in the matrix.[14] From these values of *K*, the standard free energy difference at 463 K can be obtained:[15] ΔG°_{463}(**2a**/**1**) = $-RT \ln K_{463}$(**2a**/**1**) = 7 ± 2.5 kJ/mol and ΔG°_{463}(**2b**/**1**) = 13 ± 4 kJ/mol.

The agreement between experimental and theoretical relative energies of tautomers, together with the striking similarity between the experimental and theoretical spectra, provides strong evidence for the presence of the imino–oxo form of 1MC in the weakly interacting hydrophobic environment of the argon matrix and in the gas phase.

Acknowledgment. We thank Dr. Leszek Lapinski for directing our attention again to weak bands in the IR spectra of some cytosines and Dr. David Powell for assistance with analysis, and we are especially grateful to Dr. Krystyna Szczepaniak for critical discussions. Partial support by NIH Research Grant No. 32988 and from the Army High Performance Computing Research Center (Minneapolis, MN) and the Mississippi Center for Supercomputing Research (University, MS) is gratefully acknowledged.

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[14] Gunde, R.; Felder, P.; Günthard, H. *Spectrochim. Acta, Part A* **1985**, *41A*, 319.

[15] Rostkowska, H.; Szczepaniak, K.; Nowak, M. J.; Leszczyński, J.; KuBulat, K.; Person, W. B. *J. Am. Chem. Soc.* **1990**, *112*, 2147.

The Gas-Phase and Solution-Phase Free Energy Surfaces for $CO_2 + OH^- \rightarrow HCO_3^-$

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Received November 7, 1991

The reaction of CO_2 with OH^- has an important biological role in the pH regulation of blood and the transportation of CO_2 in living systems.[1] Experiments show that the forward reaction (eq 1) in water encounters an activation barrier of 13.25 kcal/mol,[2]

$$CO_2 + OH^- \rightarrow HCO_3^- \qquad (1)$$

which leads to a reaction rate too slow to be physiologically useful.[1] However, nature provides an enzyme, carbonic anhydrase (CA), to speed up the reaction by 7 orders of magnitude, which makes this enzyme reaction one of the fastest known.[3] Thus, the origin of the aqueous-phase activation barrier is a key issue in furthering our understanding of the catalytic action of CA.[3] Previous theoretical studies of this reaction in the gas phase showed no activation barrier, which led to speculation that the solution-phase barrier is induced solely by solvation effects.[4,5] A study based on a continuum solvation model did show qualitatively a solvation-induced activation barrier.[6] However, this issue still remains open for three reasons: (1) the basis sets used in the previous ab initio studies are relatively small for such a charged system; (2) the effect of finite temperature was not included;[4,5] and (3) solvation effects were not considered explicitly.[6] In this note we have addressed all of these issues and have used this information to better understand catalysis by CA.

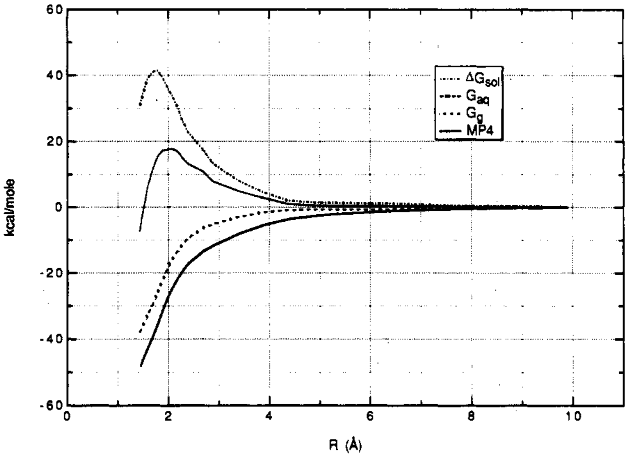

Figure 1. Calculated total energy profile (solid line, MP4), gas-phase free energy profile (dashed line, G_g), solution-phase free energy profile (dotted line, G_{aq}), and the solution-phase potential of mean force (dash–dot line, ΔG_{sol}) for $CO_2 + OH^-$.

