ATP sulfurylases from *Penicillium chrysogenum*, *P. chrysogenum* var. *dumontii*, *Aspergillus nidulans*, and *Neurospora crassa* are strongly inhibited by 3'-phosphoadenosine-5'-phosphosulfate (PAPS), the product of the second (adenosine-5'-phosphosulfate kinase-catalyzed) reaction in the two-step activation of inorganic sulfate. The *v* versus [PAPS] plots are sigmoidal. At physiological concentrations of MgATP (0.17–3 mM) and SO$_4^{2-}$ (0.4–10 mM), the *K$_{m}$* for PAPS inhibition of the *P. chrysogenum* enzyme is 35–200 μM; *K$_{m}$* is 68–310 μM. In the presence of PAPS, the *S$_{0.5}$* values for both substrates are increased and the *v* versus [MgATP] and *v* versus [SO$_4^{2-}$] or [MoO$_4^{2-}$] plots are sigmoidal. Fluorosulfonate (FSO$_3^-$) and thiosulfate (S$_2$O$_3^{2-}$) (non-reactive sulfate analogs) inhibit the enzyme at substrate concentrations in the absence of PAPS, but low concentrations of the analogs activate the enzyme when PAPS is present. Thus, PAPS behaves as an allosteric inhibitor of ATP sulfurylase. In contrast, adenosine-5'-phosphosulfate (APS = product Q), the immediate product of the SO$_4^{2-}$-dependent reaction, is a linear inhibitor of the *P. chrysogenum* enzyme, with competitive with both MgATP and MoO$_4^{2-}$ (*K_m* = 36–73 μM). FSO$_3^-$ or S$_2$O$_3^{2-}$ does not activate the enzyme in the presence of PAPS. The effect of PAPS on fungal ATP sulfurylase is very similar to that observed when a single highly reactive cysteinyl SH group/subunit (SH-1) is covalently modified (Renosto, F., Martin, R. L., and Segel, I. H. (1987) *J. Biol. Chem.* 262, 16279–16288). The results suggest that in vitro SH-1 modification induces a conformational change in the enzyme that mimics the change induced in vivo by the reversible binding of PAPS. No evidence was obtained to suggest that PAPS covalently modifies SH-1.

ATP sulfurylases from rat liver (Yu, M., Martin, R. L., Jain, S., Chen, L. T., and Segel, I. H. (1989) *Arch. Biochem. Biophys.* 269, 156–174), spinach leaf, cabbage leaf, and *Saccharomyces cerevisiae* are not strongly inhibited by PAPS, do not display sigmoidal initial velocity plots in the presence of PAPS, and do not contain a highly reactive cysteinyl residue whose modification induces increased [S$_{0.5}$] values and sigmoidal velocity curves.

The allosteric effect of PAPS on the fungal ATP sulfurylase may be part of a sequential feedback process unique to a group of organisms that use PAPS for two diverging pathways, reductive assimilation and sulfate ester formation.
complex, i.e. positive cooperativity requires occupancy of the adenyl and sulfate subunits.

The dramatic effect of in vitro modification of SH-1 suggested several possible scenarios including: (a) chemical modification of SH-1 plays a regulatory role, except in vivo, the modifying agent is a natural substance whose level reflects the sulfur or energy sufficiency of the cells and (b) in vitro chemical modification induces a conformational state in the enzyme that is normally induced in vivo by a reversibly-bound allosteric effector.

Our earlier efforts were directed toward characterizing the kinetic response of ATP sulfurylase partially purified from cells grown on a high level of L-cysteine or L-methionine, i.e. under conditions where the need for sulfate activation was markedly reduced (25). L-Methionine partially repressed enzyme formation, but the enzyme that was produced displayed normal hyperbolic kinetics. L-Cysteine had no effect on the enzyme that is normally induced in vitro.

Preliminary experiments established that the assay level of AMP kinase was sufficient to convert up to 15 μM APS to PAPS in less than 3 min at 50 mM MgATP, 5 mM excess MgFg, and 150 mM added PAPS.

