

THE CRYSTAL STRUCTURE OF LIPASE FROM *MUCOR MIEHEI*

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Summary

The crystal structure of lipase from the fungus *Mucor miehei* has been determined; it has revealed the enzyme's main chain structure as well as the details of the interactions made by the individual sidechains. The enzyme contains a central 8 strand β sheet structure that extends across the full depth of the molecule. Arranged across this, in some of the segments linking the strands, are several helices which pack against the sheet structure. There is an N terminal helix which appears to sit at the centre of the convex surface created by the β sheet.

The serine (144) at the catalytic site has been identified by chemical experiment. Inspection of the structure at this serine showed it to be part of a triad: asp ... his ... ser, equivalent at the active atoms to that seen in the serine proteases. There is no similarity in the lipase main chain structure to those of the trypsin related or the subtilisin related serine proteases - thus the appearance of the asp - his - ser triad is an example of an independent solution of these side chains for a catalytic reaction.

There is a small helix situated over the catalytic residues, effectively blocking them from the surrounding solvent. This lid explains the inactivity of the enzyme in aqueous conditions. The side chains on this helix are on one side polar and on the other non-polar. This suggests that under the influence of the interface at a micelle the lid could be destabilised by non-polar interactions and be displaced, exposing the catalytic triad to the lipid at the interface.

1. Introduction

X-ray analysis is the most penetrating technique for determining molecular structure. In the last 20 years or so the crystal structures of now some 200 odd macromolecules have been defined laying the basis for much of the modern thinking in biochemistry and biology. In particular the studies on lysozyme by Phillips and colleagues (1) and on chymotrypsin by Blow and his colleagues (2) revealed the nature of the catalytic site and specificity pockets in enzymes.

There has been intense scientific and industrial interest in lipases for many years but their structural study has been delayed by various problems of purification and crystallisation. And there is the additional complexity that the enzyme is inactive in aqueous conditions and requires the existence of an oil/water interface for enzymatic competence. Thus the catalytic mechanism must be accompanied (or preceded) by a structural change that activates the enzyme. The description of conformational changes in proteins poses serious difficulties for X-ray analysis and it is clear that the analysis of the enzyme in the free and liganded state will be necessary in order to account for its activation. Nonetheless crystallographic analyses of the free (and inactive) enzyme are now in progress on the human pancreatic lipase and a fungal lipase. These are an essential first stage for a comprehensive description of the molecule's structural and catalytic behaviour (3).

2. Experimental Details

The crystals were grown from 20 mM Tris buffer at a pH of 8.05 at a concentration of 15-16 mg/ml. The hanging drop technique, using 55-75% saturated phosphate buffer as a precipitant, was the most successful approach in getting crystals. Table 1 lists the molecular and crystallographic details of the crystals.

Table 1: Crystallographic Details

Space group	Axial length	Molecular weight	Number of molecules in asymmetric unit	X-ray data resolution	No. of data	Rmerge on data (Intensities)
P2 ₁ 2 ₁ 2 ₁	a=71.6Å b=75.0Å c=55.0Å	29,472	1	1.9Å	21,165	.08

The crystal structure was determined from an electron density map calculated with phases derived from 3 heavy atom derivatives followed by solvent flattening (3). The atomic positions and thermal parameters were subsequently refined by least squares minimisation where the shifts were restrained to conform to the requirements of peptide geometry. The essential details of the refinement calculations are given in Table 2. Refinement of the initial atomic coordinates was not straightforward owing to the poor quality of the density in some of the loop regions. Molecular dynamic simulations were applied to accelerate the positioning of these structure; this approach worked very successfully (4). At convergence the accuracy of well defined atoms with $B \leq 15 \text{Å}^2$ is about $.2 \text{Å}$.

Table 2: Crystallographic Refinement Parameters and Results

Number of atoms	Number of solvent molecules	Bav. (Å^2)	Resolution of data and in refinement	R _{cryst} $R = \frac{\sum F_o - F_c }{\sum F_o }$	Δr_{ms} between applied restrained & observed distances
2055	233	23.3	7 - 19Å	.138	.029

3. The Enzyme's Structure

3.1 The main chain. The principal structural feature of the enzyme main chain folding is an eight strand β sheet. The strands 1 - 3 are antiparallel, the strands 3 to 7 are parallel, and the last two (7 and 8) are antiparallel. As can be seen in Figure 1, this structure forms a central platform on which are arranged the connecting strands, often containing helices. This central β sheet has a convex and a concave surface. On the convex side there is only one major approach and that is made by the N terminal helix. This runs diagonally right across the centre of the sheet. By contrast the convex side of the sheet structure is crossed by numerous helices and stretches of peptide linking the β strands. This behaviour is typical for β sheets which have been shown to have an intrinsic handedness (5).

