Intramolecular general base-catalyzed ester hydrolyses by the imidazolyl group
(model of protease deacylation/rigid bicyclic ring/endo-endo structure/proper stereochemistry)

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ABSTRACT Intramolecular general base catalysis by the imidazolyl group was found in the hydrolyses of endo-5-[4'(5')-imidazolyl]-bicyclo[2.2.1]hept-endo-2-yl trans-cinnamate and endo-5-[4'(5')-imidazolyl]-bicycle[2.2.2]oct-endo-2-yl trans-cinnamate, in which the imidazolyl and trans-cinnamoyl groups are bound in close proximity to each other by rigid bicyclic rings. The rate constants for the intramolecular general base-catalyzed hydrolyses at 60° are 6.4×10⁻⁷ sec⁻¹ for the former and 1.8×10⁻⁷ sec⁻¹ for the latter and the deuterium oxide solvent isotope effects are 3.0 for both.

On the other hand, no intramolecular catalytic participation of the imidazolyl group was observed in the hydrolyses of the endo-exo isomers, exo-5-[4'(5')-imidazolyl]-bicycle[2.2.1]hept-endo-2-yl trans-cinnamate and endo-5-[4'(5')-imidazolyl]-bicycle[2.2.2]oct-exo-2-yl trans-cinnamate, in which the imidazolyl groups are located far from the trans-cinnamoyl groups. Intramolecular general base-catalyzed hydrolyses by the imidazolyl groups in endo-5-[4'(5')-imidazolyl]-bicycle[2.2.1]hept-endo-2-yl trans-cinnamate and endo-5-[4'(5')-imidazolyl]-bicycle[2.2.2]oct-endo-2-yl trans-cinnamate can serve as models of serine esterase-catalyzed hydrolyses.

Recently, a considerable number of studies on intramolecular catalyses have been made by assuming the hypothesis that intramolecular catalyses can serve as models of enzymes. Some of these studies were of special importance in clarifying the mechanism of enzymatic reactions. Bruce and coworkers (1–3) found that the imidazolyl group participates in the intramolecular nucleophilic catalysis of hydrolysis of an ester or amide. Furthermore, there are other reports on the intermolecular catalysis of ester hydrolysis (4–6) or lactonization (7) by imidazole or its derivatives. However, there are no reports of intramolecular participation by imidazole in general base catalyses involving acyl-oxygen cleavage, which takes place both in acylation to form an acyl-enzyme, and in deacylation of the acyl-enzyme in serine esterase-catalyzed reactions. Both the present model systems and chymotrypsin reactions (8) proceed through acyl-oxygen fission. An intramolecular general base catalysis involving alkyl-oxygen fission in a special aliphatic system was found by Bruce et al. (9).

We report here on the hydrolyses of endo-5-[4'(5')-imidazolyl]-bicycle[2.2.1]hept-endo-2-yl trans-cinnamate (1) and endo-5-[4'(5')-imidazolyl]-bicycle[2.2.2]oct-endo-2-yl trans-cinnamate (3). The pH-rate constant profiles and D₂O solvent isotope effects are shown. Compound 1 (10) is a recently synthesized enzyme model, which has a rigid structure with the spatial arrangement of the imidazolyl and (esterified) hydroxyl groups similar to the arrangement in α-chymotrypsin (about 3 Å from one another). The structure of 3 is similar to that of 1, although slightly more labile. The hydrolyses of 1 and 3 are good models of the decylation step of acyl-enzymes, because these compounds have the proper catalytic groups but no binding sites. In decylation, binding is not important, so one can concentrate on the catalysis. Thus, the present study probes the function of the catalytic groups in enzymatic reactions.

Furthermore, for comparison, the hydrolyses of the exo-exo isomer of 1 which is exo-5-[4'(5')-imidazolyl]-bicycle[2.2.1]hept-endo-2-yl trans-cinnamate (2) and the endo-exo isomer of 3 which is endo-5-[4'(5')-imidazolyl]-bicycle[2.2.2]oct-exo-2-yl trans-cinnamate (4) were also examined as controls. These compounds, which are obviously related to 1 and 3, lack the proper stereochemistry for intramolecular reactions (see structures 1–4).

EXPERIMENTAL

Materials. Compounds 1–4, furnished by M. Utaka, were used after drying in vacuo at 80° for 2 hr. The agreement between theoretical values and observed values in elemental analyses for these compounds were quite good (10). Furthermore, the structures and purities of all compounds were confirmed by ¹H nuclear magnetic resonance and infrared spectroscopy, and melting point measurements.