Briefly, the ab initio gas-phase results were obtained as follows: geometries used in subsequent simulations, electrostatic potential (ESP) derived atomic point charges,[7] and thermodynamic corrections (at 298.15 K) obtained from normal mode analysis[8] were all determined at the RHF/6-31+G**//RHF/6-31+G** level. The reaction coordinate[9] was identified as the distance between the C atom of CO_2 and the O atom of OH^-.[4,5] This coordinate was fixed, and all other variables were optimized. The normal mode analysis was performed for all internal coordinates except for the reaction coordinate.[9-11] Final refinement of the total energy profile was accomplished at the MP4/6-311++G**//RHF/6-311++G** level.

We chose the zero-point reference of all thermodynamic state functions to be the separated $CO_2 + OH^-$ state. The gas-phase energy (*E*) and free energy (G_g) profiles are activationless (see Figure 1). This confirms the conclusion from previous studies.[4,5] The minimum energy structure for HCO_3^- is located at R = 1.45 Å with an *E* of −48.1 kcal/mol. The *H* and *TS* corrections are 1.2 and −8.7 kcal/mol, respectively. In conjunction with experimental solvation data,[12] these results enabled us to estimate the changes in thermodynamic functions for eq 1 in both the gas and aqueous phases.[13]

---

[1] (a) Maren, T. H. *Physiol. Rev.* **1967**, *47*, 595. (b) Davis, R. E. *Biol. Rev. Cambridge Philos. Soc.* **1951**, *26*, 87. (c) Roughton, F. J. W. *Harvey Lect.* **1943**, *39*, 96.

[2] Pinsent, B. R. W.; Pearson, L.; Roughton, F. J. W. *Trans. Faraday Soc.* **1956**, *52*, 1512.

[3] For recent reviews, see: (a) Silverman, D. N.; Lindskog, S. *Acc. Chem. Res.* **1988**, *21*, 30. (b) Silverman, D. N.; Vincent, S. H. *Crit. Rev. Biochem.* **1983**, *14*, 207. (c) Lipscomb, W. N. *Annu. Rev. Biochem.* **1983**, *52*, 17. (d) Lindskog, S. In *Zinc Enzymes*; Spiro, T. G., Ed.; John Wiley & Sons: New York, 1983. (e) Pocker, Y.; Sarkanen, S. *Adv. Enzymol.* **1978**, *47*, 149. (f) Bertini, I.; Luchinat, C.; Scozzafava, A. *Struct. Bonding (Berlin)* **1981**, *48*, 45. See also ref 24.

[4] Jonsson, B.; Karlstrom, G.; Wennerstrom, H. *J. Am. Chem. Soc.* **1978**, *100*, 1658.

[5] Liang, J. Y.; Lipscomb, W. N. *J. Am. Chem. Soc.* **1986**, *108*, 5051.

[6] Miertus, S.; Kysel, O.; Krajcik, K. *Chem. Zvesti* **1981**, *35*, 3.

[7] (a) Williams, D. E.; Yan, J. M. *Adv. At. Mol. Phys.* **1988**, *23*, 87. (b) Chirlian, L. E.; Francl, M. M. *J. Comput. Chem.* **1987**, *8*, 894. (c) Singh, U. C.; Kollman, P. A. *J. Comput. Chem.* **1984**, *5*, 129.

[8] Hehre, W. J.; Radom, L.; Schleyer, P. v. R.; Pople, J. A. *Ab initio Molecular Orbital theory*; John Wiley & Sons, Inc.: New York, 1986.

[9] McCullough, E. A., Jr.; Silver, D. M. *J. Chem. Phys.* **1975**, *62*, 4050. For a good discussion on the differences between an intrinsic reaction path and a minimum energy reaction path, see: Baskin, C. P.; Bender, C. F.; Bauschlicher, C. W.; Schaefer, H. F., III. *J. Am. Chem. Soc.* **1974**, *96*, 2709.

