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Catalysis by Desolvation: The Catalytic Prowess of SAM-Dependent Halide-Alkylating Enzymes

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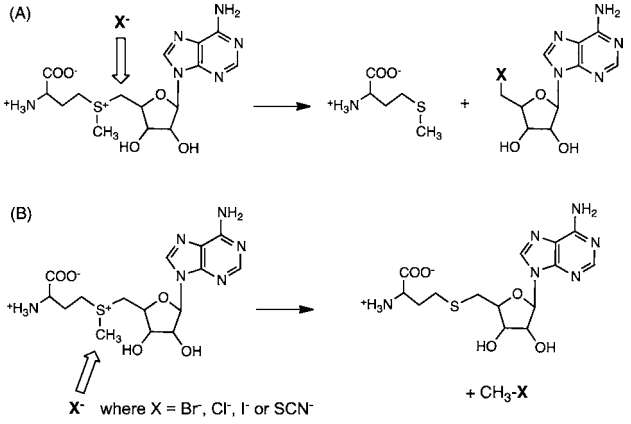
Supporting Information

ABSTRACT: In the biological fixation of halide ions, several enzymes have been found to catalyze alkyl transfer from *S*-adenosylmethionine to halide ions. It proves possible to measure the rates of reaction of the trimethylsulfonium ion with I^-, Br^-, Cl^-, F^-, HO^-, and H_2O in water at elevated temperatures. Comparison of the resulting second-order rate constants, extrapolated to 25 °C, with the values of k_{cat}/K_m reported for fluorinase and chlorinase indicates that these enzymes enhance the rates of alkyl halide formation by factors of 2×10^{15}- and 1×10^{17}-fold, respectively. These rate enhancements, achieved without the assistance of cofactors, metal ions, or general acid–base catalysis, are the largest that have been reported for an enzyme that acts on two substrates.

In the biological fixation of halide ions, several enzymes have been found to catalyze alkyl transfer from *S*-adenosylmethionine to halide ions. Among these enzymes are a fluorinase (FDAS, EC 2.5.1.63) from *Streptomyces cattleya*[1] and a chlorinase (SalL, EC 2.5.1.94) from the marine bacterium *Salinispora tropica*,[2] which catalyze attack by halide ions on *S*-adenosylmethionine (SAM^+) to generate 5′-halogenated derivatives of adenosine (Scheme 1A). Another group of halide-fixing enzymes, that are probably responsible for the appearance of halomethanes in the atmosphere, catalyzes a halide attack at the *S*-methyl group of SAM^+, with displacement of *S*-adenosylhomocysteine (Scheme 1B).[3] These enzymes are pure protein catalysts that act on their substrates without the assistance of metals or other cofactors.[4]

Scheme 1. Reactions Catalyzed by Two Groups of Cofactor-Independent Halogenases



In alkyl transfer from a sulfonium ion to a halide ion, general acid–base catalysis is improbable or impossible. To appreciate the kinetic barriers that are surmounted by enzymes of this kind, it would be desirable to compare the rates of these enzyme-catalyzed reactions with the rates of the uncatalyzed reactions in water in the absence of a catalyst. That information would also be expected to be useful in calibrating alternative approaches, such as QM/MM, to the simulation of enzyme rate enhancements based on structural information. In the experiments described here, we sought to obtain that information using the symmetrical trimethylsulfonium ion (Me_3S^+) as an alkyl donor.[5]

In these kinetic experiments, reaction mixtures containing Me_3S^+:BF_4^- (0.02 M) and the potassium salt of I^-, Br^-, Cl^-, F^-, or HO^- (0 to 1.0 M) were sealed under vacuum in quartz tubes and incubated at temperatures ranging from 65 to 200 °C in ovens equipped with ASTM thermometers. After various time periods, the reaction was stopped by cooling, and the product mixture was diluted 5-fold with D_2O containing added pyrazine as an integration standard. Analysis of product mixtures by ^1H NMR showed conversion of Me_3S^+ to methanol and dimethyl sulfide, with no other detectable products (Figure S1). The progress of reaction was followed by monitoring the decline of the integrated signal intensity of Me_3S^+, which was closely matched in each case by the appearance of methanol. The halomethane products of these reactions were not observed and were not expected to accumulate because they are hydrolyzed at least 100-fold more rapidly than the decomposition of Me_3S^+ under the conditions of these experiments. Thus, the rate constant for attack by Cl^- in the present experiments was 1.0×10^{-5} M^{-1} s^{-1} at 150 °C, where the rate constant for CH_3Cl hydrolysis is 4.4×10^{-3} s^{-1}.[6]

