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Remarkable Remote Chiral Recognition in a Reaction Mediated by a Catalytic Antibody

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When reactions of enantiomers occur in an asymmetric environment, the distance between the reaction and the chiral centers must generally be minimized for maximum enantiomeric differences.[1] In most enzyme-catalyzed reactions, too, asymmetric induction (and discrimination between enantiomeric inhibitors) typically falls off as the distance between the reaction site and the chiral center increases when unnatural substrates are studied.[2] We report antibody-catalyzed hydrolyses of enantiomeric substrates which display significant reactivity differences even though the reaction and chiral centers are separated by 7 bonds. We also explore the source of this enantiodifferentiation.

We have been studying an efficient catalytic antibody (D2.3) that hydrolyzes the esters **1**[3] and **2**.[4] The crystal structures of D2.3 Fab with the achiral 4-nitrobenzyl phosphonate hapten **3** (a TSA, transition state analogue, for hydrolysis of **1**) used to elicit this antibody,[5] as well as crystal structures of the Fab fragment alone and with other ligands, e.g., **4**[6] and **5**,[7] have been determined. The 4-nitrophenyl groups in all of the structures are deeply buried in the active site and the glycine residue points away from the reaction center toward the outside of the antibody (Figure 1).

Since only differences in the positions and conformations of the ligands are found in the structures of D2.3 complexes,[6,7] we considered substrate structural changes that might improve binding (lower K_M) and/or reactivity (higher k_{cat}). Modifications included locations near the combining site opening, where the substrate−antibody complex might better tolerate modified substrates. Our results with enantiomeric substrates **6D** and **6L**,[8] where the glycine in **2** is replaced by D- and L-alanine, respectively, and with the corresponding phosphonate TS analogues, **7D** and **7L**, including the crystal structures of the latter with D2.3 Fab, are noteworthy.

D2.3 preferentially binds the L-substrate **6L** (K_M: **6L**/**6D**, 10/45, μM) and catalyzes its hydrolysis (k_{cat}: **6L**/**6D**, 2.6/0.6, min^{-1}), resulting in an overall 20-fold improvement in catalytic efficiency. Such chiral recognition at a group seven rotatable, single bonds from a reaction site is remarkable. These results may appear more surprising since the hapten eliciting the antibody lacks a chiral center and, thus, chiral discrimination was not "programmed".[9] Furthermore, the alanine methyl groups reside in the open structure of the Fab (Figure 1), where the observed temperature factors are significantly larger than in the rest of the structure (the mean square vibrational amplitude for the glycine in **3** is 0.52 Å^2, whereas it is 0.29 Å^2 for the *p*-nitrophenolate), and protein−ligand interactions might not have been expected to be effective in enantiomer discrimination.

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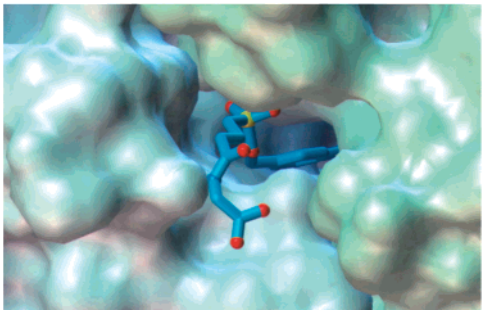
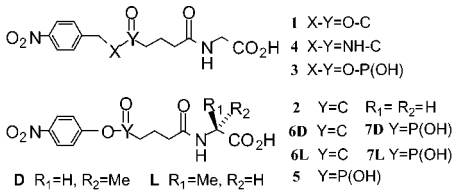


Figure 1. The reaction (antibody combining) site of D2.3 complexed with hapten **3**, a TSA for the hydrolysis of **1**. This space-filling model of the X-ray structure[5] illustrates the greater available space near the glycine residue that protrudes from the cavity. An approximate diagonal from the upper left to the lower right separates the heavy chain residues (darker green) on the right from the light chain residues (lighter green) on the left.

Scheme 1



To elucidate how antibody D2.3 controls reactivity differences, the phosphonates **7D** and **7L** (i.e., the TSAs for the reactions studied) were prepared and complexed with D2.3 Fab. Crystals of D2.3-Fab.**7D** and of D2.3-Fab.**7L** afforded 1.9 Å resolution X-ray diffraction, allowing structure determinations, the average precision of atomic positions being 0.25 Å.

