On the Intramolecularity of the Vitamin K Model Oxidation

Paul Dowd* and Seung Wook Ham

Department of Chemistry, University of Pittsburgh
Pittsburgh, Pennsylvania 15260

Received August 5, 1991

Vitamin K is an obligatory cofactor for a carboxylase that transforms selected glutamates in proteins of the blood clotting cascade into γ-carboxyglutamate residues.[1] This step is essential for blood clotting. We recently presented a model that used the spontaneous oxidation of an α-naphthoxide to demonstrate the principle of *base-strength amplification*, whereby in a biological system (or elsewhere) a weak, biologically accessible base can be transformed into an otherwise inaccessible, strong base using the energy of oxidation to drive the elevation of base strength.[2] In the context of the vitamin K dependent carboxylation, a strong base is needed to remove the γ-proton of glutamate in preparation for carboxylation.

In the model reaction, the α-naphthoxide **1** was transformed to the keto epoxy alcohol **2** (Scheme I). We suggested that the oxidation proceeds through a peroxy anion **3** leading to a dioxetane intermediate **4**. Since one can also formulate the transformation of **1** to **2** in Scheme I as an intermolecular reaction, it was important to test whether the model oxidation proceeds by an intermolecular or an intramolecular pathway. The outcome is expected to be of general applicability and may, for example, have a bearing on oxygenation paths in the vitamin E series[3] as well as other phenoxide oxidations.[4]

The experiment was carried out by treating the α-naphthoxide **1** with a 55:45 mixture of $^{16}O_2$ and $^{18}O_2$ and analyzing the mass spectrum of the product keto epoxy alcohol **2**.[5] If the reaction sequence is intramolecular, the molecular ion of the product **2** will consist of two ion clusters at m/z 204 and 208 in a 55:45 ratio corresponding to incorporation of two atoms of ^{16}O or two atoms of ^{18}O (Scheme II). If the reaction is intermolecular, the incorporation of oxygen will be random so the mass spectrum of ^{18}O-labeled product **2** will be characterized by a molecular ion triplet with peaks at m/z 204, 206, and 208 in the approximate ratio 55:100:45. The two patterns are readily distinguished from one another. The mass spectrum of the product **2** showed (Figure 1a) strong peaks at m/z 204 and 208 with a minor peak at m/z 206, the latter corresponding to the incorporation of one atom of ^{18}O. The peak at m/z 206 can be accounted for to the extent of 90% by the 8.9% of $^{16}O-^{18}O$ in the starting gas mixture (m/z 34 in Figure 1b). The remainder of the intensity of the m/z peak is well accounted for by the M$^+$ + 2 contribution from the m/z 204 peak.[6,7] The observed ratio of 56:44 for the m/z 204 and 208 peaks indicates that the oxidation is *completely intramolecular*
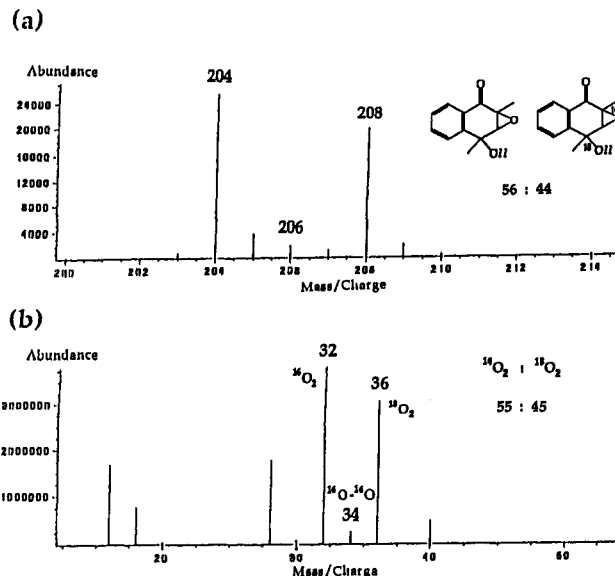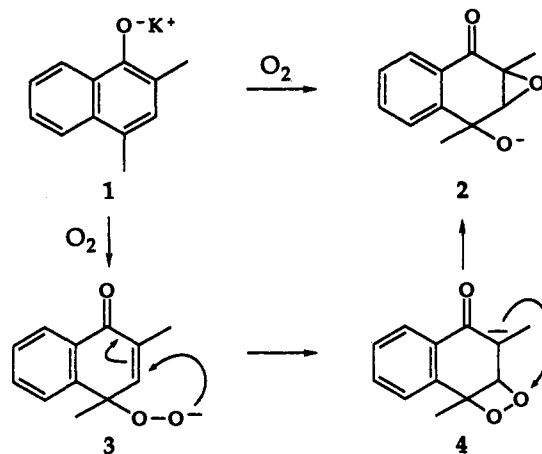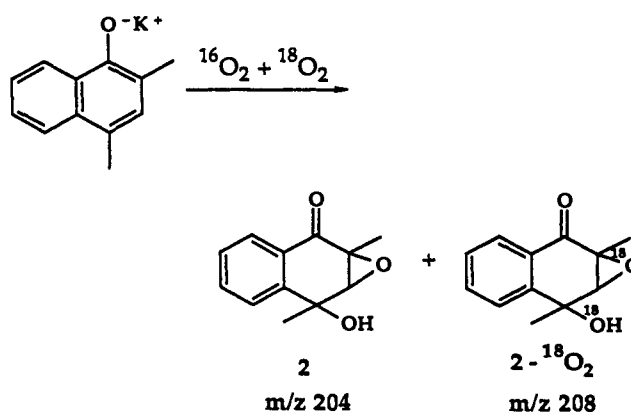

