Communication

The Burden Borne by Urease

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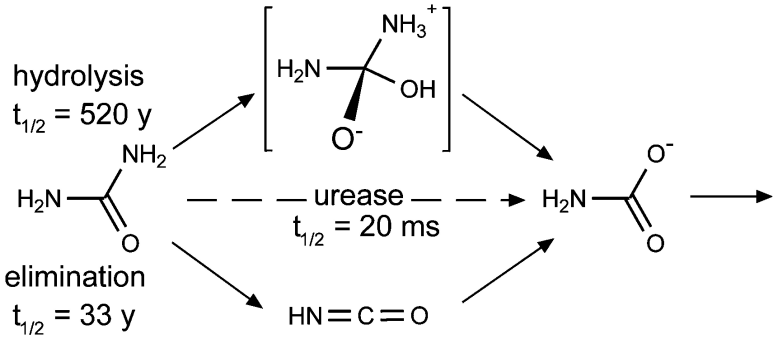

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The Burden Borne by Urease

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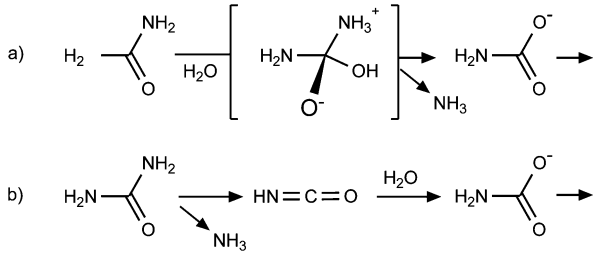
To appreciate the power of an enzyme as a catalyst, and its potential sensitivity to inhibition by an ideal transition-state analogue, it is useful to know the rate of the spontaneous reaction in the absence of a catalyst. Conventional peptide bonds undergo spontaneous hydrolysis in neutral aqueous solution with a half-time of ~500 years.[1] Numerous proteases cleave these bonds with half-times in the neighborhood of 10 ms,[1b] increasing the rate of water attack on the peptide bond by a factor of roughly 10^{12}. It would be of interest to know how these rate enhancements compare with those produced by other hydrolases that may be related in their mechanism of action. But, in the case of urease, well-characterized in most respects, the meaning of that comparison is clouded by a disparity between the mechanisms of the catalyzed and uncatalyzed reactions. At the active site of urease, urea appears to undergo nucleophilic attack by water (Scheme 1a),[2] but in solution,[3] and in the presence of an artificial dinickel catalyst,[4] urea decomposes by elimination of ammonia (Scheme 1b). The half-time for uncatalyzed elimination is 40 y at 25 °C.[3] It is clear that the half-time for spontaneous hydrolysis of urea must be substantially greater than 40 y, but the actual rate of spontaneous hydrolysis is unknown because that reaction has never been detected experimentally under any conditions.[5]

Scheme 1. Alternate Mechanisms of Urea Decomposition in Water



The results of quantum mechanical simulations, undertaken in an attempt to address that question, have been interpreted as indicating that urea hydrolysis may be very slow indeed. At 25 °C, $t_{1/2}$ was predicted to be ~10^{19} y.[6] If that inference is correct, then urease surpasses proteases, and all other enzymes,[7] in its power to enhance the rate of reaction. In view of the similarity between urea[8] and conventional amides[9] in their bond lengths and bond angles, and their implied similarity in resonance energies, that conclusion seemed surprising, and to warrant an experimental test.

If urea hydrolysis were extremely difficult, then a fully methylated derivative of urea that can undergo hydrolysis, but from which elimination cannot occur, would be expected to be extremely stable to decomposition in water. In the present work, we tested that possibility by examining the hydrolysis of 1,1,3,3-tetramethylurea (Me_4U), comparing its rate constant and heat of activation with those of related compounds.

In a typical experiment, samples of the reactant (0.01 M) dissolved in buffer (potassium ^{2}H-formate, $^2H_3$-acetate, phosphate, borate, or carbonate, 0.1 M) were sealed under vacuum in quartz tubes and heated for timed intervals in a Thermolyne 47900 furnace. After cooling, the tube's contents were diluted with ^2H_2O to which pyrazine had been added as an integration standard for analysis by ^{1}H NMR. Each compound yielded only the products expected as a result of its hydrolysis, and apparent first-order rate constants for reaction were determined from the integrated intensities of carbon-bound protons of the substrate remaining, plotted as a semilogarithmic function of time. The rate of hydrolysis of Me_4U was invariant between pH 4 and 10, as had been observed earlier for peptide hydrolysis.[1] When the logarithm of the rate of Me_4U hydrolysis was plotted as a function of the reciprocal of the absolute temperature (Figure 1), extrapolation of the results yielded a rate constant of 4.2×10^{-12} s^{-1} at 25 °C and an E_{act} of 22.9 kcal/mol. These findings, and the results of similar experiments on related compounds, are summarized in Table 1. The comparisons in Figure 2 show that the thermodynamics of activation for hydrolysis of Me_4U are similar to the values for hydrolysis of compounds related in structure, but are very different from the values for NH_3 elimination from urea.