In Figure 1, apparent first-order rate constants for the decomposition of Me_3S^+ at 150 °C are plotted as a function of the concentration of NaCl. The intercept on the vertical axis corresponds to $k_{water} = (8 \pm 2) \times 10^{-7}$ s^{-1} at 150 °C, in satisfactory agreement with the rate constant of $(8.6 \pm 0.9) \times 10^{-7}$ s^{-1} observed for decomposition of Me_3S^+ in the absence of added chloride ion (Figure S4). Second-order rate constants were corrected by subtracting the rate of background hydrolysis (k_{water}, always <10% of the total rate) from the observed rate of

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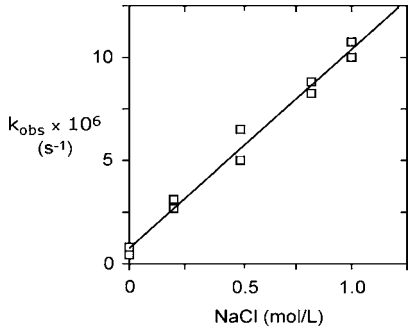

Figure 1. Decomposition of Me_3S^+ in water at 150 °C, plotted as a function of the concentration of NaCl at constant ionic strength (1.0, maintained by addition of $NaBF_4$).

decomposition and dividing that result by the concentration of halide or hydroxide ion.

Activation parameters were obtained by fitting those second-order rate constants, calculated from the results obtained at 6 or more temperatures over the range from 65 to 200 °C, to the Arrhenius equation. Rate constants at 25 °C were estimated by extrapolation of linear plots of k_2 as a logarithmic function of the reciprocal of absolute temperature (Figure 2) (see also

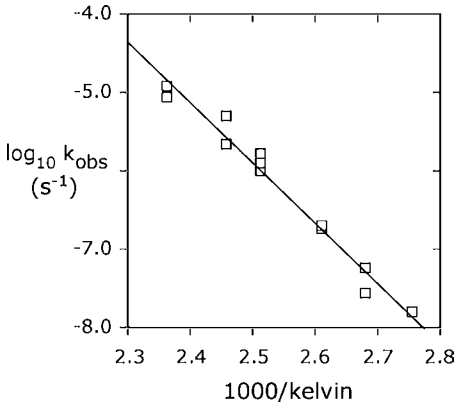

Figure 2. Decomposition of Me_3S^+ in water in the presence of NaCl (1.0 M) plotted as a function of absolute temperature over the range from 90 to 150 °C. Linear regression yields k_2 = 3.7 (±1.5) × 10^{-13} M^{-1} s^{-1}, with $\Delta H^{\ddagger}$ = 34.5 (±1.5) kcal/mol and $T\Delta S^{\ddagger}$ = 0.2 (±2.0) kcal/mol at 25 °C.

Figures S4−S8). The resulting second-order rate constants and activation parameters for the decomposition of Me_3S^+ in the presence of halide ions, hydroxide ion, and water are shown in Table 1.

In accord with earlier studies of nucleophilic substitution in water[10,11] and with earlier evidence that Me_3S^+ decomposes by an S_N2 mechanism at 100 °C in ethanol,[12] a logarithmic plot of k_2 as a function of nucleophilicity indicates that halide reactions with Me_3S^+ involve strong nucleophilic participation (Figure S9). Structural and kinetic evidence suggests that enzymatic alkyl transfer to halide ions likewise proceeds by S_N2 substitution.[13]

In free solution, nucleophilic attack is equally likely to occur at each of the three methyl groups of Me_3S^+, whereas only a single alkyl group is transferred in the enzymatic reactions of SAM^+. Division of the observed second-order rate constants (k_2) in Table 1, reduced by a factor of 3 to incorporate that statistical correction, into the second-order rate constants (k_{cat}/K_m) reported for SAM in the presence of saturating nucleophile indicates that fluorinase[8] and chlorinase[2] enhance the rates of alkyl halide formation by factors of 2.4 × 10^{15}- and 1.2 × 10^{17}-fold, respectively, at 25 °C.[14] If we assume that substrate water is not saturating, as appears to be the case for other hydrolytic enzymes,[15] then the rate of hydrolysis of SAM^+ is enhanced by the DUF62 enzyme[9] to an even greater extent (1.8 × 10^{20}-fold).[16] For steric reasons, more elaborate sulfonium ions might be expected to react somewhat more slowly than Me_3S^+. If that is the case, then the rate enhancements achieved by these enzymes are even larger than these values. Moreover, they exceed rate enhancements that have been reported for other bisubstrate reactions that have been studied in this way, which include several *O*-phosphorylating kinases (ranging from 3 × 10^{12}- to 5 × 10^{14}-fold)[17] and the peptidyltransferase center of the ribosome (3 × 10^7-fold).[18]

