

Facile Hydrogen-Deuterium Exchange at the 5'-Position of an Analogue of S-Adenosyl-L-methionine

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S-3',4'-anhydroadenosyl-L-methionine is an analogue of the S-adenosyl-L-methionine coenzyme. Here we report on a rapid solvent exchange of the methylene protons at the 5'-position of this analogue. The rate of H/D exchange was measured by nuclear magnetic resonance spectroscopy under buffered conditions in deuterium oxide. The reaction is specific base catalyzed and displays a second-order rate constant of $2 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$, which corresponds to a rate enhancement of 10^{12} compared to solvent exchange of α -methylene protons in acyclic, aliphatic sulfonium ions. No other carbon bonded hydrogens in the molecule exchange with solvent under the experimental conditions. Allylic stabilization of a carbanionic-like transition state for the solvent exchange process can account for these results. Solvent exchange under these mild conditions provides a simple way to prepare a 5'-²H-labeled form of the coenzyme analogue. © 2002 Elsevier Science (USA)

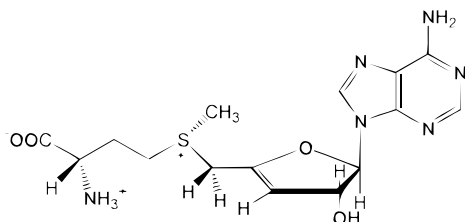
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INTRODUCTION

S-Adenosyl-L-methionine¹ (SAM) is a versatile coenzyme, which traditionally is associated with biological methyl transfer reactions. This coenzyme has other roles in nature, and its function in certain radical-based enzymatic reactions has been actively investigated in the recent past (see 1 and 2 for recent reviews). Our mechanistic studies on lysine 2,3-aminomutase led us to synthesize 3',4'-anhydroadenosyl-L-methionine (*anSAM*) (Scheme 1), an analogue of SAM that was designed to stabilize the 5'-deoxyadenosyl radical, a putative intermediate in the reaction of this enzyme. We have recently reported on the observation and characterization of the allylic 5'-deoxy-3',4'-anhydroadenosyl radical formed under turnover conditions in the reaction of lysine 2,3-aminomutase (3,4).

During the course of these studies we discovered that hydrogens at the 5'-position

¹ The *R*- and *S* nomenclature refers to the absolute configuration at the sulfur atom and is used as such throughout the text. The *L*-descriptor is only used to express the configuration at the α -carbon of methionine, following the standard convention for amino acids.



SCHEME 1.

of *anSAM* undergo equilibration with solvent protons at neutral pH. Although α -protons in sulfonium compounds such as SAM are somewhat acidic due to the inherent stability of the sulfurylide, exchange with solvent at neutral pH has not been observed. Kinetics of H/D-exchange has been observed with simple sulfonium salts, but only under extreme basic conditions and/or at elevated temperature (5,6). However, introduction of electron stabilizing functional groups, such as phenyl, carbonyl and carbonyl esters in the β -position of sulfonium ions leads to dramatic increases in acidity of the α -protons, and pK_a values below 10 have been reported (6). This paper describes the kinetics of H/D-exchange for the 5'-protons of *anSAM* and provides comparison with other carbon acids.

MATERIALS AND METHODS

Deuterium oxide (99.9% D), potassium deuterioxide (40% wt, 98+% D), and CD_3CO_2D (99.5% D) were from Aldrich. *anSAM* was synthesized and purified as the formate salt as described elsewhere (2). SAM (*p*-toluenesulfonate salt) was purchased from Sigma and purified in the same manner as *anSAM*. All other chemicals were reagent grade and were used without further purification.

Solution pH was measured with a Corning pH meter (Model 430) equipped with an Accumet, combination microelectrode that was standardized at pH 4.00 or 7.02 at 20°C. Values of pD were obtained by adding 0.40 to the observed pH meter reading (7). The concentration of OD^- at any pD reading was measured according to Eq. [1], where $pK_w = 10^{-15.13}$ is the ion product of D_2O at 20 °C (8), and $\gamma_{OD} = 0.79$ is the activity coefficient of OD^- at ionic strength $I = 1$ (9). Acetate- $C-d_3$ buffers (1 M) in D_2O were prepared by adjustment with KOD. The concentration of $CD_3CO_2^-$ at each pD was calculated using a $pK_a = 5.09$ of the acid at $I = 1$ in D_2O (10,11). The ionic strength was maintained at $I = 1$ by adding appropriate amount of KCl to each buffer.