[10] Peng, Z.; Merz, K. M., Jr. Modifications of *Gaussian 88*, unpublished work.

[11] Garrett, B. C.; Truhlar, D. G. *J. Chem. Phys.* **1979**, *70*, 1593. Garrett, B. C.; Truhlar, D. G. *J. Am. Chem. Soc.* **1979**, *101*, 4534. Miller, W. H.; Handy, N. C.; Adams, J. E. *J. Chem. Phys.* **1980**, *72*, 99. For a more recent review, see: Miller, W. H. In *Perspectives in Quantum Chemistry*; Jortner, J., Pullman, B., Eds., Kluwer Academic Publishers: Hingham, MA, 1989; pp 57–82 and references therein.

[12] (a) Wilhelm, E.; Battino, R.; Wilcock, R. J. *Chem. Rev.* **1977**, *77*, 219. (b) Ben-Naim, A.; Marcus, Y. *J. Chem. Phys.* **1984**, *81*, 2016. (c) Marcus, Y. *Ion Solvation*; John Wiley & Sons Ltd.: Chichester, 1985; pp 107. (d) Marcus, Y.; Loewenschuss, A. *Annu. Rep. Prog. Chem., Sect. C* **1984 (1985)**, 81–135. (e) Marcus, Y. *J. Chem. Soc., Faraday Trans. 1* **1987**, *83*, 339. From these references, we were able to obtain the experimental free energies of solvation at 298.15 K for HCO_3^-, OH^-, and CO_2 as −81.5, −112.9, and 0.11 kcal/mol, respectively.

[13] The thermodynamic functions (see eq 1) in the gas phase are estimated to be ΔG_g = −38.2 ± 0.7 kcal/mol, ΔH_g = −46.9 kcal/mol (−49 kcal/mol as estimated by Liang and Lipscomb[5]), and ΔS_g = −29.2 cal/(mol K). The corresponding values in the aqueous phase are ΔG_{aq} = −7.05 kcal/mol, ΔH_{aq} = −10.2 kcal/mol, and ΔS_{aq} = −10.9 cal/(mol K).

0002-7863/92/1514-2733$03.00/0

Molecular dynamics[14] free energy perturbation[15] (MD-FEP) simulations were performed in order to obtain a free energy of solvation curve (ΔG_{sol}). Bond, angle, and torsion parameters were chosen to make the $OH^- \cdots CO_2$ complexes rigid, the atomic point charges came from the ESP fitting procedure, and the Lennard-Jones parameters were tuned so that the experimental free energies of solvation of HCO_3^-, OH^-, and CO_2 were reproduced.[12] The TIP3P water model was used,[16] and the nonbonded cutoff[14,15] was 8.0 Å. SHAKE[17] was used to constrain all bond lengths, and the time step was 1.5 fs. Periodic boundary conditions were employed,[14,15] and the temperature and pressure were maintained at 298.15 K and 1.0 atm.[14d] A typical run involved minimization of the system, followed by 45.0 ps of equilibration. Electrostatic decoupling[18] was used in conjunction with a 180-ps slow-growth MD-FEP simulation. Both forward and backward runs were conducted to estimate the error, and longer test runs (360 ps) indicated that the free energies using the 180-ps runs were converged. Sixteen runs were carried out to assemble the free energy profile, and ~250 water molecules were used at the starting point (1.45 Å) and ~800 water molecules at the last point (9.9 Å). Procedures similar to ours have been utilized by others to study reactions in solution.[11,19,20]

