J|A|C|S
COMMUNICATIONS

Published on Web 10/07/2003

^{34}S Isotope Effect on Sulfate Ester Hydrolysis: Mechanistic Implications

Benjamin T. Burlingham,[†] Lisa M. Pratt,[‡] Ernest R. Davidson,[†] Vernon J. Shiner, Jr.,[†] Jon Fong,[‡] and Theodore S. Widlanski*,[†]

Department of Chemistry and Department of Geology, Indiana University, Bloomington, Indiana 47405

Received August 1, 2002; E-mail: twidlans@indiana.edu

Sulfate and phosphate monoesters are among the most biologically ubiquitous esters. Because of this, a variety of techniques have been developed to study the hydrolysis of these compounds, including elegant methods for determining bridging and nonbridging ^{18}O isotope effects by remote labeling.[1,2] Notably absent from this panoply of techniques are methods for determining isotopic discrimination associated with the central heavy atom, be it phosphorus or sulfur. In this communication we describe the first determination of a ^{34}S isotope effect on sulfate ester hydrolysis and examine the mechanistic implications of this result in light of available data and theoretical predictions.

Highly accurate isotopic ratios may be determined by use of stable isotope ratio mass spectrometry (IRMS). This technique may be used to determine the isotopic composition of a given species, provided that it can be converted to a gas, such as N_2, CO_2, etc.[3] It is also possible to use this technique to determine isotopic ratios associated with the sulfur of SO_2, provided that a dedicated isotope ratio mass spectrometer and inlet system is available. Since the conversion of sulfate to sulfur dioxide is fairly routine,[4] and has been used for some time to study isotopic constitutions in minerals, a simple extension of this technique would be to determine ^{32}S/^{34}S discrimination as a function of extent conversion during the hydrolysis of sulfate esters. The measured isotope effect would then provide an important piece of data for evaluating the mechanism of sulfate ester hydrolysis.

In testing the feasibility of this technique, two sulfate esters were of initial interest to us. ^{18}O isotope effects for *p*-nitrophenyl sulfate (*p*-NPS) **(1)** have been measured,[5] and we thought that ^{34}S isotope data would be of additional benefit in testing the proposed mechanism. Because of our interest in studying the enzyme-catalyzed mechanism of sulfate hydrolysis, we also chose to study *p*-acetylphenyl sulfate (*p*-APS), which has been used routinely in sulfatase assays.[6,7]

The accurate determination of heavy atom kinetic isotope effects (KIEs) requires the availability of highly pure starting sulfate ester, free of both free phenol and inorganic sulfate. A number of syntheses of these compounds have been reported,[7−9] but we thought that a modification in the purification procedure would lead to a more facile isolation of pure compound. Mixing the phenol of interest with sulfur trioxide−pyridinium complex in pyridine yielded the pyridinium salts of **1** and **2**. These salts could be purified by silica gel chromatography (2% triethylamine/acetonitrile) to give the triethylammonium salts, free of inorganic sulfate. Ion exchange gave the potassium salts of **1** and **2** as white solids. No phenol was detectable by ^{1}H NMR, and analysis by UV/vis spectroscopy at 405 and 325 nm for **1** and **2**, respectively, suggested that there was less than 0.5% phenol. Elemental analysis confirms this level of purity (see Supporting Information.)

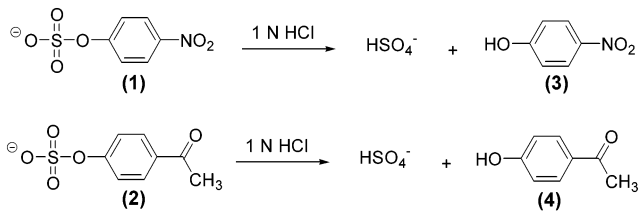

Figure 1. Acid hydrolysis of aryl sulfate monoesters.

