Molecular Mechanism of Enantiorecognition by Esterases

Zygmunt S. Derewenda* and Yunyi Wei

Medical Research Council of Canada
Group in Protein Structure and Function
Department of Biochemistry, University of Alberta
Edmonton, Alberta, T6G 2H7 Canada

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One of the most significant trends in the pharmaceutical industry is the increasing presence of single-isomer forms of chiral drugs. Annual sales of enantiopure drugs have reached $35 billion.[1] This creates special demands for novel enantioselective synthetic technologies. Biotransformations constitute a frequently used technique, but direct applications of highly enantioselective, stable, and cheap enzymes offer an attractive alternative. Esterases, including both interfacially activated lipases and short fatty acid acylhydrolases,[2] are particularly popular among pharmaceutical chemists; for example, these enzymes are used in the preparation of the blood pressure drugs cilazapril and naltiazem,[3] the preparation of the antiinflammatory drug ketorolac,[4] and the synthesis of the C13 side chain of the antitumor agent taxol.[5] To date, the mechanism of enantiorecognition by these enzymes was inferred from substrate mapping, and the resulting empirical rules were used to predict which enantiomer of a secondary alcohol will be a fast-reacting species.[6] We here propose a general model for enantiorecognition by esterases based on several newly determined crystal structures of these enzymes. We show that regardless of their tertiary fold, esterases carry out a nucleophilic attack on the *si* face of the ester bond.

While three-dimensional structures of other various hydrolases have been known for decades, it was only recently that the stereochemical details of the reactive sites of esterases were elucidated by crystallographic studies.[7,8] In all hitherto studied cases, the enzymes exhibited a common structural scaffold

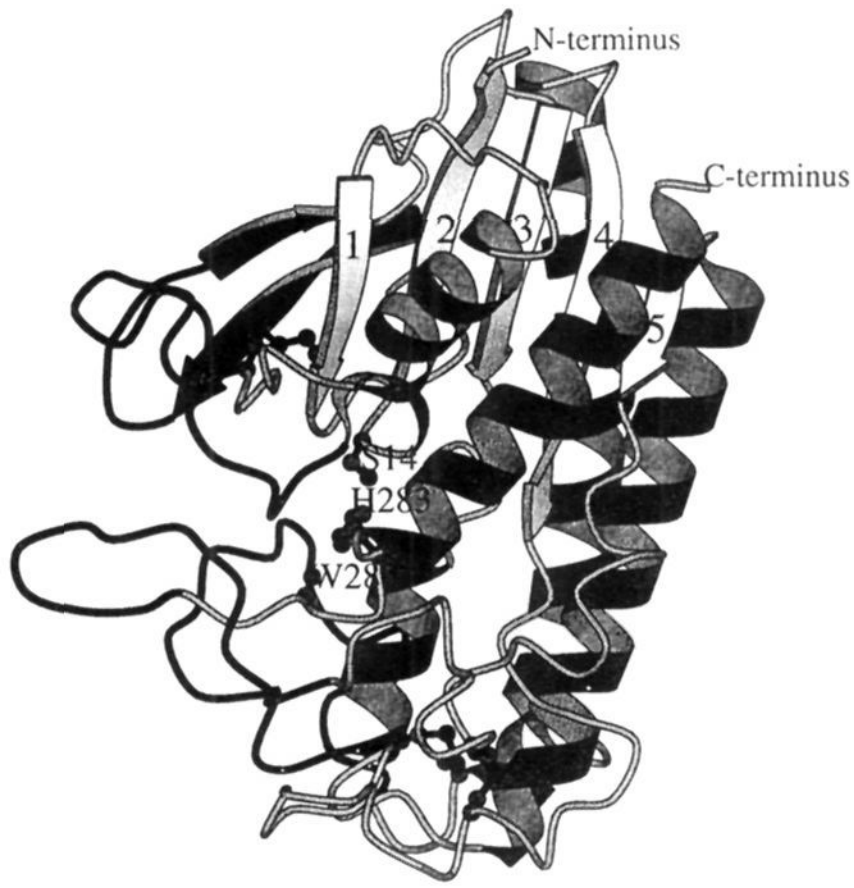

Figure 1. Diagramatic representation of the *S. scabies* esterase showing the location of the catalytic groups and the overall tertiary fold.[16]

defined as the α/β hydrolase fold;[9] a recently reported structure of a thioesterase also falls into this category.[10] It has been suggested that this conserved fold of α/β hydrolases imposes a common stereoselectivity pattern on the entire superfamily.[11]