$$[OD^-] = \frac{10^{pD - pK_w}}{\gamma_{OD}}. \quad [1]$$

Kinetic runs were initiated by preparing 15–20 mM solutions of *anSAM* or SAM in 0.5 ml of acetate- d_3 buffer. The samples were quickly transferred to NMR tubes, and 1H NMR spectra were recorded using a 200 MHz Bruker instrument. The reaction progress was monitored by collecting data at different timepoints, where 32–256

scans were accumulated depending on the rate of the reaction. Samples were kept in a constant temperature bath at $20 \pm 0.2^\circ\text{C}$ between data acquisition. The spectra were referenced to the HDO resonance at 4.8 ppm and the singlet peak for 5'-H₂ at 4.39 ppm was integrated after a baseline correction routine. A doublet signal for 3'-H at 5.88 ppm was chosen as an internal reference of intensity. The normalized intensity was plotted and fitted to a single-exponential equation using *Kaleidagraph* (Synergy software) to yield the pseudo-first-order rate constants. Each reaction was monitored for at least 2 half-lives and the standard errors for the observed rate constants were <10% in every case. After each kinetic run, a solution acidity measurement was performed using the microelectrode to adjust for minor changes in pD (< 0.1), especially in the basic range where the buffering capacity was low.

RESULTS AND DISCUSSION

Kinetics of H/D exchange. The reaction progress for H/D exchange at pD = 6.32 is shown in the ¹H NMR spectra in Fig. 1. The intensity of the resonance for the 5'-methylene protons at 4.39 ppm diminishes with time and follows a single exponential decay as shown in Fig. 2. The intensity of all other peaks remain constant, which shows that other C-H protons are not exchanging with solvent, and that the compound is not decomposing to a significant extent during the timecourse of the experiment. This was especially pertinent for the other α -protons to the sulfur; a methylene group at 2.29 ppm and a methyl group at 2.96 ppm (data not shown). Similar observations were noted at all pD values. Notice that the minor impurities in the spectra mainly represent the *R*-isomer of *an*SAM. The epimerization rate for the sulfonium ion, either appears to be slow or the intensities represent the equilibrium ratio of the *S*- and *R*-isomer. In comparison, the rate of sulfur epimerization for SAM is pH independent and displays a first-order rate constant of $1.8 \times 10^{-6} \text{ s}^{-1}$ at 37°C (12). Because no activation parameters are known for that process, a direct comparison cannot be made to our observations at 20°C . Given the relatively long half-life at 37°C for SAM ($t_{1/2} = 107 \text{ h}$), epimerization should not be a major contributor during the timecourse of the exchange reaction for *an*SAM as is evident in the spectra in Fig. 1.

The exchange reactions obey the rate law described in Eq. [2], where k_{ex} is the observed rate constant and k_{OD} , k_{B} , and k_{w} are the individual second-order rate constants for the deuterioxide, buffer, and solvent catalyzed reactions, respectively.

$$k_{\text{ex}} = k_{\text{OD}}[\text{OD}^-] + k_{\text{B}}[\text{B}] + k_{\text{w}} \quad [2]$$

The contribution from general base catalysis (k_{B}) was measured by performing the exchange reaction at two different buffer concentrations, at pD 5.60. The observed rate constants per hydrogen atom (k_{ex}) were $(6.9 \pm 0.6) \times 10^{-6} \text{ s}^{-1}$ (0.5 M acetate-C-d₃, $I = 1$) and $(7.1 \pm 0.7) \times 10^{-6} \text{ s}^{-1}$ (1.0 M acetate-C-d₃, $I = 1$), respectively. These values are identical within error and show that contribution by a buffer catalyzed reaction can be neglected in the analysis. Figure 3 shows a plot of k_{ex} against deuterioxide concentration. The y-intercept is close to zero, or $(3.8 \pm 9) \times 10^{-7} \text{ s}^{-1}$, which further demonstrates that a contribution, either from a buffer (k_{B}) or a solvent catalyzed reaction (k_{w}) is small compared to catalysis by deuterioxide. The rate law for the exchange reaction, therefore, can be simplified to $k_{\text{ex}} = k_{\text{OD}}[\text{OD}^-]$. The second-order