(a)

(b)

Figure 1. (a) Mass spectrum of the keto epoxy alcohol **2** produced upon oxidation under an $^{18}O_2 + ^{16}O_2$ atmosphere, showing unlabeled product **2** at m/z 204 and doubly labeled product **2** at m/z 208. The ratio for the m/z 204 and 208 peaks is 56:44, in good accord with expectation. The peak at m/z 206 corresponds to the incorporation of only one atom of ^{18}O. This peak is fully accounted for by the small amount of $^{18}O-^{16}O$ evident in the mass spectrum of the $^{16}O_2 + ^{18}O_2$ gas mixture at m/z 34 in Figure 1b and by the M$^+$ + 2 contribution from the m/z 204 peak. (b) Mass spectrum of the 55:45 mixture of $^{16}O_2 + ^{18}O_2$ removed from the gas space above the labeling reaction.

Scheme I

Scheme II

(1) Recent reviews: (a) Suttie, J. W. *Biofactors* **1988**, *1*, 55. (b) Suttie, J. W. *Annu. Rev. Biochem.* **1985**, *54*, 459. (c) Olson, R. E. *Annu. Rev. Nutr.* **1984**, *4*, 281.

(2) Ham, S. W.; Dowd, P. *J. Am. Chem. Soc.* **1990**, *112*, 1660. Dowd, P.; Ham, S. W.; Geib, S. J. *J. Am. Chem. Soc.* **1991**, *113*, 7734.

(3) Clough, R. L.; Yee, B. G.; Foote, C. S. *J. Am. Chem. Soc.* **1979**, *101*, 683. Foote, C. S.; Clough, R. L.; Yee, B. G. In *Tocopherol, Oxygen and Biomembranes*; de Duve, C., Hayashi, O., Eds.; Elsevier/North Holland Biomedical Press: Amsterdam, 1978; pp 13-31. Liebler, D. C.; Baker, P. F.; Kaysen, K. L. *J. Am. Chem. Soc.* **1990**, *112*, 6995. Matsuo, M.; Matsumoto, S.; Iitaka, Y.; Etsuo, N. *J. Am. Chem. Soc.* **1989**, *111*, 7179. Matsumoto, S.; Mitsuyoshi, M.; Iitaka, Y. *J. Chem. Res. Synop.* **1987**, 58. Matsuo, M.; Matsumoto, S.; Iitaka, Y. In *The Role of Oxygen in Chemistry and Biochemistry*; Ando, W., Moro-oka, Y., Eds.; Elsevier Science Publishers: Amsterdam, 1988; pp 503-508.

(4) Nishinaga, A.; Itahara, T.; Shimizu, T.; Matsuuura, T. *J. Am. Chem. Soc.* **1978**, *100*, 1820.

(5) The keto epoxy alcohol **2** was obtained in yields of 65-80%.[2]

(6) Beynon, J. H. *Mass Spectrometry and its Applications to Organic Chemistry*; Elsevier Publishing Company: Amsterdam, 1960; p 524.

(7) The relative intensities of the peaks in the mass spectrum can be measured with a precision of ±1%.

and is therefore entirely consistent with the dioxetane mechanism proposed in Scheme I. This result supports the hypothesis that

a dioxetane intermediate may provide the key to understanding the mechanism of action of vitamin K.