To date, all crystallographically studied esterases are serine hydrolases which resemble the proteolytic enzymes of the chymotrypsin and subtilisin families in that they contain catalytic triads made up of a serine, a histidine, and a carboxylic acid. In all cases, the tetrahedral transition states, which occur during the reactions, are stabilized by the so-called oxyanion holes. We have now determined the crystal structure of a novel esterase purified from *Streptomyces scabies*, both in its native form and complexed with covalent inhibitors.[12] The molecule exhibits a three-dimensional architecture very different from the α/β hydrolase fold (Figure 1). The deeply buried active site contains a serine (Ser14) and a histidine (His 283), whose conformation is stabilized by a hydrogen bond with a main chain carbonyl oxygen, and not a carboxylic acid. The implications of this unique constellation for the reaction mechanism are discussed elsewhere.[12] A comparison of this active site with those in other hydrolytic enzymes revealed an important regularity: irrespective of the three-dimensional fold, the line of approach of the nucleophile toward the carbonyl carbon of the substrate is conserved, and the ester bonds are attacked on the *si* face (Chart 1). Thus, the reactivity of specific esters of secondary alcohols should be easily predicted from the relative solvent accessibilities of the *re* and *si* faces of the respective enantiomers. While

* Address correspondence and reprint requests to Dr. Zygmunt S. Derewenda, MRC of Canada Group in Protein Structure and Function, Department of Biochemistry, University of Alberta, 4-74 Medical Sciences Building, Edmonton, Alberta, Canada T6G 2H7. Tel: (403) 492-2136. FAX: (403) 492-0886.

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(2) Biochemical literature makes a distinction between lipases, which undergo activation at an oil−water interface, and esterases, which act on soluble esters. This distinction is less relevant in organic synthesis when the enzymes are frequently used in organic media and catalyze enantioselective transesterification reactions. We are therefore using the term *esterase* throughout the manuscript in its broadest sense, i.e., to denote any enzyme capable of hydrolyzing an oxyester linkage.

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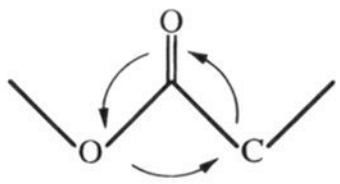
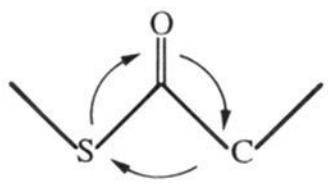
Chart 1. Preferred Orientation of the Oxy- and Thioester Groups for Enzymatic Hydrolysis



Oxyester Linkage *si* Face



Thioester Linkage *re* Face

our proposal is in full agreement with the experimental data reported by others,[11] it provides a simple and more general rationalization of the observed enantioselectivities.

How do the enzymes differentiate between the *re* and *si* faces of the ester bonds? Figure 2a shows the stereochemistry of the two key active site amino acids (serine and histidine) in the *Rhizomucor miehei* lipase.[13] The relative dispositions of the histidyl imidazol and the nucleophilic serine define the preferred orientation of the incoming substrate ester linkage, with the position of the substrate's carbonyl group fixed by the location of the oxyanion hole. Thus, three points (the hydroxyl group of the active site serine, the Nϵ2 atom of the histidine, and the oxyanion hole) define a plane complementary to the favored face of the approaching scissile peptide or ester group. Figure 2b shows the active site of the *S. scabies* esterase, where in spite of the 180° relative rotation of the imidazole, the stereochemistry favoring the *si* face of the substrate ester is well preserved.

