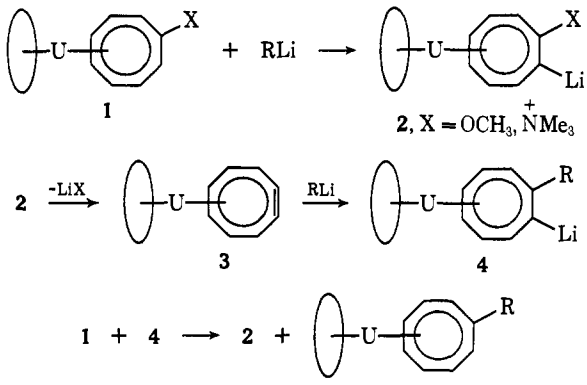
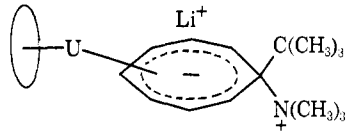


ring appears to be a less probable mechanism; in particular, the intermediate or transition state involved in the facile reaction of the trimethylammonium salt with *tert*-butyllithium **5** would appear to be highly strained.



5

Although other mechanisms can be written that involve **3**, such as direct reaction with RLi, the carbanion chain mechanism is supported by stoichiometry. Only a 1.1:1 molar ratio of RLi to ligand is required to effect >95% conversion to product. Furthermore, analogy is available in the apparent involvement of a "ferrocyne" intermediate in some reactions of chloroferrocene with bases.[11]

These reactions add to the growing chemistry of organoactinide compounds.[12] They provide important chemical evidence of the strength and covalency of the metal–ligand bond in uranocene compounds and promise to provide a useful new route to other uranocene derivatives.

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Cysteine-Catalyzed Hydrogen Isotope Exchange at the 5 Position of Uridylic Acid

Sir:

Recent reports from several laboratories have shown that some sulfur-containing agents such as 2-mercaptoethylamine,[1] bisulfite,[2,3] and glutathione[4] are effective in catalyzing the hydrogen isotope exchange of pyrimidine nucleosides at the 5 position. This type of study is of considerable interest because such a method is potentially useful for labeling nucleic acids. The chemically induced hydrogen isotope exchange of uridine has also received attention as a model for enzymic alkylation of uracil derivatives, since thymidylate synthetase is known to catalyze the hydrogen isotope exchange as well as methylation.[5] We wish to report that cysteine is a very efficient catalyst for the hydrogen isotope exchange of uridine 5′-phosphate at position 5, and that it exerts the catalytic effect by a cooperative function of the SH and amino groups.

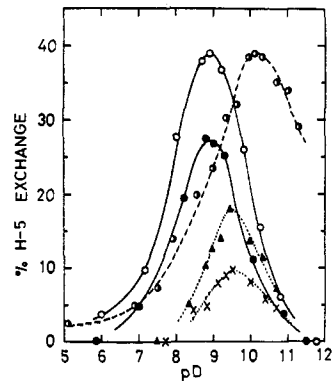

Figure 1. pD profile of the hydrogen–deuterium exchange at position 5 of uridine 5′-phosphate and of 3-methyluridine. Incubation was at 37° for 24 hr. Each D_2O solution (1 ml) contained 100 mg of disodium uridine 5′-phosphate or 80 mg of 3-methyluridine. pD was adjusted by the addition of NaOD or DCl, and no pD change was detected during the incubation. Exchanges of uridine 5′-phosphate: (○) cysteine (0.5 *M*); (●) 2-mercaptoethylamine (0.5 *M*); (×) 2-mercaptoethanol (1.2 *M*); (▲) 2-mercaptoethanol (1.2 *M*) + trimethylamine (1 *M*). Exchange of 3-methyluridine: (◑) cysteine (0.5 *M*).

Incubation of uridine 5′-phosphate in D_2O solution containing 1.0 *M* L-cysteine at 37° and pD 8.8 resulted in 100% hydrogen–deuterium exchange at the 5 position after 7 days, while no significant change was detected in the absence of cysteine under the same conditions. The extent of the exchange was determined by nmr as detailed elsewhere[3] utilizing the change in shape of the proton signal at the 6 position.[1] The pD dependence of the exchange catalyzed either by cysteine, 2-mercaptoethylamine, or 2-mercaptoethanol is presented in Figure 1. The cysteine-catalyzed exchange of uridine 5′-phosphate at pD 9.0 and 37° proceeded by pseudo-first-order kinetics. The apparent rate constants at various concentrations of cysteine were: concentration of cysteine/rate constant (hr^{-1}), 0.25 *M*/0.819×10^{-2}; 0.5 *M*/2.29×10^{-2}; 0.75 *M*/3.98×10^{-2}; 1.0 *M*/6.05×10^{-2}. The method of cysteine catalysis, which can be carried out in nearly neutral solutions, appears to be more effective compared with other known methods.[6-11] Thus, most of the reported procedures for the exchange involve reactions at higher temperatures, and the bisulfite–amine method which can be done at 37° is still less efficient than the cysteine catalysis when compared at an equal reagent concentration.

3-Methyluridine also underwent the hydrogen–deu-

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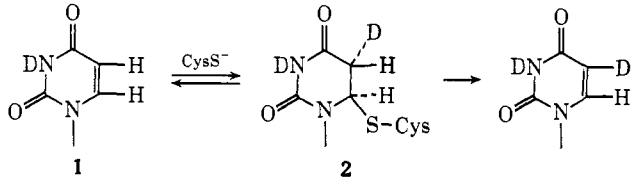
terium exchange at the 5 position by the catalysis with cysteine (Figure 1). This fact together with the pD–rate profiles shown in Figure 1 indicate that the reaction involves the unprotonated form of uridine and the mercapto anion as the reactive species (pK_a values for $H_3{}^+NCH_2CH(COO^-)SH \rightleftarrows H_3{}^+NCH_2CH(COO^-)S^-$ 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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