Host-Catalyzed Isoxazole Ring Opening: A Rationally Designed Artificial Enzyme

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Received November 20, 1995

The recent surge of interest in the field of host−guest chemistry[1] has resulted in several applications of host molecules as organic catalysts.[2] In this communication we report the artificial enzyme behavior of xylene-bridged hosts **1** and **2** (Figure 1). Both compounds catalyze the base-promoted decomposition of 5-nitrobenzisoxazole **3** to cyanophenol **4** with unusually large rate accelerations (Figure 2), via a Michaelis-like complex. The fragmentation was originally investigated by Kemp, who established it as an E_2 elimination which could be performed by simple amines.[3] More recently, Hilvert and co-workers have reported catalytic antibodies which perform the reaction with impressive rate enhancements and turnover numbers.[4]

Hosts **1** and **2** bind phenols tightly in nonpolar organic solvents.[5] When either host is mixed with isoxazole **3**[6] in $CDCl_3$, rapid conversion to **4** takes place, resulting in the formation of the host−product complex, as observed by ^{1}H-NMR.[7] A kinetic isotope effect of 5.2 (k_H/k_D) is observed in the reaction with host **1**,[8] and cyanophenol **4** is the only observable product.

$$H + G \underset{}{\overset{K_a}{\rightleftharpoons}} H\bullet G \xrightarrow{k} H\bullet P \overset{K_p}{\rightleftharpoons} H + P \qquad (1)$$

The kinetics of the system are described by eq 1; there is a fast pre-equilibrium, followed by a slow intracavity reaction, product release, and subsequent sequestration of the host as the product complex. The kinetic parameters (association constants for substrate and product, intracavity rate constant) were obtained by nonlinear least-squares fitting of time−concentration data to this model (Figure 3), using the DNRP-RKF program developed by Duggleby.[9] The parameters for the reaction with proximal xylene host **1** are $K_a = 96 \pm 12$ M^{-1}, $k = (6.3 \pm 0.3) \times 10^{-4}$ s^{-1}, and $K_p = 6 \times 10^{11}$ M^{-1}. The large value for K_p is consistent with our previous results.[10] The derived values for both association constants were corroborated by independent titrations.[11,12] The parameters for the distal xylene host **2** are $K_a = 13 \pm 1$ M^{-1}, $k = (7.6 \pm 0.5) \times 10^{-4}$ s^{-1}, and

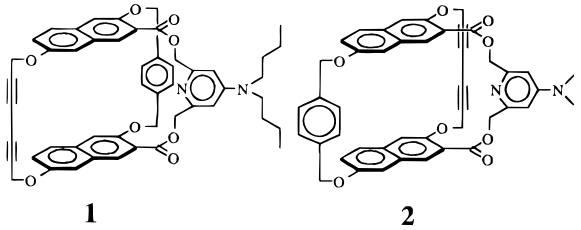

Figure 1. The "enzymes".

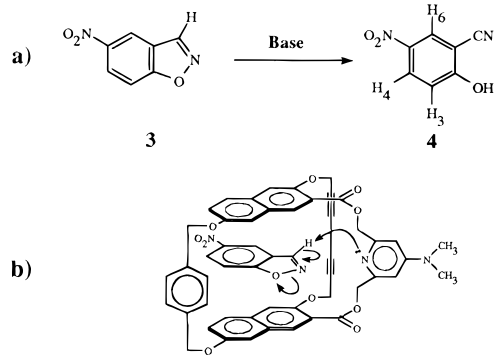

Figure 2. The system: (a) general reaction and (b) mechanism of intracavity process.

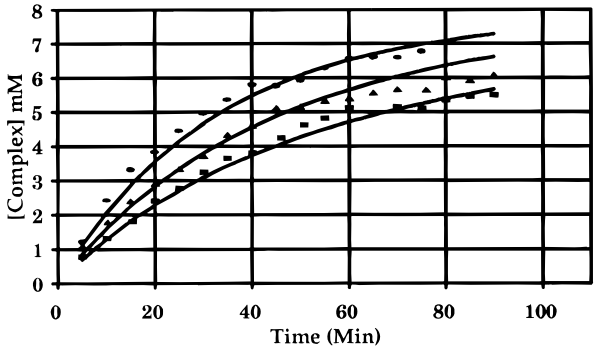

Figure 3. Plots of [**1**·**4**] versus time for the reaction of proximal xylene host **1** (7 mM) and isoxazole **3** in $CDCl_3$ at room temperature. The initial isoxazole concentrations were: (●) 45, (▲) 16, and (■) 10 mM. Solid lines represent derived fits as described in text.