Reverse reaction (ATP synthesis) activity was determined spectrophotometrically in an assay coupled to hexokinase and glucose-6-phosphate dehydrogenase (23) and by the 35S release assay (22, 23). The ATP sulfurylase concentrations used in spectrophotometric assays were limited to yield a maximum ΔA540 × min⁻¹ of 0.02. Pure ATP sulfurylase did not catalyze ATP or SO₄²⁻ formation from APS in the presence of MgPP₇.

Enzyme activities are reported in terms of units × mg protein⁻¹ where 1 unit corresponds to the formation of 1 μmol of primary product/min. Protein concentrations of solutions of the purified enzymes were determined from absorbances at 280 and 260 nm (30 or 235 and 280 nm (31).

Chemicals—35S-Labeled and unlabeled PAPS were prepared and isolated as previously described (29). ATP, coupling enzymes, and required substrates, and most of the reduced organic sulfur compounds were obtained from Sigma. Choline-O-sulfate was prepared from choline and H₂SO₄ as described by Bellenger et al. (32). Cysteine-S-sulfate (S-sulfocysteine) was prepared from L-cystine and Na₂SO₄, as described by Segel and Johnson (33). Inorganic compounds were generally Fisher or Mallinckrodt products. Na₂S was obtained from Sigma. Stock Na₂S solutions were prepared from crystals that were washed with water until they were 50–75% dissolved and then dried dry prior to weighing. Stock Na₂SO₄ solutions were prepared by dissolving the crystals in Tris-Cl buffer (0.05 M, pH 8.0) containing 1 mM sodium ascorbate and 1 mM Na₂EDTA.

RESULTS

Properties of ATP Sulfurylases from Various Sources—Table I lists the subunit Mr, values and specific activities of the ATP sulfurylases used in the present study. Each preparation yielded a single major band on Coomassie Blue-stained sodium dodecyl sulfate gels with less than 5% of the dye-staining material present as minor bands in the fungal and yeast preparations, and less than 25% in the rat liver and two plant preparations (e.g. Fig. 1 of Ref. 28). The kinetic properties of the enzymes from P. chrysogenum (21, 22, 34), P. duponti (34), and rat liver (28) have been described previously. Rabbit serum antibodies to P. chrysogenum ATP sulfurylase cross-reacted on Western blots with the enzymes from the other filamentous fungi and from yeast but not with the enzymes from plants or rat liver. Antibodies to spinach leaf ATP sulfurylase cross-reacted with the cabbage leaf enzyme but not with the others. Antibodies to the rat liver enzyme did not cross-react with any of the other enzymes.

Effect of End Products and Intermediates of the Sulfate Assimilation Pathway on ATP Sulfurylase—The following compounds were tested singly and in combination with one other (1 mM each) for their effect on the forward ATP
sulfurylase reaction (P. chrysogenum enzyme) at near $K_m$ levels of MgATP (0.2 mM) and SO$_3^-$ (0.5 mM): L-cysteine, L-methionine, L-homocysteine (thiolactone preincubated at pH 11 and then neutralized), L-cystathionine, choline-O-sulfate, L-cysteine-S-sulfate, Na$_2$SO$_3$, Na$_2$SO$_4$, and Na$_2$S. S-Adenosylmethionine was tested at 50 mM. Only S$_2$O$_3^-$ exhibited significant inhibition (62%), which was expected given that thiosulfate is a nonreactive sulfate analog (21, 22). The effect of sulfite was difficult to assess because sulfite promoted the reaction in the absence of added sulfate. The rate increased with time suggesting that the effect was caused by contaminating sulfate and sulfate formed by the continuous oxidation of sulfite. The estimated inhibition of sulfate activation by 1 mM SO$_3^-$ was 10–25%. This inhibition was not surprising because a variety of similar anions (FSO$_3^-$, ClO$_3^-$, CIO$_3^-$, NO$_2^-$ as well as $\text{O}_2^-$) are inhibitors. Free sulfite may be an intermediate in assimilatory sulfate reduction but is unlikely to be present in vivo at millimolar levels. We conclude that none of the non-nucleotide intermediates or end products of sulfate assimilation acts as a feedback inhibitor of ATP sulfurylase. On the other hand, both APS and PAPS were highly inhibitory (see below).

