Microbial Lipases: Structures, Function and Industrial Applications

Lipid Group/Industrial Biochemistry and Biotechnology Group Joint Colloquium Organized and Edited by
A. R. Macrae (Unilever Research, Sharnbrook) and Sponsored by Unilever. 659th Meeting held at Queen Mary
and Westfield College, University of London, 4–6 September 1996

Lipase stereo- and regio-selectivity towards tri- and di-acylglycerols
E. Rogalska, S. Nury, J. Douchet and R. Verger
Laboratoire de Lipolyse Enzymatique UPR 9025 de l'IFR 1 du CNRS 31, chemin Joseph Aiguier,
13402 Marseille cedex 20, France

Introduction
The specificity of lipase action on triacylglycerols can be defined at the following three levels: in terms of their typoselectivity towards a given fatty acid; in terms of their regioselectivity, i.e. the ability of a lipase to hydrolyse preferentially carboxylic ester bonds located in external positions sn-1 and sn-3 (primary esters) as opposed to those in the internal position sn-2 (secondary ester); and in terms of their stereoselectivity, i.e. the ability to discriminate between two enantiomers in the case of a racemic substrate, and between two stereoheterotopic but homomorphic (enantiotopic) groups in the case of prochiral acylglycerols (position sn-1 compared with sn-3).

Stereoselectivity
Chirality is a general attribute of objects and figures, and by definition does not depend in any way on the material world, although it has important links with it. In 1904, Lord Kelvin said 'I call any geometrical figure, or group of points, chiral, and say it has chirality if its image in a plane mirror, ideally realized, cannot be brought to coincide with itself' [1]. Chirality therefore means the absence of mirror symmetry.

Enantiomer differentiation
Several examples have been described that illustrate an enzyme's ability to recognize enantiomers [2–4]. This phenomenon can be explained by looking at the various diastereoisomeric interactions that occur between enantiomers and the active (chiral) site of an enzyme.

Although many enzymes have a high degree of enantioselectivity, this is not always the case [3]. As far as lipases are concerned, the two enantiomers present in a racemic mixture usually behave as suitable substrates, and the enantioselectivity will depend on their chemical properties and on the origin of the lipases and the experimental conditions used [5–8].

Enantiotopic differentiation
The most spectacular aspect of enzyme stereoselectivity is the fact that some enzymes are able to differentiate between the two enantiotropic groups or stereoheterotropic faces present in a prochiral molecule. All the lipases tested so far on prochiral triacylglycerols have been found to have this discriminatory ability, apart from porcine pancreatic lipase, which did not distinguish between the prochiral carboxylic ester groups of trioctanoin and triolein [9,10].

The mechanisms underlying lipase stereoselectivity
Some of the stereochemical aspects of lipase hydrolysis of acylglycerols are quite unusual. Under normal physiological conditions, many other hydrolases, such as phospholipases, proteases, glycosidases and nucleases, metabolize only one of the substrate’s antipodes, whereas lipases are often capable of recognizing enantiomers as well as enantiotopic groups of prochiral molecules. The crystallographic structures that have been recently determined [11–31] so far do
not help to explain the stereochemical data [10]. Although all these lipases have very different stereoselectivities, their basic architecture is quite similar: the residues constituting the catalytic triad and the structural motif (β-εSer-ζ) characteristic of the nucleophilic centre generally match [18–21]. Cygler et al. [29] described a mechanism possibly explaining how *Candida rugosa* lipase is able to distinguish between the two enantiomers of a menthol phosphonic ester. This was the first time that the structure of a lipase crystallized with two chiral inhibitors had been resolved, although the *C. rugosa* lipase structure had previously been determined without any inhibitors by the same group [25,31]. With respect to the stereochemistry of lipases, Cygler et al. [29] reached the conclusion that the enantiopreference shown by *C. rugosa* lipase among the secondary alcohol esters is determined not by an alcohol-binding site other than the catalytic site but by conformational changes in the catalytic triad. However, the inhibitors used in the study in question were structurally very different from the natural substrates of lipases. Egloff et al. [30] recently used a racemic mixture of methyl n-undecylphosphonate and p-nitrophenol, as well as each of the two enantiomers in the medium prepared for co-crystallization of the human pancreatic lipase–colipase complex. The two enantiomers were differently oriented in the cleft of the active site. The results of these two studies unfortunately cannot be extrapolated to all lipases and to all experimental conditions.