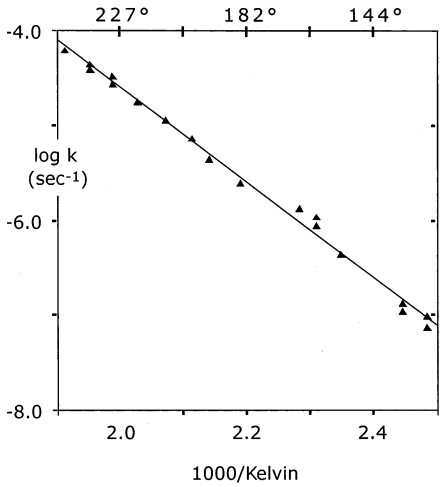


Figure 1. Pseudo-first-order rate constants for the decomposition of Me_4U in potassium phosphate buffer (0.1 M, pH 6.8) plotted as a logarithmic function of the reciprocal of absolute temperature (Kelvin).

Is urea likely to resemble Me_4U in its susceptibility to hydrolysis? N-methylation and N-dimethylation are known to influence the free energies of solvation of acetamides by water,[14] and those differences would be expected to change to some extent in the transition states for hydrolysis. But, solvation and electronic effects are not likely to be major, as indicated by the fact that acetamide undergoes spontaneous hydrolysis 2.8-fold more rapidly than *N*,*N*-dimethylacetamide at 25 °C (Table 1). If that factor[15] is applied to the observed rate constant for hydrolysis of Me_4U, then the pseudo-first-order rate constant for spontaneous hydrolysis of urea would be 1.17×10^{-11} s^{-1} at 25 °C, quite similar to the average of the values listed for related compounds in Table 1. That value (which

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Table 1. Temperature Effects on the Spontaneous Hydrolysis of Ureas and Amides in Neutral Solution at 25 °C

	$k_{hydrolysis}$ (25 °C) (s^{-1})	$\Delta H^{\ddagger}$ (kcal/mol)	$T\Delta S^{\ddagger}$ (25 °C) (kcal/mol)	$t_{1/2}$ (25 °C)
		Ureas		
urea (elimination)[3]	6.5×10^{-10}	+31.9	+2.0	33 y
sym-dimethylurea[10]	1.0×10^{-10}	+27.9	−3.1	220 y
tetramethylurea[10]	4.2×10^{-12}	+22.3	−10.6	5200 y
		Amides		
formamide[11]	1.1×10^{-10}	+21.9	−9.0	200 y
acetamide[10]	5.1×10^{-11}	+23.8	−7.6	430 y
methylacetamide[10]	4.6×10^{-10}	+22.5	−7.6	48 y
dimethylacetamide[10]	1.8×10^{-11}	+22.5	−9.5	1200 y
Ac-gly-/-gly[1b]	4.4×10^{-11}	+24.4	−7.1	500 y
Ac-gly-/-glyNHMe[1b]	3.6×10^{-11}	+22.9	−8.6	610 y
cytidine[12]	2.7×10^{-10}	+22.1	−8.3	80 y
		Urea (extrapolated, see text)		
urea (hydrolysis)[10]	*1.2×10^{-11}*	*+22.9*	*−8.2*	*520 y*
		Enzymatic		
urease (jack bean)[13]	3.6×10^{4}	+10.5	−0.7	0.02 s

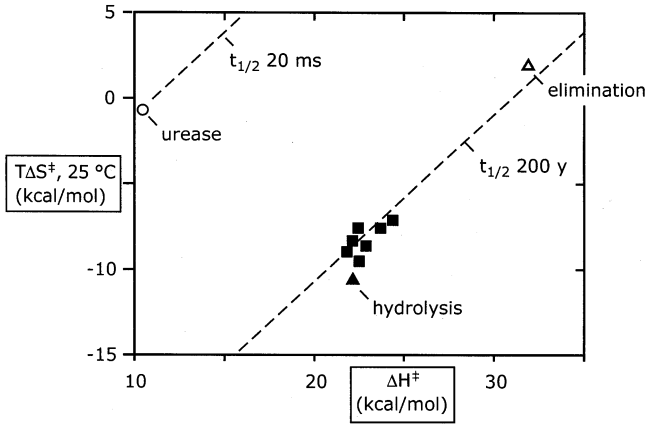

Figure 2. Entropies and enthalpies of activation for the decomposition of urea (open square), Me_4U (closed triangle), other carboxamides (closed squares) and the urease:urea complex (open circle), in potassium phosphate buffer (0.1 M, pH 6.8) at 25 °C (Table 1). The diagonal lines represent half-times of 20 ms and 200 years at 25 °C.

exceeds a reported value based on recent simulations[6] by a factor of $\sim 10^{13}$) is equivalent to 2% of the rate constant for elimination of ammonia from urea at 25 °C (Table 1), suggesting that urea hydrolysis occurs at a rate that is only just below our present limits of detection.

