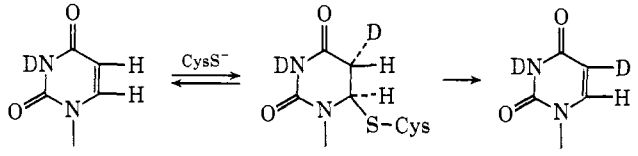
terium exchange at the 5 position by the catalysis with cysteine (Figure 1). This fact together with the pD–rate profiles shown in Figure 1 indicate that the reaction involves the unprotonated form of uridine and the mercapto anion as the reactive species (pK_a values for $H_3{}^+NCH_2CH(COO^-)SH \rightleftarrows H_3{}^+NCH_2CH(COO^-)S^-$ and $HOCH_2CH_2SH \rightleftarrows HOCH_2CH_2S^-$ are 8.7[12] and 9.4,[13] respectively). A cationic amino group at the β position of the SH in the molecule facilitates the dissociation of the SH, thereby making the compound more effective than other mercaptans such as 2-mercaptoethanol at a rather lower pH region. Furthermore, when the effectiveness of cysteine was compared with that of 2-mercaptoethanol at pD 10.0 where the SH of either reagent mostly dissociates, the per cent H exchange found for 3-methyluridine by 24-hr incubation was 38.5% with 0.5 *M* cysteine, and only 9.9% with 0.5 *M* 2-mercaptoethanol. Therefore, the amino group itself appears to play a role in the catalysis. This seems reasonable in view of the recent finding of an accelerating effect of amines on the bisulfite-catalyzed hydrogen isotope exchange at position 5 of uridine.[3] The effect of supplemented trialkylamines on the cysteine catalysis at pD 9.0 was investigated and a marked accelerating effect was observed: [catalyst], k_{obsd} at 37° (hr^{-1}), [1.0 *M* trimethylamine + 0.5 *M* cysteine], 7.67×10^{-2}; [1.0 *M* triethylamine + 0.5 *M* cysteine], 4.04×10^{-2}. In analogy to the established mechanism of the bisulfite–amine catalysis of the hydrogen isotope exchange of uridine, the cysteine reaction may be represented as illustrated in Scheme I. A

Scheme I



1 2

possible way of the action of an amine is to shift the equilibrium, **1** ⇄ **2**, to the adduct side, for it is known that amines shift the equilibrium, uridine ⇄ 5,6-dihydrouridine 6-sulfonate, to the adduct side.[3] The fact that the increase of the concentration of cysteine results in a greater increase in the exchange rate than that expected from the first-order kinetics (see above) is consistent with the participation of a second molecule of cysteine (as an amine) in the rate determining step. Another possible role of the amino group of cysteine is to abstract the hydrogen at position 5 of **2**, conceivably through an intramolecular process. Trimethylamine supplemented to 2-mercaptoethanol did enhance the rate of the exchange, but the enhanced rate was still considerably smaller than that observed for the catalysis by the cysteine type compound having an intramolecular amino group. Thus, the k_{obsd} values at pD 9.5 and 37° were 0.251×10^{-2} hr^{-1} with 0.5 *M* trimethylamine + 0.5 *M* 2-mercaptoethanol and 0.151×10^{-2} hr^{-1} with 0.5 *M* 2-mercaptoethanol (see also Figure 1). Furthermore, in consistency with the proposed mechanism, either *N*-acetylcysteine or *S*-methylcysteine, or an equimolar mixture of the two agents, was essentially ineffective as the catalyst. Glutathione (0.3 *M*) was not effective at 37° and pD 9, in contrast to the reported catalytic effect at 80°.[4]

Based on the glutathione experiment, Kalman[4] has suggested a mechanism for thymidylate synthetase which involves intermediary formation of a 5,6-dihydrouracil-6-mercapto compound by the addition of an enzyme SH group, which is known to be essential for the enzymic action,[14] across the 5,6-double bond of uracil. Recently, Santi and Sakai[15] have proposed the presence of an amino group at the active site of this enzyme on the basis of inhibition by 5-formyl-2′-deoxyuridylic acid. The above-described finding of a cooperative function of the SH and the amino groups in cysteine demonstrates that such enzymic mechanism is possible.

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An Active-Site Titrant for Arylsulfate Sulfohydrolase

Sir:

A dearth of knowledge exists concerning the nature of the active sites of arylsulfate sulfohydrolases as well as their physiological significance.[1,2] We report herein data to implicate *o*-nitrophenyl oxalate as the first known active-site titrant for arylsulfate sulfohydrolase II (EC 3.1.6.1) from *Asper. oryzae*.

Titration of the enzymatic active site with *o*-nitrophenyl oxalate[3] was determined by assaying for residual activity with 2-chloro-4-nitrophenyl and/or *p*-nitrophenyl sulfate as substrates in 0.4 *M* acetate buffer, pH 4.8, 37°. Sedimentation equilibrium measurements and gel electrophoresis indicate that the enzyme is a dimer composed of two identical subunits of *ca.* 45,000 molecular weight.[4] Extrapolation of residual activity as a function of inhibitor concentration reveals that completely inactivated protein has a 2.1:1 *o*-nitrophenyl oxalate:arylsulfate sulfohydrolase stoichiometry (Figure 1).