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(6) Prepared in 89% yield from salicylaldehyde using the procedure of Kemp and Casey.[3a]

(7) All studies described in this work were performed in $CDCl_3$ at 25 °C. The spectrum was identical to that obtained by complexation of a known sample of **4**.[3a]

(8) The 7-D analog of **3** was prepared according to Kemp and Casey.[3a]

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(10) The error in K_p is large (i.e., >100 × K_p), and the value given is only meant to demonstrate that K_p is large (see ref 12). Performing the kinetic analysis with K_p fixed at values over several orders of magnitude above a certain threshold value (ca. 10 000) has very little effect on the calculated values for the other parameters. This is not surprising since it is difficult to distinguish values this large from infinity using NMR techniques. This is also why we are hesitant to ascribe detailed physical significance to the apparently large difference in K_p for **1** and **2**, although previous work[5,14] has demonstrated the general superiority of the proximal-xylene-bridged hosts. For a further discussion of large K_a values, see ref 14c and: Wilcox, C. S. In *Frontiers in Supramolecular Organic Chemistry and Photochemistry*; Schneider, H. J., Dürr, H., Eds.; VCH Publishers: New York, 1991; pp 123−143.

(11) The value for K_a was obtained by rapid, dilute titration to avoid formation of phenol **4**.

(12) Standard methods which follow weighted average shifts could not be used for K_p due to slow exchange at 25 °C. An estimate of $K_p \geq 10\,000$ was obtained by direct integration of both free and bound species at low temperature. Since there is hardly any free guest, the error is large.

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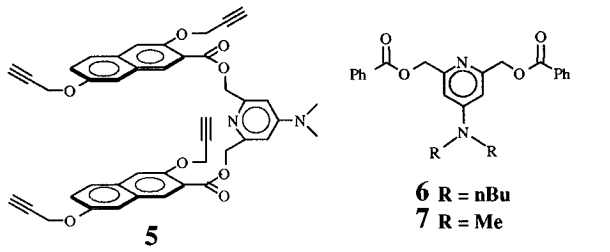

Figure 4. Control compounds.

Table 1. Relative Rates of Isoxazole Ring Opening in $CDCl_3$ at 25 °C

	host **1**	host **2**	**5**	**6**	**7**
k^a (s^{-1} M^{-1})	6.1×10^{-2}	9.8×10^{-3}	1.5×10^{-5}	1.9×10^{-4}	1.0×10^{-5}
k_{rel}	5869	950	1.4	18	1

[a] For hosts **1** and **2** the second-order rate constant $k = k$(cavity) (K_a), where k(cavity) is the derived first-order rate constant obtained from the kinetic analysis.

Table 2. Free and Complexed Chemical Shifts of Cyanophenol **4**

proton	free	**1·4**	$\Delta\delta$	**2·4**	$\Delta\delta$
H_3	7.14	5.13	2.01	5.72	1.42
H_4	8.38	3.76	4.62	6.48	1.90
H_5	8.48	6.89	1.59	4.63	3.85

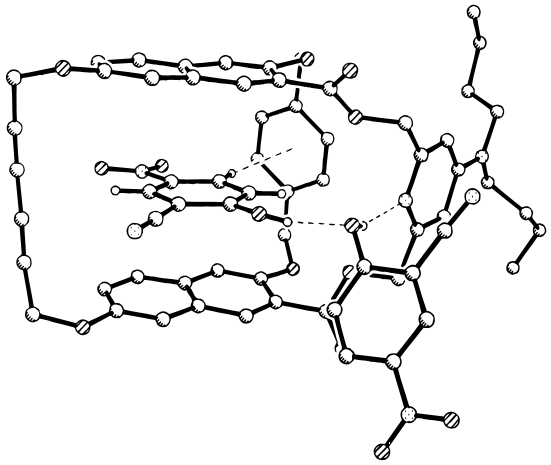

Figure 5. X-ray structure of **1·4**. Only the guest hydrogens are included. The phenolic proton is hydrogen bonded to an external guest molecule, which is in turn hydrogen bonded to the pyridine nitrogen. The external guest is an artifact of the crystallization process, and its presence during the reaction is not consistent with the observed data.

$K_p = (3.7 \pm 1.5) \times 10^4$ M^{-1}. Strong product inhibition is observed, in agreement with the derived higher K_a for the product. The degree of rate acceleration was measured by obtaining the second-order rate constants for the reaction of **3** with model pyridines **5**−**7** (Figure 4).[13] The results are also listed in Table 1.

The structure of the host−product complexes were examined by both ^{1}H-NMR and X-ray crystallography. The chemical shifts of free and bound species (Table 2) are consistent with guest intercalation into the host cavity, as seen previously.[14] In both complexes the guest proton nearest to the xylene spacer is shifted upfield by more than 3.8 ppm. The crystal structure of the **1·4** complex (Figure 5) confirms this binding model. The guest is clearly within the cavity and is almost perfectly positioned for both stacking interactions[15] with the napthalene rings and an edge−face interaction[16] with the xylene bridge.[17] The guest orientation reveals that product inhibition occurs after initial dissociation[18] and is thus unrelated to the catalytic process itself.