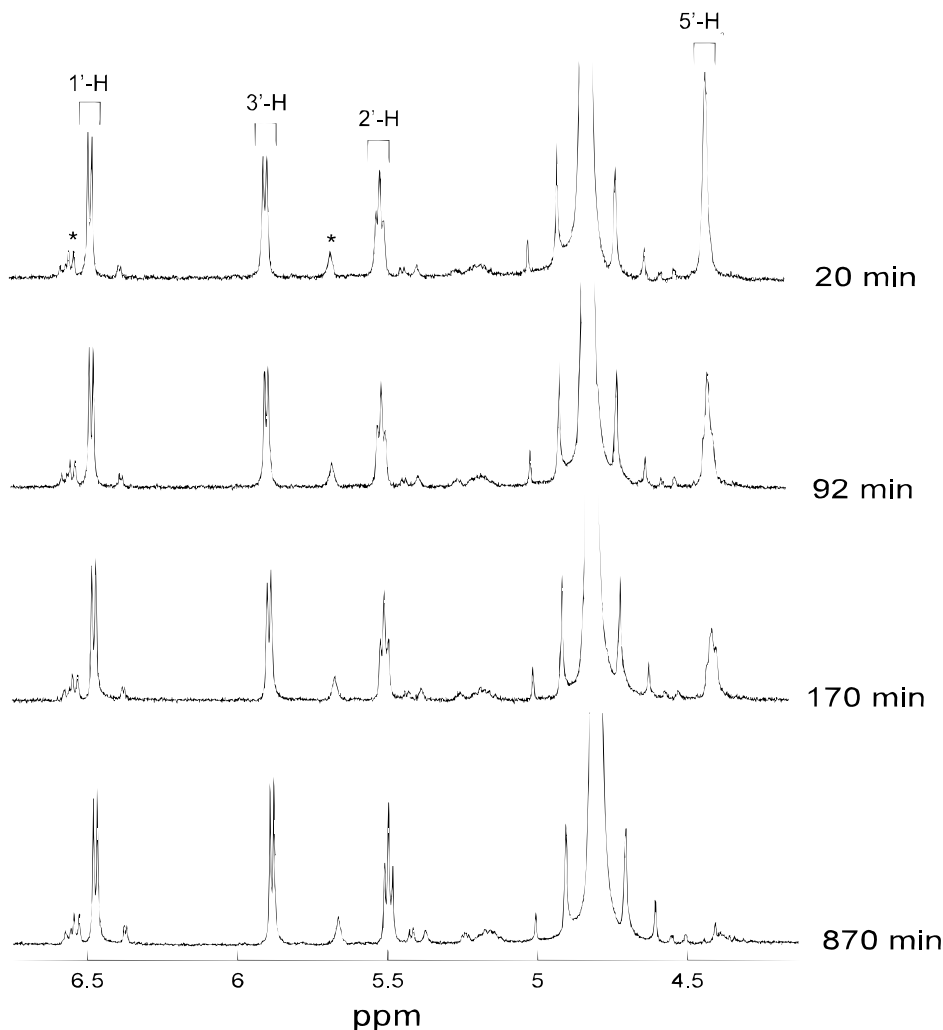


FIG. 1. ^1H NMR spectra at different timepoints during the exchange reaction of *anSAM* at pD 6.32, 20°C , and $I = 1$. The assignments are indicated above each peak in the spectrum. See reference (4) for complete assignments of all peaks in the ^1H NMR spectrum of *anSAM*. Peaks due to impurities that are most likely from the *R*-isomer of the compound are labeled with asterisks.

rate constant for the specific base catalyzed reaction is obtained from the slope of the plot in Fig. 3 and yields a value of $k_{\text{OD}} = (1.97 \pm 0.08) \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$.

Comparison with SAM. Experiments with SAM under the same conditions as described above, do not result in solvent exchange for the $5'$ -hydrogens or any other carbon bonded hydrogen atoms in the molecule (data not shown). The acidity of the $5'$ -hydrogens has, however, been demonstrated by the hydrolytic lability of the compound under basic conditions (13,14). A proton abstraction from the $5'$ -position leads to the cleavage of the glycosidic bond through an elimination reaction that