The ^{34}S isotope discrimination during acid-catalyzed sulfate ester hydrolysis[10,11] (Figure 1) was determined as follows. A 10 mM solution of the sulfate ester was incubated in 1.0 N HCl at 22 °C, and the extent of reaction was determined spectrophotometrically. At various times, 5 μL aliquots were dissolved in 995 μL of 2 N NaOH, and the amount of free phenoxide was determined by measuring the absorbance change at 405 and 325 nm for **3** and **4**, respectively. The sample was then treated with excess $BaCl_2$, leading to the rapid precipitation of the inorganic sulfate product as $BaSO_4$. The barium sulfate was then converted to SO_2, and the isotope ratio was determined by mass spectrometry. Three independent runs were conducted for each compound, and inorganic sulfate samples were collected at ∼50% extent of reaction. Experimental isotopic fractionation value determinations for each run were done in triplicate. The KIE determined for *p*-NPS (**1**) is 1.0154 (±0.0002), and the KIE determined for *p*-APS is 1.0172 (±0.0003). The *p*-acetyl compound was further analyzed at ∼30 and ∼70% reaction, and the data are in agreement with the values determined at 50% reaction (See Supporting Information.)

On the basis of the available data, a number of different reaction mechanisms have been proposed for acid-catalyzed sulfate ester hydrolysis. Any mechanism must account for the relatively small ^{18}O leaving group isotope effect measured by Hengge's group.[5] A priori, such an observation suggests an associative type of mechanism (Figure 2C), in which formation of a pentavalent intermediate of some kind (either what is shown in 2C, or some kind of protomer or tautomer) is partially rate determining, and cleavage of the S−O bond takes place subsequently. However, this mechanism is inconsistent with the bulk of previously available data. A concerted process has also been proposed (Figure 2A).[5] In this mechanism, proton transfer from the sulfate ester to the leaving-group oxygen is mediated by an intervening water molecule, and attack on sulfate is concerted (though not necessarily synchronous) with the breaking of the S−O bond. Protonation of the leaving-group oxygen suppresses the intrinsic oxygen isotope effect associated with S−O cleavage. This mechanism may also account for the small, but experimentally significant, difference in ^{18}O nonbridging isotope effects associated with sulfate hydrolysis under differing HCl concentrations, and for the difference in secondary ^{18}O isotope effects for the acidic and basic hydrolysis reactions. On the basis of symmetry arguments, mechanism 2A may be found to be incomplete. To avoid generating a zwitterionic protonated sulfate

[†] Chemistry Department.
[‡] Geology Department.

10.1021/ja0279747 CCC: $25.00

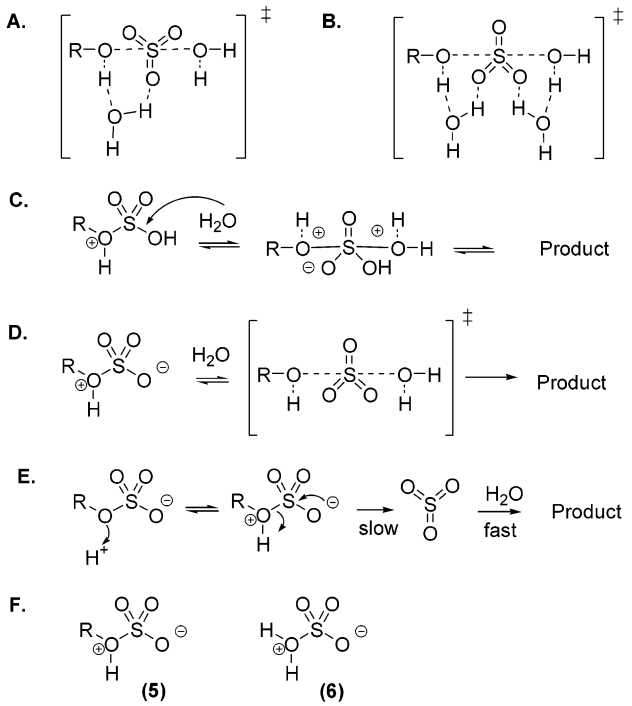

Figure 2. Possible mechanisms for the hydrolysis of sulfate esters. (A) This transition state has been previously proposed to account for ^{18}O KIE's. (B) Modification of the transition state in A; this figure takes into account the demands of microscopic reversibility. (C) Associative mechanism utilizes a pentavalent intermediate. (D) Concerted mechanism. (E) Stepwise mechanism. (F) Two analogous zwitterionic species.