The above observations demonstrate that hosts **1** and **2** are competent artificial enzymes. They promote reaction via a host−substrate complex, analogous to enzymic Michaelis complexes. Comparison of second-order rate constants (Table 1) reveals that both hosts accelerate the reaction approximately 1000-fold, with effective molarities of 63 and 76 M.[19] The decreased efficiency of acyclic models, even those as similar as **5**, and the severe product inhibition demonstrate that a well-defined, appropriately functionalized cavity is essential.

The source of enzymatic rate accelerations has been the subject of much controversy in the literature.[20−23] The accelerations observed here, though small in comparison to those observed with Hilvert's catalytic antibodies,[24] are nonetheless significant. They are unusual in that large rate accelerations are observed despite the absence of strained or conformationally rigid ground states normally associated with intramolecular catalysis. The host compensates with two primary weapons. The first is the complexation-induced proximity of the pyridine nitrogen to the proton which must be removed. This allows for rate enhancement in accord with Menger's spatiotemporal effect.[21,25] In addition, the napthalene rings are available for dispersion-mediated selective stabilization of the highly polarizable transition state. Although the importance of such an aromatic microenvironment has been noted by both Kemp[3d] and Hilvert,[4] the reaction of **5** in benzene was only slightly accelerated compared to that in chloroform.[26] The achievement of such large increases in rate with so few tools represents a triumph for rationally designed artificial enzyme construction.[27]

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(13) Obtained from plots of substrate concentration versus observed pseudo-first-order rate constants.

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(15) The distances between the xylene and napthalene planes (3.38, 3.32 Å) are reminiscent of those in base pair stacking (≈3.4 Å): Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984.

(16) (a) Cox, E. G.; Cruikshank, D. W. J.; Smith, J. A. C. *Proc. R. Soc. London* **1958**, *A347*, 1. (b) Blundell, T.; Singh, J.; Thornton, J. M.; Burley, S. K.; Petsko, G. A. *Science* **1986**, *234*, 1005. (c) Jorgensen, W. L.; Severance, D. L. *J. Am. Chem. Soc.* **1990**, *112*, 4768−4774.

(17) The distance between the guest and xylene centroids is 4.95 Å. An optimal value of 5.0 Å was calculated by Jorgensen.[16c] Similar experimental values have also been observed: Paliwal, S.; Geib, S.; Wilcox, C. S. *J. Am. Chem. Soc.* **1994**, *116*, 4497−4498 and references therein.

(18) In order to maintain a reasonable transition state for the elimination, the initial product complex must have the cyano group pointing toward, rather than away from, the pyridine. Simple rotation within the cavity is sterically prohibited on the basis of an examination of CPK models.

(19) The effective molarity = k(cavity)/k_{obs}, where k_{obs} is the second-order rate constant measured for control compound **7**.

(20) (a) Page, M. I.; Jencks, W. P. *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *68*, 1678−1683. (b) Jencks, W. P. *Adv. Enz.* **1975**, *43*, 219−402. (c) Bruice, T. C. *Annu. Rev. Biochem.* **1976**, *45*, 331. (d) Alberg, W. J.; Knowles, J. R. *Angew. Chem., Int. Ed. Engl.* **1977**, *16*, 285. (e) Kirby A. J. *Adv. Phys. Org. Chem.* **1980**, *17*, 183−278. (f) Jencks, W. P. *Acc. Chem. Res.* **1980**, *13*, 161. (g) Hansen, D. E.; Raines, R. T. *J. Chem. Educ.* **1990**, *67*, 483−488.

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(22) For an alternative view, see: (a) Houk, K. N.; Tucker, J. A.; Dorigo, A. E. *Acc. Chem. Res.* **1990**, *23*, 107−113. (b) Eurenius, K. P.; Houk, K. N. *J. Am. Chem. Soc.* **1994**, *116*, 9943.

(23) For recent work which questions Menger's original example, see: Curran, T. P.; Borysenko, C. W.; Abelleira, S. M.; Messier, R. J. *J. Org. Chem.* **1994**, *59*, 3522−3529.

(24) Hilvert sees accelerations on the order of 10^8 and effective molarities of 20 000−40 000. Part of this difference may be due to the nature of the base employed. The carboxylate in the active site may have an advantage over the neutral pyridine of the hosts, given the nonpolar nature of the environments.

(25) While this notion is appealing, it is unclear exactly how much more time the substrate spends "close" to the nitrogen, since the isoxazole binding is so weak.

(26) The rate is increased eight times in benzene, far short of the increase observed with **1** or **2**.

(27) We thank Professor W. W. Cleland for helpful discussions and Dr. Douglas R. Powell for obtaining the X-ray structure of **1·4**. A.J. K. thanks the NIH for a Chemistry−Biology Interface traineeship (T32-GM08505) and the DOE for a predoctoral fellowship.