Kinetics. The hydrolyses of 1–4 were monitored by the decrease in absorbance at 310 nm. Stock solutions of 1–4 were prepared in dioxane. The reactions were carried out at 60 ± 1°, where the ionic strength I was 0.1 M (KCl) in doubly distilled H₂O containing about 0.5% dioxane (vol/vol). Carbonate and bicine [N,N-bis(2-hydroxyethyl)glycine] buffers were used above and below pH 9.0, respectively. The pH change during the reactions did not exceed ±0.03 pH unit. For the D₂O experiments, pD was determined using the equation: pD = meter reading + 0.4 (11). The initial concentrations of 1–4 were 0.1–0.8 mM and all the reactions followed first-order kinetics for two half-lives. The specific rate constants, kobs, were determined by the method of Guggenheim (12).

At least two measurements were made to determine the specific rate constant at a given concentration of substrate and the individual specific rate constants are accurate to within about ±5%.

Abbreviations: 1, endo-5-[4'(5')-imidazolyl]-bicycle[2.2.1]hept-endo-2-yl trans-cinnamate; 2, exo-5-[4'(5')-imidazolyl]-bicycle[2.2.1]hept-endo-2-yl trans-cinnamate; 3, endo-5-[4'(5')-imidazolyl]-bicycle[2.2.2]oct-endo-2-yl trans-cinnamate; 4, endo-5-[4'(5')-imidazolyl]-bicycle[2.2.2]oct-exo-2-yl trans-cinnamate.
The solutions in which the hydrolyses of 1–4 at pH 7.5 and 60° were almost complete exhibited absorption maxima at 270 nm, which is attributable to trans-cinnamate anion. The concentrations of resulting trans-cinnamate anion were identical with the initial concentrations of 1–4.

RESULTS AND DISCUSSION

The specific rate constants, \( k_{obs} \), of the hydrolyses of 1–4 remained constant when the concentrations of 1–4 were varied from 0.1 to 0.8 mM. Thus, the hydrolyses here include the term due to intramolecular catalyses by the imidazolyl group, which should be independent of the concentrations of the substrates, as well as the term due to intermolecular alkaline hydrolyses. Intermolecular catalyses by the imidazolyl groups, whose participation in hydrolyses should depend on the concentrations of the substrates, were not observed in the range of substrate concentrations employed. The hydrolyses of 1–4 catalyzed by carbonate or bicinque buffer are negligibly small under the experimental conditions, because the changes of buffer concentrations from 0.02 to 0.08 M hardly changed the \( k_{obs} \).

Figs. 1 and 2 show the pH-rate constant profiles for the hydrolyses of 1 and 3, the endo-endo compounds, both in H\(_2\)O and in D\(_2\)O as well as those for the hydrolyses of the exo-endo isomer of 1 (2) and the endo-exo isomer of 3 (4) in H\(_2\)O. The pH-rate constant profiles for 1 and 3 are composed of two regions: (i) a curve reaching a plateau around pH 7.9 (pD 8.4) and

\[ k_{obs} = k_c (K_a + [H^+]) + k_{OH} (K_w/[H^+]) \]  

(1)

where \( k_c \) and \( k_{OH} \) are the rate constants for intramolecular catalysis by the neutral imidazolyl group and that for intermolecular alkaline hydrolysis, respectively; \( K_a \) refers to the imidazolyl group. The ion product, \( K_w \), for H\(_2\)O at 60°, was determined to be \( 10^{-13} \) by extrapolation of the results in the literature (13), while that for D\(_2\)O was taken as \( 0.154 \times 10^{-15} \), by assuming that the ratio of the value in D\(_2\)O to that in H\(_2\)O at 60° is equal to that at 25° (14).