Temperature Dependence of the Kinetic Isotope Effect for a Gas-Phase S_N2 Reaction: $Cl^- + CH_3Br$

A. A. Viggiano, John S. Paschkewitz, Robert A. Morris, and John F. Paulson

Phillips Laboratory, Geophysics Directorate
Ionospheric Effects Division (LID)
Hanscom Air Force Base, Massachusetts 01731-5000

Angels Gonzalez-Lafont

Unidad Quimica Fisica, Departamento Quimica
Universidad Autonoma de Barcelona
Bellaterra 08193, Spain

Donald G. Truhlar*

Department of Chemistry and Supercomputer Institute
University of Minnesota
Minneapolis, Minnesota 55455-0431

The study of ion reactions in the gas phase continues to provide a host of new insights into organic reactivity.[1] Kinetic isotope effects (KIEs) are traditionally considered the most sensitive experimental probes of transition-state structure for reactions at well-defined temperatures.[2] Very recently several KIEs have been measured at room temperature for gas-phase S_N2 and E2 reactions.[3] In this paper we report a measurement of the temperature dependence of the deuterium KIE for the reaction $Cl^-(g) + CH_3Br(g) \rightarrow CH_3Cl(g) + Br^-(g)$ and a molecular modeling study to interpret the results.

The experiments were performed in a variable-temperature selected ion flow drift tube (VT-SIFDT). The measurements of the absolute rate constants are standard, and the apparatus is described elsewhere.[4] While the error limit on the absolute rate constants is ±25%, the KIE was measured much more accurately. All conditions (flow rates, pressure, temperature, tuning, etc.) were kept identical when changing isotopes, and the experiments on CH_3Br and CD_3Br were done very close to one another in time.

Table I. Reaction Rates and Kinetic Isotope Effects for $Cl^-(g)$ + $CH_3Br(g)$

	k_H, cm^3 molecule^{-1} s^{-1}		k_H/k_D	
T, K	expt[a]	theory[b]	expt	theory[b]
207	3.1×10^{-11}	5.3×10^{-11}	0.81 ± 0.03	0.88
300	2.4×10^{-11}	2.4×10^{-11}	0.81 ± 0.03	0.93
538	1.4×10^{-11}	1.6×10^{-11}	0.89 ± 0.06	0.97
564	1.5×10^{-11}	1.6×10^{-11}		0.97

[a] Twenty-five percent uncertainty in absolute value, 15% for relative values. [b] Canonical variational transition state theory and conventional transition-state theory yield the same results to the precision shown in these cases. Also the maximum of the vibrationally adiabatic ground-state potential curve is below the zero-point energy of reactants for both isotopic versions of the reaction so the semiclassical transmission coefficient[10] is unity. There is only one maximum in the free energy of activation profile (at any of these temperatures for either isotope) in the region between the ion–dipole complexes, and the free energy of activation associated with the central barrier is considerably larger than that associated with ion–molecule association; thus there would not be a significant difference if we used the canonical unified statistical theory[10a] instead of canonical variational theory.

As a result, the errors that contribute to the determination of the KIE are in the determination of the decay of the Cl^- signal with added neutral reactant and in the ratio of the gas correction factors for CH_3Br and CD_3Br used in measuring the reactant gas flow rates. The gas correction factor depends only on the heat capacity of the gas,[5] which can be calculated using the known vibrational frequencies of CH_3Br^6 and CD_3Br.[7] At least five determinations of the decay rate were made for each gas at each temperature. A future paper will report temperature-dependent drift-tube studies of this reaction in order to study the effect of the internal temperature of the CH_3Br and CD_3Br on the reactivity.

The molecular modeling effort is based on direct dynamics calculations[8] employing canonical variational transition state theory (CVT).[9,10] The force field is modeled by neglect of diatomic differential overlap[11,12] with specific reaction parameters (NDDO-SRP)[13] molecular orbital theory. We started with the Austin model 1 (AM1)[12] parameters and the adjustment in the atomic core matrix element U_{pp}^{Cl} made previously[13] for the $Cl^-(g)$ + $CH_3Cl(g)$ reaction. Then we adjusted U_{pp}^{Br} (-76.25 eV) and U_{pp}^C (-40.705 eV) to the exoergicity (-6.3 kcal) and room temperature rate constant for the isotopically unsubstituted case. The resulting classical barrier height is -1.5 kcal, i.e., the saddle point is 1.5 kcal below the energy of reactants.

The reaction rates for the unsubstituted reaction are given in Table I. The increase in the experimental rate at 564 K is smaller than the error bars, but it is also observed in the theoretical results, which show minima at 548 and 558 K for CH_3Br and CD_3Br, respectively. Interestingly, the previous study of CH_3Br by

