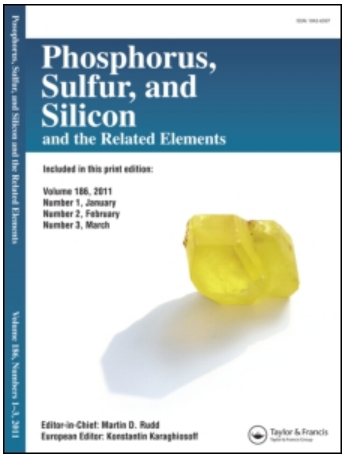

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Structures of Esterase-Like Catalytic Antibodies

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We report structures of complexes of two esterase-like catalytic antibodies elicited against phosphonate transition-state analogues (TSAs) of the reaction they catalyse; the complexes studied are with these TSAs and species along the reaction pathway. The TSAs are deeply sequestered in a pocket located in the combining site at the interface of the two antibody polypeptide chains; the deepest part of this pocket is almost completely hydrophobic. The larger number and stronger hydrogen bonds between the antibodies and the TSAs as compared to those established with the corresponding substrates account for the catalytic activity; transition state stabilisation is through oxyanion holes elicited in response to the negatively charged phosphonate oxygens.

Keywords: Antibody; catalysis; hydrolysis; phosphonate; transition-state

INTRODUCTION

Ester hydrolysis is the first reaction to have been unambiguously enhanced by an antibody; this reaction is catalysed by the majority of catalytic antibodies reported to date. As a consequence, the kinetic information is most abundant on this reaction and the largest number of X-ray structural studies are available for antibodies that catalyse ester hydrolysis. Almost all of the haptens used to mimic the transient states in ester hydrolysis and to elicit catalytic antibodies

comprise the phosphonate structure. We report structures of the complexes of two catalytic antibodies with species along the reaction pathway. This allows us to establish their catalytic mechanism and to define the triangular hapten-substrate-antibody relationship.

STRUCTURES

The antibodies in the first family we studied catalyse the hydrolysis of p-nitrophenyl esters such as **1** (Figure 1) and were elicited by immunisation against the transition state analogue (TSA) hapten **2**[1]. In the structure of the complex of one of these antibodies with the TSA **3**, two themes of the structures of aromatic ester hydrolase antibodies have appeared. First, the ligand is deeply embedded in a pocket at the interface of the two polypeptide chains that constitute the antibody, the deepest portion of this pocket being almost completely hydrophobic. Second, the phosphonate portion of the TSA establishes hydrogen bonds with the Fab that cannot all be made, or be made as efficiently, with the planar uncharged ester substrate[2]. Given the limited resolution of the crystallographic data, the residues of the oxyanion hole cannot be identified unambiguously among those that establish hydrogen bonds with the TSA phosphonate.

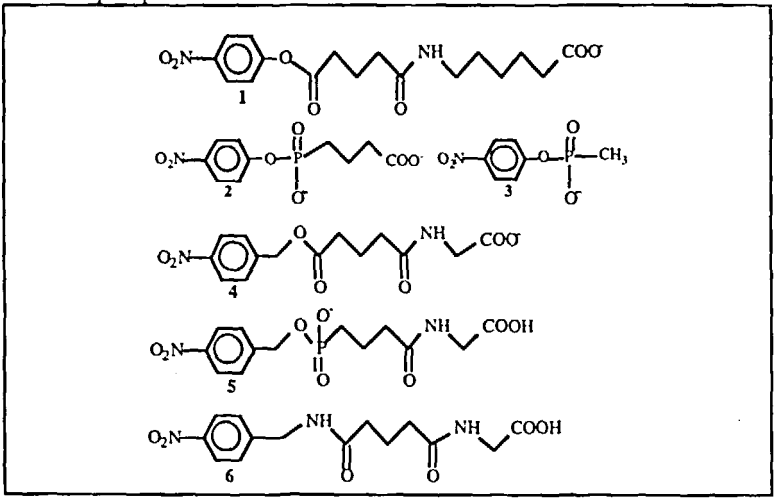

FIGURE 1 Structures of the compounds used in this study. Esters **1** and **4** are substrates. p-nitrophenyl phosphonate **2** is the TSA hapten used to elicit the first family of antibodies. Crystal structures examined are those of antibodies complexed with p-nitrophenyl phosphonate **3**, with p-nitrobenzylphosphonate **5** and with p-nitrobenzyl amide **6** which is a stable analogue of the substrate ester **4**.

The antibodies of the second family catalyse the more difficult hydrolysis of p-nitrobenzyl esters such as **4** and were elicited against the hapten TSA **5**[3]. Nine catalysts were identified through screening of all hybridomas recovered in one fusion and the structures of the three most efficient of them were determined to a resolution of up to 1.9 Å[4]. The same characteristic features identified in the first family we studied occur in this family too: the hapten TSA is buried in a pocket at the interface of the antibody heavy and light chain and the deepest part of the pocket is almost completely hydrophobic. In this case the phosphonate oxygens establish hydrogen bonds with side chain atoms of three residues in the combining site, two in the heavy chain and one in the light chain (Figure 2).

MECHANISM

The structures of the complexes with the TSA do not establish, even when determined to a high structural resolution, which of the groups in the antibody combining site that hydrogen bond to the phosphonate oxygens constitute the oxyanion hole. The reason is that only one of the phosphonate oxygens mimics the negatively charged oxygen in the oxyanion intermediate, and which of the two phosphonyl oxygens this is cannot be decided on the sole basis of the structure of the complex with the TSA. The structure of the complex of the most efficient of the antibodies in the second family with the stable amide substrate analogue **6** shows that the carbonyl oxygen in **6** that is equivalent to that of the to be hydrolysed ester bond is within hydrogen bonding distance of the hydroxyl of residue Tyr H100d[5] (Figure 2). This strongly suggests that this Tyrosine constitutes the oxyanion hole together with an Asparagine of the light chain (Asn L34).

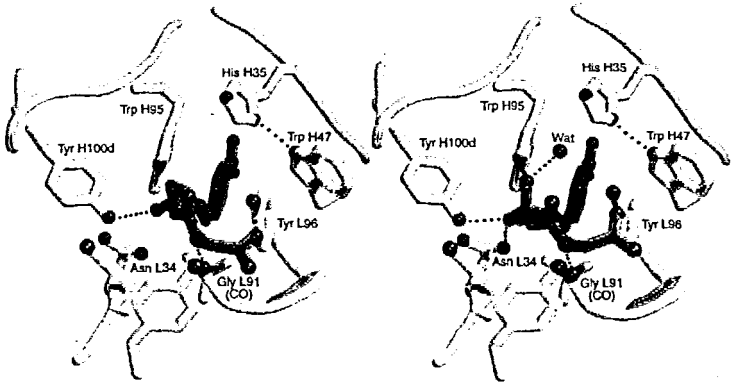

FIGURE 2 Complexes of the esterase-like antibody D2.3 with an amide which is a stable substrate analogue (left) and a phosphonate TSA (right). Hydrogen bonds are shown as dotted lines.

The following mechanism emerges for the hydrolysis of p-nitrobenzyl esters by this family of antibodies. The substrate binds and the carbonyl oxygen of the ester bond is polarised and prepared for attack by a hydroxide ion through a hydrogen bond with a Tyrosine residue of the heavy chain. A channel allows access of hydroxide ions to the reaction centre. As the carbonyl carbon becomes tetrahedral when the oxyanion forms, the negatively charged oxygen gets additional stabilisation through a second hydrogen bond with an Asparagine of the light chain. Product is then formed and the acid is released first, followed by p-nitrobenzyl alcohol[5].

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