In another series of experiments, the purified enzyme (6 $\mu$g) was incubated in 500 $\mu$L of 40 mM Tris-Cl buffer, pH 8.0, containing 25 $\mu$L of crude cell-free extract and 1 mM oxidized glutathione, or L-cysteine-S-sulfate, or homocysteine thiolactone, or 100 $\mu$M S-adenosylmethionine (potential modifiers). Residual activity in a diluted sample was measured at subsaturating MgATP and SO$_3^-$ after 30 min. No evidence for SH-1 modification (i.e. enzyme "inactivation") was obtained.

**Product Inhibition by APS**—Inhibition by APS, the physiological reaction product, was expected and has long been recognized as the obstacle to linear SO$_3^-$-dependent assays of the forward reaction in the absence of APS kinase (5, 21, 35). The APS inhibition patterns of the P. chrysogenum enzyme were determined using the molybdolysis assay. In the presence of APS, the $v$ versus [MgATP] and $v$ versus [MoO$_4^{2-}$] curves are hyperbolic. The linear reciprocal plots and slope replots (Fig. 1) show APS to be a linear competitive inhibitor. For varied MgATP, $K_{i,app} = 60$ nM at 100 $\mu$M MoO$_4^{2-}$ and 400 nM at 20 mM MoO$_4^{2-}$. For varied MoO$_4^{2-}$, $K_{i,app} = 80$ nM at 50 $\mu$M MgATP and 400 nM at 5 mM MgATP. Competitive inhibition by APS against both MgATP and MoO$_4^{2-}$ is qualitatively consistent with either a rapid equilibrium-ordered mechanism (with APS competitive with the leading substrate), or a rapid equilibrium random addition of substrates (with APS excluding both MgATP and MoO$_4^{2-}$) (36). However, in the ordered mechanism, $K_{i,app}$ for APS competition with the leading substrate would be independent of the cosubstrate concentration, which is clearly not the case here. The results support a random addition of MgATP and MoO$_4^{2-}$ and are in agreement with conclusions drawn from reaction progress (APS accumulation) data as analyzed by "average velocity" linear plots (21, 35) and from determinations of $k_{cat}/K_m$ with alternative inorganic substrates (22).
The kinetic constants for the molybdolysis reaction at pH 8.0, 30 °C, 5 mM excess Mg²⁺ were as follows: $K_{m\text{A}} = 23 \mu M$; $K_a = 500 \mu M$; $K_{\text{mth}} = 93 \mu M$; $K_b = 2020 \mu M$; $V_{\text{max}} = 22$ units $\times \text{mg protein}^{-1}$ where $A = \text{MgATP}$ and $B = \text{MoO}_4^{2-}$ (data not shown). Except for the lower value of $K_{m\text{A}}$, the constants are nearly identical to those reported earlier (34). If we assume a random addition of MgATP and $\text{MoO}_4^{2-}$ and that APS excludes both substrates, the $K_{\text{app}}$ values are related to the limiting $K_{i\text{A}}$ for APS binding by $K_{\text{app}} = K_{i\text{A}}[1 + ([S]/K_s)]$ where $[S]$ is the concentration of the non-varied substrate ($A$ or $B$) and $K_s$ is the $K_i$ or $E_i$ dissociation constant (i.e. $K_a$ or $K_n$) (36). The data of Fig. 1 yield, respectively, calculated $K_{i\text{A}}$ values of (a) 60 nM, (b) 37 nM, (c) 73 nM, and (d) 36 nM which are in reasonable agreement with the value of 40 nM obtained from initial velocity measurements of the reverse reaction using a fixed-time radiochemical assay (22).

The spinach leaf enzyme has not been completely characterized, but it appears to be similar to the fungal enzyme with respect to inhibition by APS. Velocities were measured during the period 1 to 2 min after starting the reaction. a, varied [MgATP] at subsaturating $\text{SO}_4^{2-}$; b, Hill plot of data shown in a. $V_{\text{max,app}}$ was assumed to be 4 units $\times \text{mg protein}^{-1}$ for all PAPS concentrations. c, varied [SO₄²⁻] at subsaturating MgATP; d, varied [SO₄²⁻] at "high" (15 mM) MgATP. e, Hill plots of data shown in d.

