Enantioselectivity vs. kinetic resolution in antibody catalysis: formation of the (S) product despite preferential binding of the (R) intermediate

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Antibody 14D9, which catalyzes the stereoselective transformation of achiral enol ethers into the corresponding (S)-ketal, resolves a racemic mixture of structurally similar chiral enol ethers by selective conversion of the (R)-enol ether into the (R)-ketal, raising the possibility that the (S) transition state is preferentially stabilized by the antibody despite a better binding of the (R) intermediate. This mechanistic issue could be resolved if the affinity of 14D9 to each of the two enantiomeric forms of 1 could be compared. These enantiomeric intermediates occur not only along the reaction pathway leading from 2 to ketal 4, but also along the similar conversion of the isomeric enol ether 5 to 4 (Scheme 2). Therefore, one could obtain the desired information about the relative stability of (S)-1 and (R)-1 by studying the kinetic resolution of 5 by 14D9. Conversion of (S)-5 and (R)-5 into ketals (S)-4 and (R)-4, respectively, proceeds via the enantiomeric intermediates (S)-4 and (R)-4. If the antibody catalyzed the protonolysis of (S)-5 preferentially over (R)-5 this would imply that 14D9 binds (S)-1 more tightly than (R)-1. This would be consistent with the energy diagram shown in Fig. 1(A) for enol ether 2. Conversely, if catalytic protonolysis of (R)-5 was faster than that of (S)-5, this would support the alternative energy profile described in Fig. 1(B).

Substrate 5 was prepared from methyl 4-bromomethylbenzoate and ethyl 2-methyl-3-oxobutanoate.6,7 The initial alkylation product was decarboxylated and the resultant ketone was converted to the corresponding 1,3-dioxolane. The latter was opened with (Me3Si)2NH and TMSI to produce a mixture of three isomeric enol ethers in almost equal proportions. These isomers were separated by column chromatography and each was subjected to aminolysis with ethanolamine. The two

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This is an intriguing question related to the origin of the enantioselectivity in the rate-limiting protonation step. Intuitively, one would expect that (S) selectivity arises from preferential binding of the antibody to both the (S) transition state, (S)-TS and the structurally similar (S) intermediate, (S)-I [Fig. 1A]. Nevertheless, we cannot rule out \textit{a priori} the alternative possibility, in which the antibody still binds selectively to (S)-TS but binds preferentially to the opposite enantiomeric intermediate (R)-I [Fig. 1B].

![Scheme 1](image-url)

**Scheme 1**

**Fig. 1** Alternative free energy diagrams for the antibody-catalyzed enantioselective protonation of enol ether 2

conditions, see Table 1.

Fig. 2. Lineweaver–Burk plot of reaction rates for the formation of ketone 3 from (C) racemic enol ether 5, (D) (S)-5 and (E) (R)-5. For the reactions conditions, see Table 1.

Table 1 Kinetic parameters for the antibody 14D9-catalyzed hydrolysis of 5.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>$K_m$/μM</th>
<th>$k_{cat}$/min$^{-1}$</th>
<th>$k_{cat}/K_m$</th>
<th>$K_{cat}/K_m$/μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>rac-5</td>
<td>480 ± 200</td>
<td>(4.3 ± 1.9)× 10$^{-2}$</td>
<td>1.55× 10$^{-5}$</td>
<td>2760</td>
</tr>
<tr>
<td>(R)-5</td>
<td>210 ± 70</td>
<td>(4.6 ± 1.5)× 10$^{-2}$</td>
<td>1.55× 10$^{-5}$</td>
<td>2900</td>
</tr>
<tr>
<td>(S)-5</td>
<td>470 ± 230</td>
<td>(3.4 ± 1.7)× 10$^{-3}$</td>
<td>1.55× 10$^{-5}$</td>
<td>220</td>
</tr>
</tbody>
</table>

Reactions were carried out in 100 mM NaCl and 50 mM 1,3-bis[tris(hydroxymethyl)methylamino]propane (bistris), pH 8.0, 25 °C. Ketal 4 (ca. 20%) was formed in all cases.

Scheme 2

![Scheme 2](image)

The 31-fold selectivity in the kinetic resolution of 5 suggests that the natural binding selectivity of antibody 14D9 favors intermediate (R)-I. The (R) selectivity in the hydrolysis of 5 stands in stark contrast to the (S) selectivity observed in the 14D9-catalyzed hydrolysis of several enol ethers such as 2, and supports the mechanistic option shown in Scheme 2(b).

Earlier experiments indicate that the high catalytic efficiency observed with enol ethers such as 2 ($k_{cat}/k_{on} = 10^{10}$–$10^{14}$) is caused by a carboxylic acid residue acting as a general acid catalyst within the antibody’s binding pocket. Thus, the strong preference for protonation on the re-face of enol ether 2 to produce the (S) products is the result of the relative positioning of this general acid with respect to the bound substrate. The evidence presented here suggests that 14D9 binds (R)-I tighter than (S)-I. It also raises the intriguing possibility that moving the catalytic residue in the antibody binding pocket by mutagenesis could create a new catalyst that will convert prochiral enol ethers to (R) products. Moreover, such a modified antibody is expected to be a more efficient catalyst. Future experiments will address this possibility.

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Notes and References

8. Separation of the enantiomers was carried out using a normal phase chiral column (Chiralcel OD, Chiral Technologies Inc.) with hexane–PrOH (80:20) at a flow rate of 1 ml min$^{-1}$; the retention times of (S)-5 and (R)-5 are 9.13 and 12.23 min, respectively.

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