The resulting ΔG_{sol} and total G_{aq} profiles are shown in Figure 1. The maximum in the ΔG_{sol} profile occurs at 1.8 Å, which is also the minimum point for the dipole moment of the complex along the reaction path. G_{aq} has a pure solvation-induced free energy barrier of 17 ± 2 kcal/mol at $R = 2.0$ Å. We analyzed the original experimental data[2] and obtained an experimental free energy barrier of 13.5 ± 0.2 kcal/mol at 298.15 K.[21] In light of the fact that we neglected solvent polarization[22] in this charged system, the agreement between experiment and theory is remarkably good. The position of the solution-phase transition state is similar to that observed by others for the reactions of OH^- with formaldehyde and formamide.[19,20d] Our results indicate that the energetic cost for the reorganization of the water molecules as the reaction proceeds is on the order of 40 kcal/mol. This solvation penalty combined with the activationless gas-phase potential yields the observed free energy of activation barrier. An interesting experiment that could be done in order to verify this conclusion would be to measure the $CO_2 + OH^-$ reaction rate in solvents less polar than water. Our results predict that the reaction would have a higher rate constant under these conditions.

In this work we have obtained molecular-level insights into the hydration of CO_2 by hydroxide. Given this information, we can now better understand how CA catalyzes the hydration of CO_2. CA accelerates this reaction by providing a relatively anhydrous environment,[23] which is accomplished by placing the active site in a hydrophobic region.[3,4] Furthermore, the reaction only requires one OH^- (unlike the reaction of CO_2 with water, which apparently requires two water molecules[24]), and CA provides this at physiological pH with the aid of a zinc ion, which reduces the pK_a of zinc-bound water.[3,25] Finally, the CA active site places the CO_2 and OH^- in such a way that the reaction is greatly facilitated.[26] Whether CA renders the hydration of CO_2 by OH^- activationless like the gas-phase reaction is an interesting issue and is the subject of current investigation.[27]

Acknowledgment. We thank the NIH for supporting this work (GM44974) and the Pittsburgh Supercomputer Center for generous allocations of CRAY Y-MP computer time. Helpful discussions with Przemyslaw Maslak, Dr. Y. J. Zheng, and Dr. K. V. Damodaran are also acknowledged.

Supplementary Material Available: Table of derived parameters for hydroxide, carbon dioxide, and bicarbonate (1 page). Ordering information is given on any current masthead page.

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(14) For general references on MD techniques, see: (a) Brooks, C. L., III; Karplus, M.; Pettit, B. M.; Proteins: A Theoretical Perspective of Dynamics, Structures, and Thermodynamics. In *Advances in Chemical Physics*; John Wiley & Sons: New York, 1988; Vol. LXII. (b) McCammon, J. A.; Harvey, S. C. *Dynamics of Proteins and Nucleic Acids*; Cambridge University Press: London, 1987. (c) Allen, M. P.; Tildesley, D. J. *Computer Simulation of Liquids*; Clarendon Press: Oxford, 1987. (d) van Gunsteren, W. F.; Berendsen, H. J. C. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 1023 and references therein.

(15) For general references on FEP, see: (a) Mezei, M.; Beveridge, D. L. *Ann. N.Y. Acad. Sci.* **1986**, *482*, 1. (b) van Gunsteren, W. F. *Protein Eng.* **1988**, *2*, 5. (c) Jorgensen, W. L. *Acc. Chem. Res.* **1989**, *22*, 184. (d) Beveridge, D. L.; DiCapus, F. M. *Annu. Rev. Biophys. Chem.* **1989**, *18*, 431. (e) Kollman, P. A.; Merz, K. M., Jr. *Acc. Chem. Res.* **1990**, *23*, 246.

(16) Jorgensen, W. L.; Chandrasekhar, J.; Madura, J. D.; Impey, R. W.; Klein, M. L. *J. Chem. Phys.* **1983**, *79*, 926.

(17) van Gunsteren, W. F.; Berensen, H. J. C. *Mol. Phys.* **1977**, *34*, 1311.

(18) Singh, U. C.; Brown, F. K.; Bash, P. A.; Kollman, P. A. *J. Am. Chem. Soc.* **1987**, *109*, 1607.

(19) Weiner, S. J.; Singh, U. C.; Kollman, P. A. *J. Am. Chem. Soc.* **1985**, *107*, 2219.