Fig. 2. Velocity curves of $P$. chrysogenum ATP sulfurylase (APS synthesis reaction) in the presence of varied PAPS and a constant subsaturating cosubstrate level. The reaction was started by adding the enzyme. Velocities were measured during the period 1 to 2 min after starting the reaction. a, varied [MgATP] at subsaturating $\text{SO}_4^{2-}$; b, Hill plot of data shown in a. $V_{\text{max,app}}$ was assumed to be 4 units $\times \text{mg protein}^{-1}$ for all PAPS concentrations. c, varied [SO₄²⁻] at subsaturating MgATP; d, varied [SO₄²⁻] at "high" (15 mM) MgATP. e, Hill plots of data shown in d.

Feedback Inhibition by PAPS—In earlier initial velocity kinetics measurements of the $\text{SO}_4^{2-}$-dependent ATP sulfurylase reaction coupled to excess APS kinase, the level of accumulating PAPS generally did not exceed 5 $\mu M$ at any substrate concentration (21, 22). Also, at 1 mM MgATP and 1 mM SO₄²⁻ ($>K_m$ but not saturating), the reaction progress curve was linear up to the accumulation of 20 $\mu M$ PAPS (21). Consequently, the inhibition exerted by PAPS was not detected.

Fig. 2a and b show the effect of 50–125 $\mu M$ PAPS on the initial velocity of the APS synthesis reaction catalyzed by the $P$. chrysogenum enzyme. MgATP was varied at subsaturating (0.5 mM) SO₄²⁻. The curves are clearly sigmoidal in the presence of PAPS and show a $V_{\text{max,app}}$ effect at high PAPS.

The velocity curves for varied [SO₄²⁻] at subsaturating (0.25 mM) ATP (Fig. 2e) are also sigmoidal at low substrate concentrations. Again, there is an effect of PAPS on $V_{\text{max,app}}$. However, at a much higher fixed MgATP concentration (15 mM), the $v$ versus [SO₄²⁻] curve approaches the control $V_{\text{max}}$ (Fig. 2d).

PAPS also induced sigmoidal velocity curves in the molybdolysis reaction, both in the presence and absence of PP⁺-ase and APS kinase (data not shown). The curves were qualitatively similar to those shown for the APS synthesis reaction. In reaction mixtures lacking APS kinase, the reaction was started by adding the enzyme in order to avoid a preincubation period that would allow APS to accumulate from contaminating $\text{SO}_4^{2-}$. The induction of sigmoidicity by PAPS in the myokinase-coupled molybdolysis reaction eliminates an (hypothetical) interaction of the two sulfite activating enzymes as the site of PAPS action (37).

Fig. 3 shows the effect of PAPS on the APS synthesis reaction of the $P$. chrysogenum enzyme at MgATP and SO₄²⁻ concentrations found in vivo. PAPS is a complete inhibitor, i.e. $i$ is driven to zero at all substrate concentrations as the concentration of PAPS approaches infinity. At 170 $\mu M$ MgATP and 400 $\mu M$ SO₄²⁻, the $[I]_{o\text{s}}$ for PAPS is 35 $\mu M$ (Fig. 3a). Under the same conditions, $[I]_{o\text{s}}$ for PAP was 1.8 mM.
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Thus, the inhibition is highly specific for the phosphosulfate group and cannot be attributed to contaminating PAP.

Stability of PAPS in the Reaction Mixture—The destruction of an inhibitor by contaminating enzymes can lead to non-hyperbolic velocity curves, so it was necessary to assess the stability of PAPS in the complete reaction mixture. This was accomplished by two methods. In the first, 6 or 16 μM [35S]PAPS was incubated in the complete assay mixture. [35S]SO$_3^-$ was measured (22, 38) at zero time and after 20 min. No increase in [35S]SO$_3^-$ over the original 11% contamination was observed. Thus, the primary and coupling enzymes did not contain measurable PAPS sulfohydrolase activity. In the second method, PAPS (27 μM) was incubated with ATP sulfurylase, hexokinase, and glucose-6-phosphate dehydrogenase and the required coupling enzyme substrates, including PF, (23). No reaction was observed. After 20 min, nuclease P-1 was added. The decrease in NADPH was equivalent to the concentration of PAPS originally added. Thus, the ATP sulfurylase and coupling enzymes did not contain measurable 3'-phosphatase activity.