3.2 The surface structure. The ability of lipase to function at an oil/water interface means that its surface will be endowed with the capacity to interact with polar and non-polar molecules. Moreover the interactions with the non-polar surfaces (at micelles for example) must be associated with a structural change in the enzyme through which it achieves its catalytic power. Thus the distribution of polar and non-polar side groups externally (and possibly internally) govern both folding and the enzyme's potential for

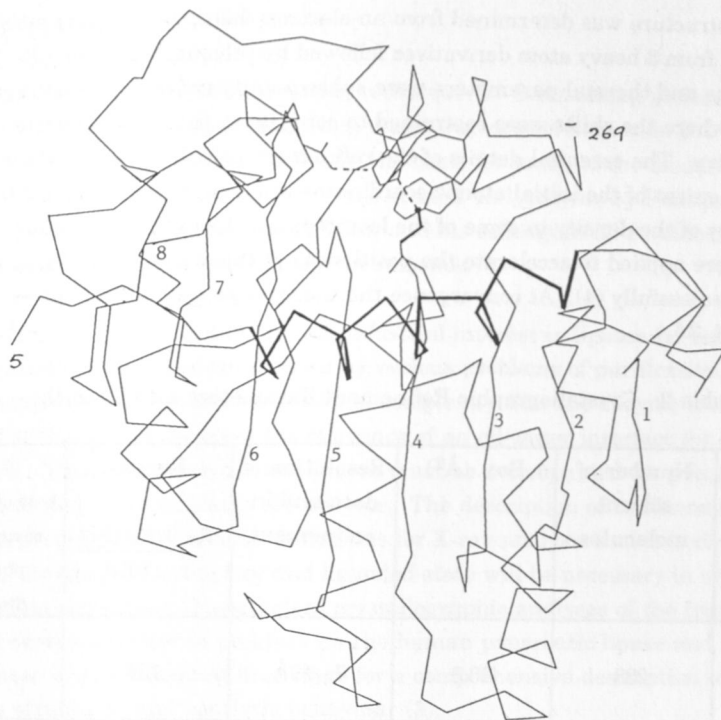


Figure 1

The peptide backbone structure of lipase from *M. Miehei*. Residues 1-4 are disordered and are not determined. The main chain is represented by the C α atoms and the helix 11 to 29 is drawn with thick lines. The active side chains ser 144, his 257 and asp 203 and the tryptophan 88 are also shown with their connecting H bonds. For clarity the ser 144 side chain is drawn in thick lines. The view is roughly perpendicular to the axis of the N terminal helix. It shows the location of the ser 144 at the end of a β sheet, the his 257 on the terminal segment and the asp 203 to be in a stretch of helix forming part of a large loop between two of the C terminal strands.

interaction. Generally the enzyme's surface is polar but examination shows that there is an extensive region of non-polar residues near the site of the catalytic residues. One side of this is bounded by the small helix 85 - 92 covering the catalytic site (see below), and on the other by the residues linking the adjacent strands. The cross section of this region is about 25Å by 15Å. There is a further extension of the non-polar surface to the residues linking the C terminal β strands. It seems likely that because of the extent and

location next to the active site, the non-polar properties of this region will help govern the enzyme's interaction at polar/non-polar interfaces and the subsequent cleavage of lipids. There are other smaller regions where non-polar residues are congregating but it is not obvious at this stage that they have any particular significance.

3.3 The interactions between the helices and sheet structure. There are 5 stretches of well defined helix, which pack against the central 8 stranded β sheet structure. The contacts are mostly non-polar, typical of the kind seen in the case of globular proteins. The N terminal helix which extends from 11 - 29 makes however a series of polar as well as non-polar interactions to the sheet and to other surrounding structures formed by links between the β strands which together partly bury it. As can be seen in Figures 1 and 2, the N terminal helix runs right across to the β sheet diagonally to the strands. This side of the β sheet is structurally simple, consisting only of this helix which is likely therefore to be a major contributor to the overall stability of the enzyme.