The stereoselectivity of lipases has been tested in our laboratory using monomolecular films of di- and tri-acylglycerol analogues [32], with optically pure diacylglycerols [7], racemic diacylglycerols [5,8] and prochiral triacylglycerol emulsions [9,10]. The data obtained using this

![Figure 1](image.png)

**Figure 1**

Lipase stereoselectivity on trioctanoin and triolein

method were independent of the tyroselectivity of the lipases. It was observed that, with a given substrate and under specific reaction conditions, the stereoselectivity of any lipase can be taken to be its fingerprint. With the 25 pure lipases tested in this way, the enantioselectivity ranged between 0 and 100% towards positions \( sn-1 \) and \( sn-3 \) when prochiral triacylglycerol substrates were used (Figure 1), and it was also very variable when diacylglycerol substrates were used (Figure 2). It is worth noting, however, that the general stereopreference of most of the lipases tested is similar towards both tri- and di-acylglycerol substrates. On the basis of the above experimental data, we put forward the hypothesis that the chiral recognition centre might undergo enantio-morphic organization during the stage when the interfacial E*S complex is being formed, which may be precisely the stage at which the induced-fit process takes place [10].

The results obtained with prochiral triacylglycerols [10] show that, although some lipases, such as those of \textit{Pseudomonas fluorescens}, \textit{Pseudomonas species}, \textit{Rhizomucor miehei} and \textit{C. rugosa}, show some preference for position \( sn-1 \) of triacylglycerols, considerable differences exist from one lipase to another when triolein is used as the substrate: an enantiomeric excess of 70% was recorded in \textit{Pseudomonas} species lipase, as compared with 16% in the case of \textit{C. rugosa} lipase. On the other hand, many enzymes, such as cutinase from \textit{Fusarium solani pisi}, and human, rabbit and dog gastric lipases, show a distinct stereopreference for position \( sn-3 \). In addition, the lipase from \textit{Candida antarctica} B, which is consistently stereospecific towards position \( sn-3 \) with trioctanoin, shows a reversal of stereospecificity when triolein is used as the substrate (giving an enantiomeric excess of up to 40% with position \( sn-1 \)). A similar pattern of reversal of stereoselectivity between trioctanoin and triolein has been previously observed in the case of some other lipases (such as \textit{Geotrichum candidum} A and guinea-pig pancreatic lipase). Porcine pancreatic lipase, which is known to have no stereoselectivity with triacylglycerols and their analogues [32,33], was found surprisingly enough to have a stereopreference for position \( sn-3 \) when a synthetic diacylglycerol analogue was used as the substrate [32].

Figure 2

\textbf{Lipase stereoselectivity on trioctanoin and 1,2(2,3)-dicaprin enantiomers}

Correlation between the stereoselectivities toward the tri- and di-acylglycerols is shown [7]. For abbreviations see the legend to Figure 1.
The fact should not be overlooked that the stereoselectivity of lipases, like their specific activity, can depend to a great extent on the hydrophilicity of the solvent used [34], the interfacial tension [5,8,32], the chemical composition of the interface, and even the chirality of the non-substrate lipid molecules present at the interface [8].

8 Rogalska, E., Ransac, S., Douchet, I. and Verger, R. (1994) in Closing meeting of the BRIDGE lipase T-project, pp. 44, International Workshop — Bendor Island, Bandol, France

Received 20 June 1996