Velocity Response of the SH-modified Enzyme at Low Cosubstrate Concentration—In the earlier study of the kinetic consequences of SH-1 modification (24), the experimental velocity curves were obtained at a fixed cosubstrate concentration that was near saturating. Under this condition, SH-1 modification resulted only in a small decrease in cosubstrate level. As shown in Fig. 4, saturation with only 3'-phosphatase activity.

FIG. 3. Inhibition of the APS synthesis reaction of P. chrysogenum ATP sulfurylase by PAPS at physiological concentrations of MgATP and SO$_4^{2-}$. The substrate concentrations were as follows: a, 0.17 mM MgATP and 0.4 mM SO$_4^{2-}$; b, 1 mM MgATP and 1 mM SO$_4^{2-}$; c, 1 mM MgATP and 10 mM SO$_4^{2-}$; d, 3 mM MgATP and 10 mM SO$_4^{2-}$. The actual $v_0$ values ranged from 1.4 units x mg protein$^{-1}$ (a) to 10.3 units x mg protein$^{-1}$ (d).

FIG. 4. Velocity curves for the native and the SH-modified enzyme at a fixed level of the nonvaried cosubstrate that is subsaturating for the modified enzyme. The enzyme was modified with NEM and the excess NEM removed by repeated ultrafiltration as described previously (24). a, $v$ versus [MgATP] at 5 mM excess MgCl$_2$ and 3.5 mM SO$_4^{2-}$. b, $v$ versus [SO$_4^{2-}$] at 2 mM MgATP plus 5 mM excess MgCl$_2$.

FIG. 5. Effect of nonsubstrate sulfate analogs in the absence and presence of PAPS. a, effect of increasing FSO$_3^-$ at 1.8 mM MgATP, 100 μM MoO$_4^{2-}$, and 5 mM excess Mg$^{2+}$ in the absence of PAPS. The enzyme concentration was 0.2 μg X ml$^{-1}$. The uninhibited velocity ($v_0$) was 10 units x mg protein$^{-1}$. b, effect of increasing FSO$_3^-$ at the above substrate concentrations in the presence of 150 μM PAPS. $[E]$, 1.4 μg x ml$^{-1}$; $v_0$ was 1.6 units x mg protein$^{-1}$. c, effect of increasing S$_2$O$_3^{2-}$ on the APS synthesis reaction at 1.3 mM MgATP, 0.5 mM SO$_4^{2-}$, and 5 mM excess Mg$^{2+}$ in the presence of 150 μM PAPS. $[E]$ was 9.4 μg x ml$^{-1}$; $v_0$ was 0.13 units x mg protein$^{-1}$. For comparative purposes, the data are normalized to the uninhibited velocity ($v_0$).
Aside from disclosing differences in sensitivity to PAPS of ATP sulfurylases from different sources, the experiment depicted in Fig. 6 also served as a further control showing that the sigmoidal response of the fungal enzymes was not an artifact of the assay (e.g., inhibition of a coupling enzyme by PAPS or an effect of minute levels of contaminating APS).

**Correlation Between High Sensitivity to PAPS, Induction of Sigmoidal Velocity Curves by PAPS, and the Presence of a "Regulatory" Sulfhydryl Group—ATP sulfurylases from N. crassa and A. nidulans (3 μM sites based on subunit Mr, values listed in Table I) were incubated with 250 μM DTNB at pH 8.0, 30 °C, in the presence of 5 mM MgCl₂. The reactions were followed spectrophotometrically at 412 nm. Assuming a molar absorption coefficient of 14,200 M⁻¹ cm⁻¹ for thionitrobenzoate, each enzyme was found to contain one reactive SH group.