(**6**) analogous to the zwitterionic protonated sulfate ester **(5)** (see Figure 2F) another water molecule must be added to the reaction mechanism to give a new version (Figure 2B). Such an argument hinges on whether the zwitterionic form of sulfuric acid **(6)** and the zwitterionic intermediate **5** are of similar energy, something that is currently unknown.

Although no specific S−O bond breaking data have been reported to date, data for the C−S KIEs are available. Such measurements are in the range of 1.0−1.8%. Simple calculations also put the maximum C−S KIE at 1.8%.[12] This suggests that our measured isotope effects are near the upper limit of what would be predicted for S−O bond cleavage. Mechanistically, our observation of a large ^{34}S kinetic isotope effect associated with acid-catalyzed hydrolysis suggests that cleavage of the S−O bond does take place during the rate-limiting step. This probably rules out an associative mechanism (Figure 2C), since such a mechanism requires two isotopically sensitive steps that would be of opposing magnitudes. However, it is consistent with the other mechanisms shown, all of which contain an isotope sensitive S−O bond cleavage event.

In summary, measurement of the ^{34}S isotope effect on sulfate ester hydrolysis suggests that an associative mechanism is unlikely. The isotope effect method we report should also prove useful for studying the mechanisms of sulfate hydrolyses under various conditions, other sulfuryl group transfers, and a variety of enzyme-catalyzed reactions involving sulfur-containing compounds.

Acknowledgment. This work has been funded by NIH/NCI Grant RO1CA71736 (T.S.W.) and NSF Grant EAR-978267 (L.M.P.). We thank Alvan Hengge for helpful discussions.

Supporting Information Available: Synthesis and characterization of **1** and **2**; general procedure for isotopic data acquisition; general procedures for KIE calculations (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Hengge, A. C.; Cleland, W. W. *J. Am. Chem. Soc.* **1990**, *112*, 7421−7422.
(2) Hengge, A. C.; Edens, W. A.; Elsing, H *J. Am. Chem. Soc.* **1994**, *116*, 5045−5049.
(3) O'Leary, M. H. *Methods Enzymol.* **1980**, *64*, 83−125.
(4) Giesemann, A.; Jager, H.-J.; Norman, A. L.; Krouse, H. R.; Brand, W. A. *Anal. Chem.* **1994**, *66*, 2816−2819.
(5) Hoff, R. H.; Larsen, P.; Hengge, A. C. *J. Am. Chem. Soc.* **2001**, *123*, 9338−9344.
(6) Anderson, C. J.; Lucas, L. J. H.; Widlanski, T. S. *J. Am. Chem. Soc.* **1995**, *117*, 3889−3890.
(7) Anderson, C.; Freeman, J.; Lucas, L. H.; Farley, M.; Dalhoumi, H.; Widlanski, T. S. *Biochemistry* **1997**, *36*, 2586−2594.
(8) Benkovic, S. J.; Dunikoski Jr., L. K. *Biochemistry* **1970**, *9*, 1390−1397.
(9) Burkhardt, N. G.; Lapworth, A. *J. Chem. Soc.* **1926**, 684−690.
(10) Fendler, E. J.; Fendler, J. H. *J. Org. Chem.* **1968**, *33*, 3852−3859.
(11) Kice, J. L.; Anderson, J. M. *J. Am. Chem. Soc.* **1966**, *88*, 5242−5245.
(12) Saunders, W. H.; Asperger, S. *J. Am. Chem. Soc.* **1957**, *79*, 1612−1615.

JA0279747