Table 1. Rate Constants and Thermodynamics of Activation for Reactions of Nucleophiles with Me_3S^+ in Water at 25 °C, Compared with Rates of Enzymatic Methylation with SAM^+

Nu	k_2 (M^{-1} s^{-1})	$\Delta H^{\ddagger}$ kcal/mol	$k_{cat}/K_{m(SAM^+)}$ (M^{-1} s^{-1})	RE[a]
I^-	3.1 × 10^{-11}	30.3	5000[b]	5 × 10^{14}
Br^-	3.2 × 10^{-12}	31.9	13 300[b]	1.2 × 10^{16}
Cl^-	3.7 × 10^{-13}	34.5	15 000[b]	1.2 × 10^{17}
F^-	1.9 × 10^{-13}	32.4[c]	179[d]	2.8 × 10^{15}
HO^-	2.9 × 10^{-10}	32.5	NA	NA
H_2O	5.8 × 10^{-17}[e]	38.7	7500	1.8 × 10^{20}

[a]Values of these rate enhancements (RE) were obtained by dividing the second-order rate constant $k_{cat}/K_{m(SAM^+)}$ for the enzyme reaction, in the presence of saturating halide ion, by one-third of the second-order rate constant (k_2) of the uncatalyzed reaction evaluated in the present work (see Table S1 for binding constants). [b]Chlorinase (ref 2). Although it has been described as a "chlorinase," Sa1L is almost equally active as a brominase or an iodinase (ref 2). [c]Considerably lower values (22.0 or 26.8 kcal/mol) have been estimated on the basis of QM/MM simulations (ref 7). [d]Fluorinase (ref 8). [e]Obtained by dividing the observed first-order rate constant (k_{cat}) for enzymatic hydrolysis of SAM^+ by the hydrolase DUF62 (ref 9) by the molarity of water.

What is the source of the large rate enhancements produced by chlorinase and fluorinase? In the reported crystal structures of fluorinase[1] and chlorinase,[2] water is stripped from the halide ion as it is bound; the halide ion's remaining contacts with water vanish when SAM^+ is bound during formation the ternary complex, and the halide ion is held in a position appropriate for in-line attack on SAM^+. In general, desolvation is known to enhance the rates of reactions in which charge is delocalized or neutralized in the transition state,[19] and simulations indicate that only partial desolvation is required for F^- to serve as a powerful nucleophile.[20] Halide ions and Me_3S^+ have been shown to exist predominantly as ion pairs in organic solvents, such as acetone and tetrachloroethane.[21,22] When we examined the decomposition of Me_3S^+:I^- (0.02M) in DMSO-d_6 we found that this reaction followed first-order kinetics up to at least 90% completion, consistent with decomposition of ion-paired Me_3S^+:I^- to dimethyl sulfide and iodomethane. This reaction was found to proceed 4 × 10^4-fold more rapidly in DMSO than in water at 37 °C (Figure S9). In surroundings less polar than DMSO, the accelerating effects of removing the substrates from water might approach the much larger solvent effects that have been observed for the hydrolysis of phosphate esters in cyclohexane.[23]

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In summary, the relative resistance of the trimethylsulfonium ion to hydrolysis makes it possible to measure rates of methyl transfer to I^-, Br^-, Cl^-, and F^-, in water at elevated temperatures. The resulting second-order rate constants, obtained by extrapolation of Arrhenius plots to 25 °C, imply that fluorinase and chlorinase enhance the rates of alkyl halide formation by factors of 2×10^{15}- and 1×10^{17}-fold, respectively. These rate enhancements, achieved without the assistance of metals, cofactors, or general acid−base catalysis, give some indication of how much can be accomplished by juxtaposition and desolvation of two substrates at an enzyme's active site.[24] It is also possible that, following substrate binding, the reactants are compressed as they proceed toward the transition state, as has been demonstrated in the case of catechol *O*-methyltransferase.[16]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H NMR spectrum of a reaction mixture. Plots of k_{obs} vs [$NaBF_4$] and [HCl]. Arrhenius plots for the decomposition of Me_3S^+ with H_2O, F^-, Br^-, I^-, and HO^-. Plot of log(k_2) vs nucleophilicity constant for the halides. A plot of decomposition of $Me_3S^+I^-$ in DMSO-d_6 at 50 °C. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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