In another series of experiments, each of the ATP sulfurylases listed in Table I was preincubated at 30 °C, pH 8.0, with 40 μM DTNB for up to 35 min. (The rat liver and plant leaf enzymes were previously dialyzed to remove residual DTT or β-mercaptoethanol). Remaining activity was measured at 50 μM MgATP and 100 μM MoO₄⁻, i.e., substrate concentrations in the region of the Kₘ values so that a chemical modification that affects Kₘ would be detected. Only the enzymes from the fungi were rapidly "inactivated" by DTNB. For example, t₀ for inactivation of the N. crassa enzyme by DTNB was 5 min; no activity was lost after 35 min in the absence of DTNB. The spinach enzyme retained 90% of its activity after 35 min (the same in the presence or absence of DTNB). The spinach enzyme lost activity (70% remaining activity at 30 min), but the same inactivation was observed when activity was measured at 5 mM MgATP and 20 mM MoO₄⁻. The DTNB modified ATP sulfurylases from the fungi displayed sigmoidal v versus [MgATP] curves (data not shown). The results confirm the relationship between potent inhibition by PAPS and the presence of a highly reactive SH group whose covalent modification induces increased [S]₀.₅ values and sigmoidal velocity curves in the absence of PAPS.

**Does PAPS Modify SH-I?—** The similarity between the effect of in vitro SH-1 modification by DTNB, NEM, etc. and the effect of PAPS promoted us to explore the possibility that PAPS acts as an SH-1 modifying agent. In one series of experiments, the P. chrysogenum enzyme (3.4 μM sites, 38 pM sites based on subunit Mr, values) was preincubated at pH 8.0, 30 °C with 200 μM PAPS in the absence and in the presence of MgATP (0.5 mM), or MgCl₂ (0.5 mM), or MoO₄⁻ (0.5 mM), or MgATP (0.5 mM) plus FSO₃⁻ (0.5 mM). Periodically for 30 min, samples were taken and diluted a total of 1300-fold into the molybdenyls assay mixture at subsaturating substrates, (120 μM MgATP, 200 μM MoO₄⁻, 5 mM MgCl₂, etc.). No loss in activity was observed.

As another approach, ATP sulfurylase (1 nmol), [³⁵S]PAPS (20 nmol; 5.2 × 10⁶ cpm x nmol⁻¹), NaPP (2 nmol), and MgCl₂ (1.5 μmol) were incubated in 0.3 ml (total vol) of 0.05 M Tris-Cl, pH 8.0, at 30 °C. (The PP, was included to promote the removal of any APS present in the PAPS.) After 10 min, the solution was applied to a Sephadex G-25 column (1.5 × 47 cm) and the column eluted with the Tris buffer. Two-ml fractions were collected. The enzyme eluted in fractions 12-18 (peak at fraction 14), while almost all of the applied [³⁵S]PAPS eluted in fractions 20-32 (peak at fraction 25). The pooled fractions containing the enzyme (0.84 nmol) contained 0.1 nmol of [³⁵S]PAPS. The experiment depicted in Fig. 6a was performed at fixed MgATP and MoO₄⁻ concentrations equal to Kₘ for each of the P. chrysogenum enzyme. Hence, the higher sensitivity of the N. crassa and P. duponti enzymes to PAPS could reflect either a higher affinity for PAPS or higher Kₘ and/or Kₘ values. On the other hand, the lower sensitivities of the non-fungal enzymes could not be attributed to lower Kₘ and Kₘ values; Kₘ values for these enzymes range from 130 to 610 μM; Kₘ values are 500 to 1250 μM; Kₘ values are 240 to 640 μM (21, 22, 29).³
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Moreover, in the presence of PAPS, the $v$ versus [MgATP] and $v$ versus [SO$_4^{2-}$] or [MoO$_4^{2-}$] curves are sigmoidal (Fig. 2). In the absence of PAPS, nonreactive sulfate analogs inhibit the enzyme, but in the presence of high PAPS low analog concentrations activate the enzyme (Fig. 5). Also, at a fixed substrasating cosubstrate concentration, saturation with the other cosubstrate does not yield the control $V_{nax,app}$. Saturation with both substrates overcomes the inhibition. The effects of PAPS on $P. chrysogenum$ ATP sulfurylase are essentially identical to those observed when a single, highly reactive cysteinyl residue/subunit is covalently modified in vitro (24, 26). ATP sulfurylases from several other fungi (L. dupontii, A. nidulans, N. crassa) are also highly sensitive to PAPS and display sigmoidal velocity curves in the presence of PAPS (Fig. 6). The ATP sulfurylases from these fungi also possess a reactive cysteinyl group whose modification results in increased $V_{max}$ values and sigmoidal velocity curves in the absence of PAPS. The results suggest that in vitro modification of fungal ATP sulfurylase triggers a conformational change that is very similar to that produced by the reversible binding of PAPS. In contrast, ATP sulfurylases from baker’s yeast, rat liver, cabbage leaf, and spinach leaf do not possess a regulatory cysteinyl group, are not strongly inhibited by PAPS, and do not display sigmoidal velocity curves in the presence of PAPS. The results strongly suggest that ATP sulfurylase of filamentous fungi is regulated by feedback inhibition by PAPS. As shown in Fig. 3, 35 to 200 $\mu$M PAPS causes a 50% inhibition of enzyme activity at physiological concentrations of MgATP (0.17–3 mM) and SO$_4^{2-}$ (0.4–10 mM); 90% inhibition is obtained at 68 to 310 $\mu$M PAPS. The intracellular PAPS concentration is known, but as shown with the plant ATP sulfurylase coupled to fungal APS kinase (Fig. 7), there is no thermodynamic barrier to the accumulation of PAPS to inhibitory levels.