We infer that the rate enhancement produced by urease is not markedly much greater than the rate enhancements produced by other C−N hydrolases that bring about water attack on similar bonds, typified by carboxypeptidase b[1b] and *Escherichia coli* cytidine deaminase,[11] as shown in Table 2. Within this group of enzymes, which differ in their overall folding and active-site topology, urease retains the distinction of catalyzing the hydrolysis of a very simple substrate, urea, with protease-like proficiency (Table 2). The rate enhancement that urease produces (3×10^{15}-fold) seems especially remarkable in view of the absence from urea of the relatively elaborate binding determinants that are ordinarily present in the substrates of proteases, which presumably help proteases to distinguish between the substrate in the ground state and the transition state for hydrolysis. Urease appears to be unique among hydrolases in containing two nickel atoms,[2,4] which presumably assist this enzyme in grappling effectively with this unusually simple substrate.

Table 2. Rate Enhancements and Catalytic Proficiencies of Amidohydrolases Acting on Urea, Proteins, and Nucleosides at 25 °C

	jack bean urease[10]	beef carboxypeptidase B[1b]	*E. coli* cytidine deaminase[11]
k_{non} (s^{-1})	1.2×10^{-11}	1.8×10^{-11}	2.7×10^{-10}
k_{cat} (s^{-1})	3.6×10^{4}	240	300
K_m (M)	4×10^{-3}	4×10^{-5}	1.2×10^{-4}
k_{cat}/K_m ($M^{-1}\,s^{-1}$)	9×10^{6}	6×10^{6}	2.7×10^{6}
rate enhancement = k_{cat}/k_{non}	3×10^{15}	1.3×10^{13}	1.1×10^{12}
catalytic proficiency = $(k_{cat}/K_m)/k_{non}$ (M^{-1})	8×10^{17}	3.3×10^{17}	1.0×10^{16}

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References

(1) (a) Bryant, R. A. R.; Hansen, D. E. *J. Am. Chem. Soc.* **1996**, *118*, 5498−5499. (b) Radzicka, A.; Wolfenden, R. *J. Am. Chem. Soc.* **1996**, *118*, 6105−6109. (c) Smith, M. R.; Hansen, D. E. *J. Am. Chem. Soc.* **1998**, *120*, 8910−8913.

(2) (a) Dixon, N. E.; Riddles, P. W.; Gazzola, R. L.; Blakely, R. L.; Zerner, B. *Can. J. Biochem.* **1980**, *58*, 1335−1343. (b) Jabri, E.; Carr, M. B.; Hausinger, R. P.; Karplus, P. A. *Science* **268**, 998−1004.

(3) Shaw, W. H. R.; Bordeaux, J. J. *J. Am. Chem. Soc.* **1955**, *77*, 4729−4733.

(4) (a) Barrios, A. M.; Lippard, S. J. *J. Am. Chem. Soc.* **2000**, *122*, 9172−9177. (b) Barrios, A. M.; Lippard, S. J. *Inorg. Chem.* **2001**, *40*, 1250−1255.

(5) At elevated temperatures, both urea (Lynn, K. R. *J. Phys. Chem.* **1965**, *69*, 687−689) and tetramethylurea (Homer, R. B.; Alwis, K. W. *J. Chem. Soc., Perkin II* **1976**, 362−365) undergo base-catalyzed decomposition in 1−2 M NaOH.

(6) Estiu, G.; Merz, K. M. *J. Am. Chem. Soc.* **2004**, *126*, 1832−1842. (b) Estiu, G.; Merz, K. M. *J. Am. Chem. Soc.* **2004**, *126*, 6932−6944.

(7) Lad, C.; Williams, N. H.; Wolfenden, R. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, 100, 5607−5710.

(8) (a) Vaughan, P.; Donahue, J. *Acta Crystallogr.* **1952**, *5*, 530−535. (b) Andrew, E. R.; Hyndman, D. *Discuss. Faraday Soc.* **1955**, 19, 195−200.

(9) Kimura, M.; Aoki, M. *Bull. Chem. Soc. Jpn.* **1953**, *57*, 429−435.

(10) This work.

(11) Siebocka-Tilk, H.; Sauriol, F.; Monette, M.; Brown, R. S. *Can. J. Chem.* **2002**, *80*, 1343−1350.

(12) Snider, M. J.; Gaunitz, S.; Ridgway, C.; Short, S. A.; Wolfenden, R. *Biochemistry* **2000**, *39*, 9746−9753.

(13) Laidler, K. J.; Hoare, J. P. *J. Am. Chem. Soc.* **1950**, *72*, 2489−2494.

(14) Wolfenden, R. *Biochemistry* **1978**, *17*, 201−204.

(15) In view of the presence of two dimethyl groups in Me_4U, it might be more appropriate to use the *square* of 2.8 as a correction factor. That correction would not alter the present conclusions.

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