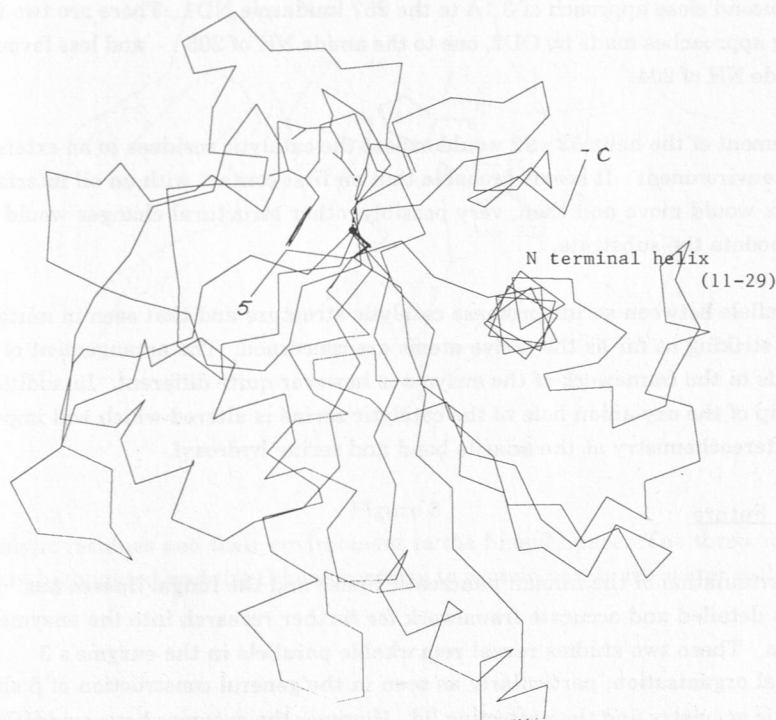


Figure 2

The lipase structure viewed in the direction of the N terminal helix. The main chain and side chains conventions are as in Figure 1 except that the helix 11-29 is not highlighted. This view illustrates the packing of the N terminal helix against the concave surface of the β sheet forming an effective cover for that structure.

The principal polar interactions between the N terminal helix and the sheet involve protein groups such as threonine, serine, tyrosine and glutamine, as well as non-polar groups. There are also buried water molecules which complete the H bonding requirements of some of the hydroxyl groups and amide groups.

3.4 The catalytic site. The catalytic residues in mucor lipase are asp 203 - his 257 - ser 144. These residues are buried by a small helix between 85 - 92 from which extend a tryptophan (88), isoleucine (89) and a leucine (92). The isoleucine 89 faces an extensive aliphatic grouping while the tryptophan (88) is at ca. 4.0Å from the active serine and effectively blocks all access to the catalytic site. The stereochemistry at the active site is illustrated in Figure 3. The serine 144 is H bonded to the histidine NE2 and as well to a well defined water molecule trapped between the tryptophan ring and H bonded closely to the main NH of residue 82 serine. As already described, the imidazole group of his 257 is H bonded (2.7Å) through NE2 to the OG of the active ser 144. There is also a H bond from the imidazole ND1 to the OD1 of asp 203. The aspartic 203 through OD2 makes a second close approach of 3.1Å to the 257 imidazole ND1. There are two further H bonding approaches made by OD2, one to the amide NH of 205 - and less favourably to the amide NH of 204.

Displacement of the helix 52 - 92 would expose the catalytic residues to an extensive non-polar environment. It seems probable that on first contact with an oil interface this small helix would move and then, very possibly, other structural changes would occur to accommodate the substrate.

The parallels between serine protease catalytic structure and that seen in mucor lipase are striking as far as the active atoms are concerned. The arrangement of the amino acids in the framework of the enzyme is however quite different. In addition the relationship of the oxy-anion hole to the catalytic serine is altered which will impose different stereochemistry at the scissile bond and serine hydroxyl.

4. The Future

The determination of the human pancreatic lipase and the fungal lipases has provided a detailed and accurate framework for further research into the enzyme's mechanism. These two studies reveal remarkable parallels in the enzyme's 3 dimensional organisation, particularly as seen in the general construction of β sheet, the catalytic geometry and the protecting lid. However the enzymes have evidently achieved their structural and functional properties independently and these similarities seem to be striking examples of convergence in evolution at the molecular level.

The enzyme's mechanism of activation remains from these studies speculative and the analysis of a covalently bound substrate analogue is essential for progress on this question. There is no doubt that this will be achieved and that it will reveal how the enzyme's architecture has equipped it to deal with both the aqueous and non-polar environments.

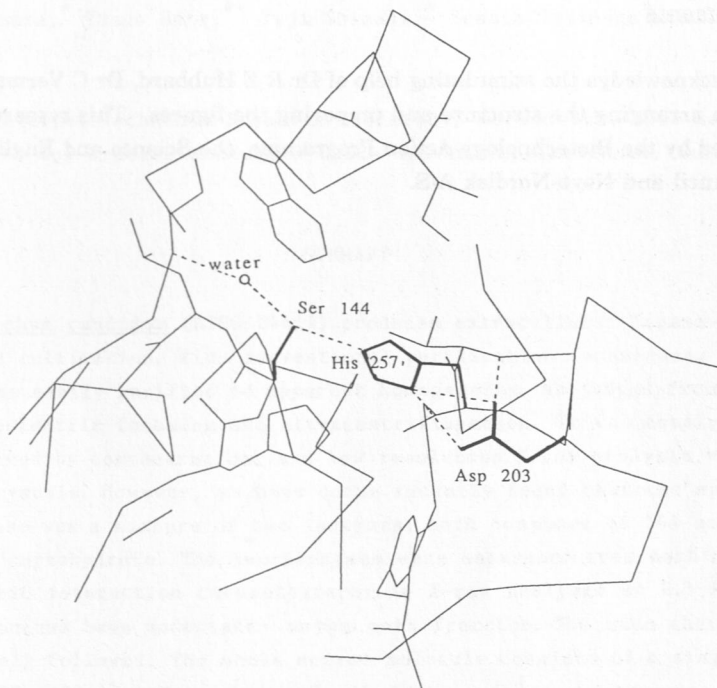


Figure 3

The catalytic residues and their environment in the fungal lipase. The three active side chains are highlighted and the H bond contacts to protein and to the water molecule are shown.

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