Table 1 lists the rate constants and \( pK_a \) values for the hydrolyses of 1 and 3. The D\(_2\)O solvent isotope effects on \( k_c \) are 3.0 for both 1 and 3. These D\(_2\)O effects definitely show that the imidazolyl groups in 1 and 3 function as intramolecular general base in the hydrolyses. The magnitude of the D\(_2\)O effects for 1 and 3 are close to that (2.5) for the decylation of trans-cinnamoyl-o-chymotrypsin (15), in which the imidazolyl group of histidine-57 functions as an intramolecular general base catalyst. Of course, the D\(_2\)O effects for 1 and 3 are much larger than those (1.0–1.1) for intermolecular nucleophile catalyses

<table>
<thead>
<tr>
<th>Compound</th>
<th>Bicyclic ring</th>
<th>Stereochemistry*</th>
<th>( k_c ) (10(^{-7}) sec(^{-1}))</th>
<th>( k_{OH} ) (10(^{-7}) M(^{-1}) sec(^{-1}))</th>
<th>( pK_a ) of imidazolyl group</th>
<th>( k_c ) (H(_2)O)</th>
<th>( k_c ) (D(_2)O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>[2.2.1]</td>
<td>endo-endo</td>
<td>6.4 ± 0.4</td>
<td>1.4 ± 0.1</td>
<td>6.8 ± 0.1</td>
<td>3.0 ± 0.5</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>[2.2.1]</td>
<td>exo-endo</td>
<td>2.1 ± 0.2†</td>
<td>1.4 ± 0.1†</td>
<td>7.4 ± 0.1†</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>[2.2.2]</td>
<td>endo-endo</td>
<td>0.00 ± 0.05</td>
<td>2.1 ± 0.2</td>
<td>6.8 ± 0.1</td>
<td>3.0 ± 0.5</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>[2.2.2]</td>
<td>endo-exo</td>
<td>1.8 ± 0.2</td>
<td>0.95 ± 0.06</td>
<td>7.4 ± 0.1†</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

* The first term and the last term, respectively, refer to the imidazolyl group and the trans-cinnamoyl group.
† In D\(_2\)O.
by imidazole in ester hydrolyses (16). The $pK_a$ of the imidazolyl group of 1 and that of 3 are equal to each other, either in H$_2$O or in D$_2$O. Besides, the kinetically determined $pK_a$ of the imidazolyl group (6.8) of 1 at 60° in H$_2$O is equal to the $pK_a$ (6.7) of 1, determined by potentiometric titration, within experimental error. The intramolecular general base catalysis by the imidazolyl group in 1 is a little more effective than in 3. Apparently, the more rigid structure of 1 is favorable for intramolecular catalysis.

There are no measurable intramolecular catalyses by the imidazolyl groups in 2 and 4, because $k_{obs}$ increased proportionally with pH over a wide pH range. Thus, the $pK_a$ of the imidazolyl groups in 2 and 4 could not be determined by the present kinetic study. Lack of intramolecular catalyses in 2 and 4 is quite reasonable, because the imidazolyl groups are too far from the trans-cinnamoyl group to function as intramolecular general base catalysts. Consequently, a precise arrangement of the imidazolyl and trans-cinnamoyl groups is essential for intramolecular general base catalysis by the imidazolyl groups in 1 and 3.

The present finding of intramolecular general base catalyses by the imidazolyl groups for the hydrolyses of trans-cinnamic acid esters is interesting, because imidazole inhibits the alkaline hydrolysis of methyl trans-cinnamate in bimolecular reactions, which has been attributed to complex formation between imidazole and methyl trans-cinnamate (17). Fixation of the imidazolyl and trans-cinnamoyl groups by rigid covalent bonds in 1 and 3 prevents complex formation between the two groups in a given molecule, and results in effective intramolecular general base catalyses. Furthermore, the intramolecular complex formation between the imidazolyl group in a molecule and the trans-cinnamoyl group in another molecule is negligible under the present experimental conditions, because the equilibrium constant for intramolecular complex formation between imidazole and methyl trans-cinnamate is small (1.0 M$^{-1}$ at 25°) (17).

Most of the intramolecular catalyses in ester hydrolyses by imidazolyl groups hitherto reported proceed through nucleophilic attack by the imidazolyl group (1–3). However, the present study definitely shows intramolecular general base catalysis by the imidazolyl group in 1 and 3 as in serine esterases. Thus, the hydrolyses of 1 and 3 can serve as good models of enzymatic reactions. The rate constants, $k_a$, for the intramolecular general base-catalyzed hydrolyses by the imidazolyl groups in 1 and 3 are much smaller than that (1.25 × 10$^{-2}$ sec$^{-1}$ at 25°, ref. 18) for the decylation of trans-cinnamoyl-α-chymotrypsin. However, it should be noted that neither 1 nor 3 has the "charge-relay" system which is present in α-chymotrypsin. We at present find that benzoic acid exhibits a considerable acceleration of the hydrolyses of 1 and 3 in dioxane-H$_2$O mixtures, and this may be a possible analog of the "charge-relay" system.

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