(1) See, e.g.: (a) *Ion-Molecule Reactions*; Franklin, J. L., Ed.; Dowdin, Hutchison, and Ross: Stroudsburg, PA, 1979. (b) Jarrold, M. F.; Bass, L. M.; Kemper, P. R.; van Koppen, A. M.; Bowers, M. T. *J. Chem. Phys.* **1983**, *78*, 3756. (c) Bohme, D. K. In *Ionic Processes in the Gas Phase*; Almoster Ferreira, M. A., Ed.; Reidel: Dordrecht, 1984; p 111. (d) Han, C.-C.; Dodd, J. A.; Brauman, J. I. *J. Phys. Chem.* **1986**, *90*, 471. (e) Olesik, S.; Baer, T.; Morrow, J. C. *J. Phys. Chem.* **1986**, *90*, 3563. (f) Henchman, M.; Hierl, P. M.; Paulson, J. F. *Adv. Chem. Ser.* **1987**, *215*, 83. (g) DePuy, C. H.; Damrauer, R.; Bowie, J. H.; Sheldon, J. C. *Acc. Chem. Res.* **1987**, *20*, 127. (h) Beauchamp, J. L. *ACS Symp. Ser.* **1987**, *333*, 11. (i) Schultz, R. H.; Elkind, J. L.; Armentrout, P. B. *J. Am. Chem. Soc.* **1988**, *110*, 411. (j) Brickhouse, M. D.; Squires, R. R. *J. Am. Chem. Soc.* **1988**, *110*, 2706. (k) Kebarle, P.; Dillow, G. W.; Hirao, K.; Choudhury, S. *Faraday Discuss. Chem. Soc.* **1988**, *85*, 23. (l) Barlow, S. E.; van Doren, J. M.; Bierbaum, V. M. *J. Am. Chem. Soc.* **1988**, *110*, 7240. (m) Brutschy, B. *J. Phys. Chem.* **1990**, *94*, 8637. (n) Grabowski, J. J.; Lum, R. C. *J. Am. Chem. Soc.* **1990**, *112*, 607. (o) DePuy, C. H.; Gronert, S.; Mullin, A.; Bierbaum, V. M. *J. Am. Chem. Soc.* **1990**, *112*, 8650.
(2) (a) Bigeleisen, J.; Wolfsberg, M. *Adv. Chem. Phys.* **1958**, *1*, 15. (b) Saunders, W. H., Jr. In *Investigation of Rates and Mechanisms of Reaction, Part I*; Bernasconi, C. F., Ed.; John Wiley & Sons: New York, 1986; p 565.
(3) Gronert, S.; DePuy, C. H.; Bierbaum, V. M. *J. Am. Chem. Soc.* **1991**, *113*, 4009.
(4) (a) Viggiano, A. A.; Morris, R. A.; Dale, F.; Paulson, J. F.; Giles, K.; Smith, D.; Su, T. *J. Chem. Phys.* **1990**, *93*, 1149. (b) Smith, D.; Adams, N. G. *Adv. At. Mol. Phys.* **1988**, *24*, 1.

(5) Instruction Manual for Models 258/259 Mass Flowmeters, MKS Instruments Inc., Andover, MA.
(6) Chase, M. W., Jr.; Davies, C. A.; Downey, J. R.; Frurip, D. J.; McDonald, R. A.; Syverud, A. N. *J. Phys. Chem. Ref. Data* **1985**, *14* (*Suppl. 1*), 1.
(7) Herzberg, G. *Molecular Spectra and Molecular Structure: II. Infrared and Raman Spectra of Polyatomic Molecules*; D. Van Nostrand Co., Inc.: New York, 1945.
(8) Truhlar, D. G.; Gordon, M. S. *Science* **1990**, *249*, 491.
(9) Garrett, B. C.; Truhlar, D. G. *J. Chem. Phys.* **1979**, *70*, 1593.
(10) (a) Truhlar, D. G.; Isaacson, A. D.; Garrett, B. C. In *Theory of Chemical Reaction Dynamics*; Baer, M., Ed.; CRC Press: Boca Raton, FL, 1985; Vol. IV, p 65. (b) Tucker, S. C.; Truhlar, D. G. In *New Theoretical Concepts for Understanding Organic Reactions*; Bertrán, J., Csizmadia, I. G., Eds.; Kluwer: Dordrecht, 1989; p 291. (c) The calculations were carried out with the computer program MORATE, version 2.5, by Truong, T. N., Lu, D.-h., Stewart, J. J. P., Garrett, B. C., Steckler, R., Isaacson, A. D., Rai, S. N., Hancock, G. C., Joseph, T., Melissas, V., and Truhlar, D. G., unpublished.
(11) (a) Pople, J. A.; Santry, D. P.; Segal, G. A. *J. Chem. Phys.* **1965**, *43*, S129. (b) Dewar, M. J. S.; Thiel, W. *J. Am. Chem. Soc.* **1977**, *99*, 4899.
(12) (a) Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902. (b) Dewar, M. J. S.; Zoebisch, E. G. *J. Mol. Struct. (THEOCHEM)* **1988**, *180*, 1.
(13) Gonzalez-Lafont, A.; Truong, T. N.; Truhlar, D. G. *J. Phys. Chem.* **1991**, *95*, 4618.