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ON THE EVALUATION OF A SMALL MOLECULE MIMIC OF CHYMOTRYPSIN

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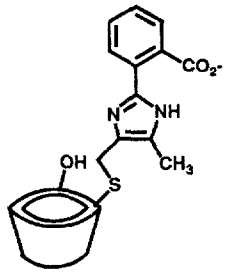
Abstract: An alternative analysis of a recently published serine protease model suggests a more benign role for its imidazole-carboxylate moiety.

A recent series of papers have described **1**, a small molecule mimic of chymotrypsin in which an imidazole-benzoate group is covalently attached to the secondary side of β-cyclodextrin.[2a-d] As such, model **1** contains the triad of catalytic groups (hydroxyl, imidazole, carboxylate) found in the active site of all serine proteases. The kinetic constants for hydrolysis of *m*-(*tert*-butyl)phenyl acetate by **1** at its pH optimum (pH = 10.7) were compared with those for hydrolysis of *p*-nitrophenyl acetate by chymotrypsin at its pH optimum of 8.0 (Table 1). This comparison lead to the



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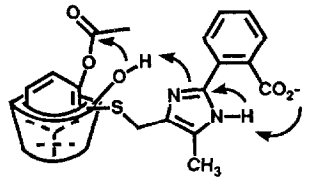

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argument[2] that **1** catalyzes the hydrolysis by using the charge relay mechanism shown in **2** because: 1) k_{cat} and K_m are very close to those for chymotrypsin and 2) the solvent isotope effect (k_{H_2O}/k_{D_2O} = 3) indicates imidazole general base catalysis. However, a simpler mechanism involving nucleophilic attack by an ionized cyclodextrin hydroxyl, *without involvement of the imidazole-carboxylate moiety*, seems most consistent with the experimental observations.

Support for this alternative mechanism comes from the observation that β-cyclodextrin alone hydrolyzes *m*-(*tert*-butyl)phenyl acetate ca. 4 times *faster* than does **1** (Table 1). The very high pH optimum (pH = 10.7) is more consistent with ionization of a secondary hydroxyl group (pK_a ca. 12, ref. 3b) than with ionization of the imidazolium moiety, which would be predicted to have a pK_a of ca. 7.[4] Of course, the cyclodextrin alkoxide cannot enjoy general base catalysis. The solvent isotope effect seen with **1** (k_{H_2O}/k_{D_2O} = 3) is identical to that reported for the hydrolysis of *m*-(*tert*-butyl)phenyl acetate by α-cyclodextrin.[5] This is believed to result from a solvent induced shift in the pK_a of the secondary hydroxyls.

Chymotrypsin model **1** has also been shown to hydrolyze 2.5 equivalents of *m*-(*tert*-butyl)-phenyl acetate in a continuous manner, which has been termed turnover.[2c] However, the release of *m*-(*tert*-butyl)phenol was monitored while the fate of the acyl portion of the substrate was not determined. Since **1** contains 13 secondary hydroxyl groups, multiple acylations are possible without true turnover catalysis by the chymotrypsin mimic.

Table: Hydrolysis of Esters by Chymotrypsin and **1**.

Catalyst	Acetate Substrate	pH	k_{cat} x 10^{-2} (s^{-1})	K_m x 10^{-5} (M)	k_{cat}/K_m ($M^{-1}s^{-1}$)	Ref.
Chymotrypsin	*p*-nitrophenyl-	8.0	1.1	4.0	275	2
1	*m*-(*tert*-butyl)phenyl-	10.7	2.8	13.3	210	2
β-Cyclodextrir	*m*-(*tert*-butyl)phenyl-	10.6	12.2	13	938	3a

It is not clear why the imidazole-benzoate group of **1** slows down the rate of hydrolysis relative to β-cyclodextrin. A change in conformation of the hydrophobic pocket or an interaction between the substrate and the imidazole-benzoate group may result in a less favorable positioning of the carbonyl group for nucleophilic attack by the secondary hydroxyl group. It is remarkable how difficult it has been to observe O-acylation catalyzed by an internal imidazole. Hydroxy-imidazoles reported to date undergo preferential N-acylation,[6] catalyze the attack of a water molecule,[7] or have undetermined mechanisms. Brown's recently reported system appears to be the only documented model of the acylation mechanism proposed to operate in the chymotrypsin active site.[8]

In conclusion, the experimental data reported for the hydrolysis of *m*-(*tert*-butyl)phenyl acetate catalyzed by **1** is most consistent with a mechanism involving nucleophilic attack by an ionized secondary hydroxyl on the substrate carbonyl without involvement of the imidazole-carboxylate moiety. Thus, an effective mimic for the chymotrypsin catalytic cycle remains elusive although substantial progress has been reported toward this goal.[9,10]

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10. The accompanying letter by Breslow and Chung reaches similar conclusions through a direct evaluation of **1**. The author thanks Prof. R. Breslow for communicating their results in advance of publication.

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