-reducing pathway in yeast appears to be regulated at the ATP-sulfurylase step through feedback inhibition by sulfide and through methionine acting as a repressor.[4]

Most organisms, including higher animals and plants, oxidize reduced sulfur to sulfate, although the aerobic chemosynthetic bacteria (Fig. 1) are the only organisms which have been observed to couple the energy released, some 180 kcal/mol, to the reduction of carbon dioxide. The anoxygenic photosynthetic bacteria use reduced sulfur compounds as photosynthetic electron donors by oxidizing them to sulfur, thiosulfate, and sulfate.[10]

Acknowledgments

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[59] Sulfate-Activating Enzymes

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Reactions

The two sulfate-activating enzymes, ATP-sulfurylase (ATP: sulfate adenylyltransferase; EC 2.7.7.4, sulfate adenylyltransferase), reaction (1), and APS kinase (ATP: adenylylsulfate 3′-phosphotransferase; EC 2.7.1.25, adenylylsulfate kinase), reaction (2), catalyze the activation of inorganic sulfate first to APS (adenosine- 5′-phosphosulfate or 5′-adenylylsulfate) and then to PAPS (adenosine 3′-phosphate 5′-phosphosulfate, or 3′-phospho-5′-adenylylsulfate, or 3′-phosphoadenosine 5′-phosphosulfate). Although the equilibrium of the sulfurylase reaction lies far to the left, the overall production of PAPS *in vivo* is promoted by the hydrolysis of the inorganic pyrophosphate, reaction (3), and the favorable APS kinase reaction.

$$\mathrm{ATP} + \mathrm{SO_4^{2-}} \xrightleftharpoons{\text{ATP-sulfurylase}} \mathrm{PP_i} + \mathrm{APS} \qquad (K_{eq} \sim 10^{-7}) \qquad (1)$$

$$\mathrm{ATP} + \mathrm{APS} \xrightleftharpoons{\text{APS kinase}} \mathrm{PAPS} + \mathrm{ADP} \qquad (K_{eq} \sim 10^{3}) \qquad (2)$$

$$\mathrm{PP_i} + \mathrm{H_2O} \xrightleftharpoons{\text{Pyrophosphatase}} 2\ \mathrm{P_i} \qquad (K_{eq} \sim 10^{3}\ M) \qquad (3)$$

$$2\ \mathrm{ATP} + \mathrm{SO_4^{2-}} \rightleftharpoons \mathrm{PAPS} + \mathrm{ADP} + 2\ \mathrm{P_i} \qquad (K_{eq} \sim 10^{-1}\ M)$$