Several lines of evidence showed that the sigmoidal velocity curves induced by PAPS were not artifacts of the assay. (a) Sigmoidal curves were obtained with MoO$_4^{2-}$ as the inorganic substrate (myokinase-coupled reaction) as well as with SO$_4^{2-}$ (APS kinase-coupled reaction). PAPS caused sigmoidal curves for the molybdoysis reaction in the presence or absence of APS kinase and PP$_i$ase. Thus, the sigmoidicity cannot be attributed to PAPS inhibition of a primary coupling enzyme. (b) APS (the only likely contaminating effector) inhibits the forward ATP sulfurylase reaction but does not induce sigmoidal velocity curves. Furthermore, if the inhibition exerted by PAPS was actually caused by contaminating APS, the spinach and rat liver (28) enzymes (which have $K_{app}$ values for APS nearly the same as those of the $P. chrysogenum$ ATP sulfurylase (22) at identical cosubstrate levels) would have been just as sensitive as the fungal enzyme to “PAPS.” (c) ATP sulfurylases from plants, yeast, and animal sources do not display sigmoidal curves in the presence of PAPS when all conditions are identical to those used to assay the fungal enzymes.

Why should strong inhibition of ATP sulfurylase by PAPS be restricted to the fungal enzymes? The answer probably lies in the unique sulfate metabolism of fungi. Euroascomycetes and Fungi Imperfecti use PAPS not only as the substrate for reductive sulfate assimilation (forming cysteine, methionine, etc.), but also as the sulfurylating agent (39) for the synthesis of choline-O-sulfate (sulfurylcholine) (40–42). This sulfate ester is presumed to be a sulfur storage compound and can account for 1.5% of the dry weight in fungal spores (43) and up to 0.26% of the mycelial dry weight (44, 45). This amount is 70 and 13 $\mu$mol/g dry weight, respectively. Mycelium growing on excess sulfate contains about 120 $\mu$mol of total organic.
sulfur/g dry weight (52). Thus, choline-O-sulfate formation represents a substantial fraction of the total sulfate metabolized by fungi. Yeast and bacteria also use PAPS for reductive sulfate assimilation, but with a few exceptions (42, 46), these organisms do not normally accumulate high levels of sulfate esters. Higher plants (47) and red algae (48) have been reported to synthesize choline-O-sulfate. Marine algae also synthesize large amounts of carbohydrate sulfate esters. But higher plants and algae use APS rather than PAPS for sulfate reduction. Animals form many types of sulfate esters but do not reduce APS or PAPS. Thus, PAPS is a definite branch point metabolite in fungi but not in any of the other organisms examined. We can envision a sequential feedback process occurring in fungi whereby excess cysteine (or another sulfate assimilation end product) feedback inhibits the PAPS reductase system. PAPS, in turn, inhibits ATP sulfurylase reducing the expensive sulfate activation sequence (3 \sim \text{P/ISO}^\ldownarrow) to a level commensurate with the need for choline-O-sulfate.4 Over the long term, excess methionine represses ATP sulfurylase to about 30% of the fully derepressed level (25, 49).