The available structural data appear to indicate that, regardless of their specific tertiary structure, oxy- and thioesterases exhibit clearly defined convergence with regard to the stereospecificities of their active sites. A similar phenomenon is observed among serine proteinases of the two nonhomologous subtilisin and chymotrypsin families.[14] However, it has been noted independently on several occasions that the catalytic triads of esterases have a chirality opposite to that found in serine proteinases.[7] Why the difference? The answer is probably in the steric restrictions imposed on the main chain dihedral angles of a polypeptide by the Ramachandran rules. It is well established that the conformational ϕ angle is largely restricted to negative values, except where amino acids are in a left-handed helical conformation or some atypical strained conformation. Thus, the *si* face of the peptide group will be less solvent accessible (Figure 3). Such steric restrictions do not apply to the much more flexible ester linkage, in which both faces may be equally accessible, and consequently proteinases can easily function as esterases.[15] Given these steric constraints, it is conceivable that esterases evolved to minimize the likelihood of fortuitous hydrolysis of peptide bonds by favoring the *si* face of the substrate which in a peptide bond is less exposed.

(13) This semischematic figure is based on atomic coordinates of the *R. miehei* lipase (1TGL) and those of the recently solved *S. scabies* esterase. The *R. miehei* lipase is a good representative of the active site geometries of all structurally known esterases belonging to the α/β hydrolase family.

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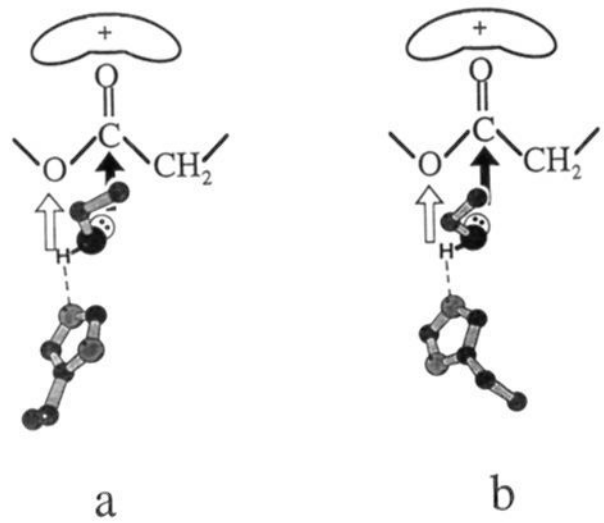

Figure 2. Semidiagrammatic representation of the reaction stereochemistry in the *R. miehei* lipase (a) and *S. scabies* esterase (b). The conformations of the histidyl and seryl residues are based on the actual atomic coordinates derived from crystal structures. Substrates are shown diagrammatically. The positively charged cloud represents the oxyanion hole, the full arrow indicates the direction of the nucleophilic attack of the lone electron pair on the seryl hydroxyl, and the open arrow indicates the line along which a proton will be transferred by the histidine onto the ester oxygen of the scissile bond. The position of the hydrogen atom is shown schematically.

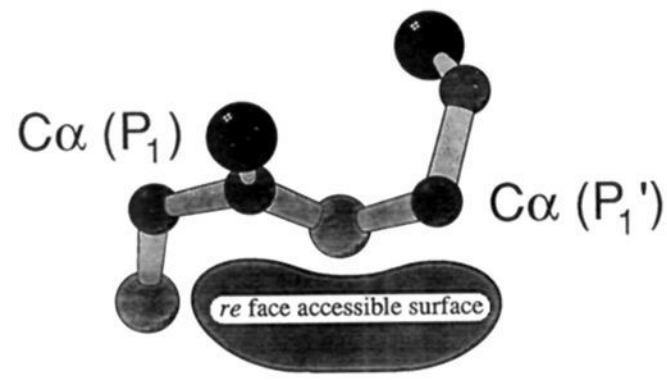

Figure 3. Typical conformation of a dipeptide with the negative value of the ϕ angle at the P_1' position. Only main chain atoms are shown: large dark spheres represent the oxygens atoms, and large lighter spheres denote the peptide nitrogens. The notations P_1 and P_1' correspond to the standard notation of amino acids bound in the active sites of proteinases. The scissile peptide bond is between these two residues.

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