Further experiments show that a competitive reversible inhibitor, *p*-nitrophenyl phosphate,[1] will protect against inactivation caused by *o*-nitrophenyl oxalate (Figure 2). The time-dependent loss of enzymatic

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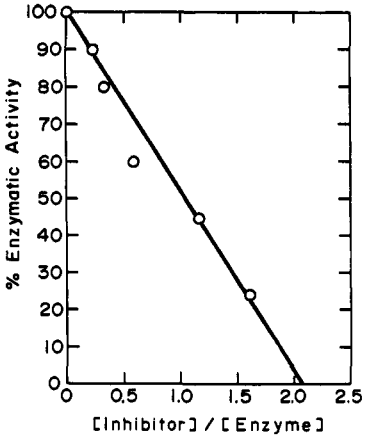

Figure 1. The activity of arylsulfate sulfohydrolase II inhibited by varying amounts of *o*-nitrophenyl oxalate as a function of the ratio of inhibitor per enzyme. The protein[5] (homogenous by disc gel electrophoresis and equilibrium ultracentrifugation criterion, specific activity = 0.7 unit/mg) was incubated with varying amounts of the oxalate ester for 20 hr at 4°.

activity as a variation of the concentration of the phosphate ester is characteristic of the minimal scheme

$$EI_2 \underset{k_{I_2}}{\overset{I_2}{\longleftarrow}} E + I_1 \overset{K_S}{\rightleftharpoons} EI_1 \qquad (1)$$

where I_1 represents *p*-nitrophenyl phosphate, I_2, *o*-nitrophenyl oxalate, and E, arylsulfate sulfohydrolase. Solution of eq 1 gives

$$\ln\left(1 - \frac{[EI_2]}{[E_0]}\right) = \frac{-k_{I_2}[I_2]t}{1 + [I]/K_s} \qquad (2)$$

where $[E_0] \cong$ initial enzyme concentration, K_s is the dissociation constant for EI_1, and k_{I_2} is the second-order rate constant for oxalate inhibition. Values of $k_{I_2} = 4.3 \times 10^2\ M^{-1}\ \text{min}^{-1}$ and $K_s = 3.5 \times 10^{-4}\ M$ were employed to calculate the solid lines of Figure 2. The increased protection afforded by higher concentrations of I_1 at a given time is in accord with *o*-nitrophenyl oxalate reacting at the active site. Similar results were obtained in the presence of excess substrate, *p*-nitrophenyl sulfate.

The reaction of the oxalate ester with the enzyme occurs with an initial rapid exponential release of *o*-nitrophenol followed by its slower liberation, the latter apparently due to both spontaneous hydrolysis and unspecified reaction with the protein. Since the difference in the two rate processes is a factor of *ca.* 16-fold at pH 4.8, the measurement of the burst may be accomplished by extrapolation of the initial OD values. The burst height (π) is directly proportional to enzyme concentration.[5,6] The results, listed in Table I, are in agreement with the data of Figure 1 and collectively imply but do not mandate an active site per subunit. It is noteworthy that k_{I_2} calculated from the initial phase of *o*-nitrophenol release is $2.0 \times 10^2\ M^{-1}\ \text{min}^{-1}$, a value *ca.* 50% less than that deduced in the above experiments at a lower I_2 concentration. This discrepancy provides minimal evidence that eq 1 be expanded to include an EI_2 complex formed prior to inactivation. The corresponding solution has a $K_{I_2}[I_2]$ term in both numerator and denominator so that the nonlinear dependency of the observed rate constant on I_2 concentration is described.

(5) Protein concentration determined by the method of Lowry (O. H. Lowry, *et al.*, *J. Biol. Chem.*, **193**, 265 (1951).

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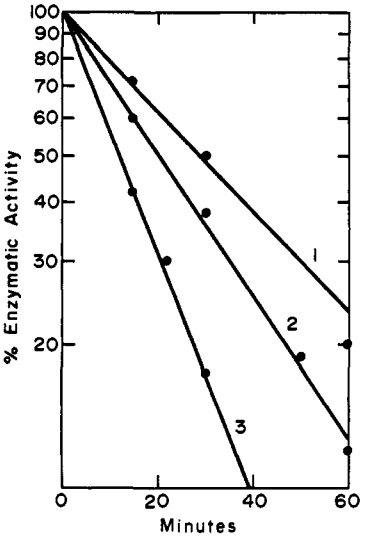

Figure 2. Protection of arylsulfate sulfohydrolase II against inactivation by *o*-nitrophenyl oxalate with *p*-nitrophenyl phosphate. *o*-Nitrophenyl oxalate, $3.3 \times 10^{-4}\ M$; *p*-nitrophenyl phosphate, plot 1, $2.0 \times 10^{-2}\ M$; 2, $9.5 \times 10^{-3}\ M$; 3, $5.1 \times 10^{-3}\ M$. All solutions are in 0.4 *M* acetate buffer, pH 4.8, 25°; enzyme specific activity $\cong$ 0.5 unit/mg.[1]

Table I. Effect of Enzyme Concentration on the Reaction with *o*-Nitrophenyl Oxalate[a]

[Enzyme] $\times 10^5\ M$	π/[enzyme]
2.6	1.8
1.1	1.8

[a] [Inhibitor] = $1.1 \times 10^{-3}\ M$; [enzyme], specific activity = 0.7 unit/mg; 0.2 *M* acetate, pH 4.8, 25°.

The discovery of this active-site titrant should expedite quantitative studies of the mechanism of action and active-site characterization of the arylsulfate sulfohydrolases.

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Chemistry of α-Alkoxy Sulfoxides. Formation of Methylene Acetals from Dimethyl Sulfoxide and Alcohols

Sir:

Among the large number of reactions undergone by DMSO[1-3] is its thermal decomposition, alone or cat-

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