Aside from the feedback inhibition of the fungal enzyme by PAPS, there is no other well-documented evidence for regulation of ATP sulfurylase activity. Previous reports of inhibition of the fungal (49) and yeast (50) ATP sulfurylase by inorganic sulfide need to be reassessed for several reasons. (a) Reagent grade Na$_2$S crystals contain small amounts of SO$_2^-$ (probably on their surface). This contamination can generate APS in prolonged, fixed-time molybdolyis assays conducted in the absence of APS kinase. As shown in Fig. 1, APS is an extremely strong inhibitor of the molybdolyis reaction. Thus, it is not surprising that an APS-insensitive ATP sulfurylase from a strain of wine yeast also appeared to be insensitive to sulfide (50). (b) Contaminating polysulfides in Na$_2$S may have covalently modified SH-1 of the fungal enzyme causing decreased activity at subsaturating substrate levels (49). Finally, (c) Na$_2$S at millimolar levels may interfere with some types of colorimetric molybdolyis assays. ATP sulfurylase has a very high affinity for APS, probably a result of evolutionary pressure to maximize $k_{cat}/k_{m}$ for an enzyme that catalyzes a reaction with a very small $k_m$ in the physiological direction (35). There is no doubt that product inhibition by APS (and PPi) limits the net forward reaction under steady-state conditions in vivo (37), but it is doubtful that this inhibition plays a regulatory role in the usual physiological sense. One reason is that MgATP and SO$_2^-$ are both competitive with APS. At in vivo levels of the substrates, the apparent $k_m$ for APS is about 1 $\mu$M. But the maximum intracellular level of APS cannot exceed 1.5 $\mu$M (37). Another reason is that APS kinase is not subject to feedback inhibition by sulfate assimilation end products (53) and, consequently, the cellular level of APS would not vary in direct response to the sulfur sufficiency of the cells.

The exact mechanism of PAPS inhibition remains to be established although the kinetic behavior of the system provides some clues. The activation produced by non-reactive sulfate analogs in the presence of PAPS (Fig. 5) indicates that PAPS binds to an allosteric site distinct from the active site (51; p. 450 of Ref. 36). A degree of normal competitive inhibition may be superimposed because of the structural similarity between PAPS and APS and the extremely high affinity of the active site for the phosphosulfate group. Within the context of a general concerted transition model, the cumulative data are compatible with the following: (a) the native enzyme exists almost entirely in the $R$ state. That is, $L$, the allosteric constant is very small so that the $T$ state is a very small fraction of the enzyme in the absence of PAPS. (Thus, control velocity curves are essentially hyperbolic.) (b) PAPS binds predominantly to an allosteric site on the $T$ state. PAPS may also compete with MgATP and SO$_2^-$ at the active site on the $R$ state, but the net effect of PAPS binding is to promote formation of the $T$ state. (c) MgATP and SO$_2^-$ individually bind to both states; the affinity of the $T$ state for MgATP is lower than that of the $R$ state, while a $T$ state E-MgATP-SO$_2^-$ complex may not form at all. Thus, in the presence of PAPS, the velocity curves are sigmoidal (mainly a result of ternary $EAB$ complex formation on the $R$ state). The nonexclusive binding of MgATP and SO$_2^-$ is responsible for the Hill coefficients being smaller than the actual number of subunits, the highly unfavorable formation of a $T$ state EAB is responsible for $V_{max,app}$ effects at subsaturating, but not at saturating cosubstrate concentrations. (d) Chemical modification of SH-1 is assumed to stabilize a $T$ state-like conformation of the enzyme in the absence of PAPS. The modified enzyme binds MgATP or SO$_2^-$ but reverses to an $R$ state conformation when both substrate subsites are occupied (24, 25).

Systematic kinetics studies on the reverse reaction combined with equilibrium binding measurements may further elucidate the mechanism of PAPS action. Structure-function studies on ATP sulfurylases from filamentous fungi that have been reported to not synthesize choline O-sulfate (e.g. Phycomyces, Mucor, some Rhizopus species) (40-42) may also prove informative.

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Inhibition of ATP Sulfurylase by PAPS

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