(20) (a) Chandrasekhar, J.; Smith, S. F.; Jorgensen, W. L. *J. Am. Chem. Soc.* **1984**, *106*, 6867; **1985**, *107*, 154. (b) Chandrasekhar, J.; Jorgensen, W. L. *J. Am. Chem. Soc.* **1985**, *107*, 2974. (c) Jorgensen, W. L.; Buckner, J. K. *J. Phys. Chem.* **1986**, *90*, 4651. (d) Madura, J. D.; Jorgensen, W. L. *J. Am. Chem. Soc.* **1986**, *108*, 2517. (e) Blake, J. F.; Jorgensen, W. L. *J. Am. Chem. Soc.* **1991**, *113*, 7430. (f) Gao, J. *J. Am. Chem. Soc.* **1991**, *113*, 7796.

(21) (a) Amdur, I.; Hamnes, G. G. *Chemical Kinetics, Principles and Selected Topics*; McGraw-Hill: New York, 1966; pp 48–58. (b) Boudart, M. *Kinetics of Chemical Process*; Prentice-Hall: Old Tappan, NJ, 1968; pp 35–46. (c) Kreevey, M. M.; Truhlar, D. G. Investigation of Rates and Mechanics of Reactions. In *Techniques of Chemistry*, 4th ed.; Bernasconi, C. F., Ed.; Wiley-Interscience: New York, 1986; Vol. VI, Part 1.

(22) (a) Caillol, J. M.; Levesque, D.; Weis, J. J.; Kusalik, P. G.; Patey, G. N. *Mol. Phys.* **1985**, *55*, 65; **1987**, *62*, 1225. (b) Howard, A. E.; Singh, U. C.; Billeter, M.; Kollman, P. A. *J. Am. Chem. Soc.* **1988**, *110*, 6984. (c) Angyan, J. G.; Colonna-Cesari, F.; Tapia, O. *Chem. Phys. Lett.* **1990**, *116*, 180. (d) Straatsman, T. P.; McCammon, J. A. *Mol. Simul.* **1990**, *5*, 181; *Chem. Phys. Lett.* **1990**, *167*, 252; *177*, 433. (e) Sprik, M.; Klein, M. L. *J. Chem. Phys.* **1988**, *89*, 7556. Sprik, M. *J. Phys. Chem.* **1991**, *95*, 2283. Watanabe, K.; Klein, M. L. *Chem. Phys.* **1989**, *131*, 157.

(23) Dewar, M. J. S.; Storch, D. M. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 2225.

(24) Merz, K. M., Jr. *J. Am. Chem. Soc.* **1990**, *112*, 7973.

(25) Merz, K. M., Jr.; Hoffmann, R.; Dewar, M. J. S. *J. Am. Chem. Soc.* **1989**, *111*, 5636.

(26) Merz, K. M., Jr. *J. Mol. Biol.* **1990**, *214*, 799.

(27) Zheng, Y. J.; Merz, K. M., Jr. Research in progress.

Modulation of the Chain Conformation of Spiropyran-Containing Poly(L-lysine) by the Combined Action of Visible Light and Solvent

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Received November 1, 1991

In a previous paper[1] we reported clear CD evidence that poly(L-glutamic acid) containing spirobenzopyran units in the side chains can undergo reversible random coil ⇄ α-helix conversions upon exposure to sunlight and dark conditions, alternately.

Here we describe the photochromic behavior of polypeptides obtained by attaching spirobenzopyran units to the side chains of poly(L-lysine). In contrast to the analogous polymers derived from poly(Glu), the spiropyran-modified poly(Lys) do not give photoresponse effects in pure hexafluoro-2-propanol (HFP). However, when appropriate amounts of triethylamine are added to the HFP solutions, exposure to sunlight produces reversible helix

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(1) Ciardelli, F.; Fabbri, D.; Pieroni, O.; Fissi, A. *J. Am. Chem. Soc.* **1989**, *111*, 3470–3472.

0002-7863/92/1514-2734\$03.00/0 © 1992 American Chemical Society