One might have expected that the crystal structures of **7D** and **7L** with D2.3 Fab would find the D- and L-alanines overlapping with glycine, such that each methyl group would roughly occupy the positions of the pro-R and pro-S hydrogens, respectively, and that their different steric interactions would help rationalize the enantiomeric preferences observed in the D2.3-catalyzed reactions of the corresponding substrates. However, instead of displaying such presumed enantiomeric electron density maps, the electron densities of the D- and L-Ala enantiomers coincide in the crystal structures, the additional electron densities of the enantiomeric methyls being accommodated at the opening of the combining site (Figure 2). The prominent H-bond between Tyr L96 and an alanine carboxylate oxygen (Figure 2A) is precisely maintained in the **7D** and **7L** structures. This hydrogen bond, the amide NH···Gly L91 H-bond, and the *p*-nitrophenyl phosphonate interactions (the same three interactions were observed in earlier structures[5−7]) apparently "lock

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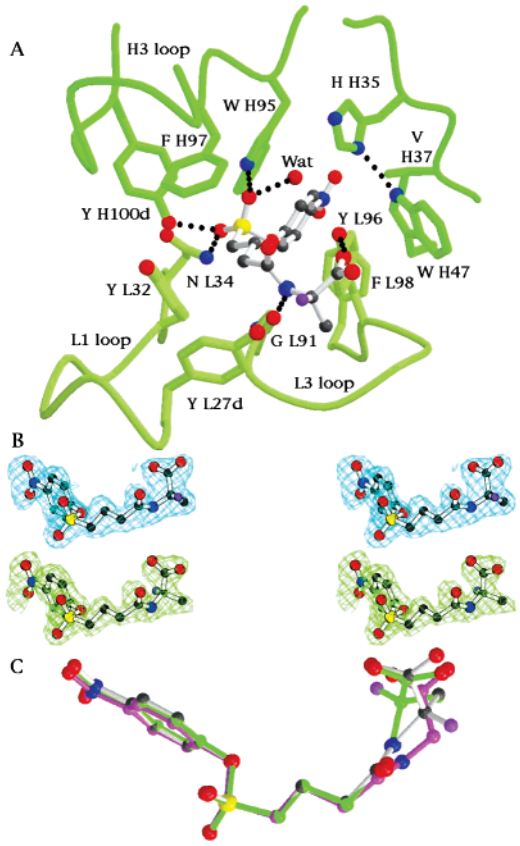

Figure 2. Complexes of D2.3 with **7D** and **7L**. Carbon is in gray, oxygen in red, nitrogen in blue, and phosphorus in yellow; hydrogen at the chiral centers of **7D** and **7L** is in purple. The chiral center hydrogen atoms are included for clarity; their positions are deduced from those of the other substituents of the alanine α-carbon. (A) Schematic view of D2.3 residues that interact with ligand **7L**. Residue numbering is according to Kabat et al.[14] The Fab Cα trace is in green (dark for the heavy chain and light for the light chain) and the ligand is in white. Hydrogen bonds are drawn as dotted lines. (B) Stereoviews of the structures of **7L** (top, blue net) and **7D** (bottom, green net) superimposed within nets representing the corresponding electron densities ($2F_{obs} - F_{calc}$ maps) in D2.3 Fab. The maps are contoured at the one standard deviation level. (C) Superimposed structures of **7D** (green) and **7L** (white) and **5**[7] (magenta) in D2.3 Fab.[15]

in" these two ligands, which now adjust to a minimum energy conformation within these constraints.

Refinement of the atomic coordinates for the structures of the complexes of the D- and L-Ala isomers **7D** and **7L** in D2.3 provides models for these haptens that fit the electron density (Figure 2B). The energy difference between the two complexes, 0.5 kcal/mol (deduced from the affinities of **7D** and **7L** for D2.3; see below), cannot be ascribed to one dominant strong intermolecular interaction; it is due to the sum of different interactions of the haptens with the antibody, associated with slightly different positions of these haptens in the complexes (Figure 2C), and to their different conformational energies when locked therein. Although the differences in the structures of **7D** and **7L** in D2.3 are subtle, these differences are manifested by consistent interactions and chemical behavior. Thus, the affinity of **7D** is 50 nM while that of **7L** is 26 nM (measured by fluorescence quenching[10]) and the K_M of **6L** (10 μM) is lower than that of **6D** (45 μM).

In catalytic antibody research, a single hapten is typically used to generate a large number of catalytic antibodies which are then studied with a substrate, i.e., the programmed reactant, homologous to the hapten. Antibody modification, via laborious site directed mutations, has rarely improved catalytic efficiency. (See refs 11 and 12 for exceptions). Modification of the substrate, which is invariably much simpler, may afford improved catalytic behavior[13] and even, as seen here, significant enantiodifferentiation at a reaction center remote from the chiral center. Analysis of the triangular relationship beween haptens, antibodies, and substrates, where any of the three is judiciously varied, may provide empirical results and/or insights, allowing improved rational design of catalytic antibodies.

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Supporting Information Available: Table of kinetic parameters for D2.3-catalyzed hydrolysis of **2**, **6D**, and **6L**, experimental details for the synthesis and characterization of **6D**, **6L**, **7D**, and **7L**, crystallization, data collection and processing, structure determinations, and refinements (PDB codes for the two complexes are, respectively, 1KN4 and 1KN2), and a table of crystallographic data and refinement statistics for D2.3-**7D** and -**7L** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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