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A Leukotriene A_4 Hydrolase-Related Aminopeptidase from Yeast Undergoes Induced Fit upon Inhibitor Binding

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Vertebrate leukotriene A_4 hydrolases are bifunctional zinc metalloenzymes with an epoxide hydrolase and an aminopeptidase activity. In contrast, highly homologous enzymes from lower organisms only have the aminopeptidase activity. From sequence comparisons, it is not clear why this difference occurs. In order to obtain more information on the evolutionary relationship between these enzymes and their activities, the structure of a closely related leucine aminopeptidase from *Saccharomyces cerevisiae* that only shows a very low epoxide hydrolase activity was determined. To investigate the molecular architecture of the active site, the structures of both the native protein and the protein in complex with the aminopeptidase inhibitor bestatin were solved. These structures show a more spacious active site, and the protected cavity in which the labile substrate leukotriene A_4 is bound in the human enzyme is partially obstructed and in other parts is more solvent accessible. Furthermore, the enzyme undergoes induced fit upon binding of the inhibitor bestatin, leading to a movement of the C-terminal domain. The main triggers for the domain movement are a conformational change of Tyr312 and a subtle change in backbone conformation of the PYGAMEN fingerprint region for peptide substrate recognition. This leads to a change in the hydrogen-bonding network pulling the C-terminal domain into a different position. Inasmuch as bestatin is a structural analogue of a leucyl dipeptide and may be regarded as a transition state mimic, our results imply that the enzyme undergoes induced fit during substrate binding and turnover.

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Abbreviations used: *sc*LTA4H, leukotriene A_4 hydrolase from *Saccharomyces cerevisiae*; GST, gluthathione *S*-transferase; PEG, polyethylene glycol; Tris, tris(hydroxymethyl)aminomethane; ES, 2-(*N*-morpholino)ethanesulfonic acid; MMT buffer, L-malic acid; MES, Tris buffer.

Introduction

The zinc metalloenzyme leukotriene A_4 (LTA_4) hydrolase (LTA4H) catalyses the hydrolysis of the unstable epoxide LTA_4 (5*S*-*trans*-5,6-oxido-7,9-*trans*-11,14-*cis* eicosatetraenoic acid) into LTB_4 (5*S*,12*R*-dihydroxy-6,14-*cis*-8,10-*trans*-eicosatetraenoic acid), a potent chemotactic agent and mediator of inflammation.[1,2] The LTA4Hs found in vertebrates are bifunctional proteins, since they also possess an anion-dependent Arg-aminopeptidase activity.[3–5] The zinc atom is essential for both activities, which take place in overlapping active sites. The human enzyme has been studied extensively, and the key elements for the catalytic activities have been identified by a combination of site-directed mutagenesis and crystallography techniques.[6]

LTA4H is part of a class of multifunctional proteins, also known as moonlighting proteins, which have more than one distinct function within a single polypeptide chain.[7] These proteins may contain dual catalytic activities or combinations of noncatalytic functions with enzymatic activities. Moonlighting proteins are a diverse class of proteins that can be found in all phyla, and their functions address a wide range of cellular and physiological processes.[8] To perform multiple functions, these proteins can utilize separate binding sites for different substrates or assume different roles upon complex formation with cofactors or other proteins. Products of gene fusions, splice variants, or proteins showing promiscuous enzymatic activity are not considered to be part of this class. It has been speculated that in order to obtain a moonlighting function, a protein needs to have an innate compatibility to obtain this function.[9] According to this hypothesis, additional mutations are required to develop the full multifunctional protein. LTA4H is a somewhat unique moonlighting protein, since its two activities are exerted via distinct but yet overlapping active sites. Thus, within one active centre, certain amino acids are common for the two activities, whereas others are specifically used in only one of the two catalytic mechanisms.[6] The function of the epoxide hydrolase activity of LTA4H is well established and provides a potent chemotactic factor, LTB_4, during the initiation of inflammation. Recently, data were presented, suggesting that the aminopeptidase activity of LTA4H cleaves and inactivates a proinflammatory peptide, Pro-Gly-Pro, during the resolution of inflammation.[10] Hence, the two activities of LTA4H seem to play specific and functionally opposite roles during two separate phases of an inflammatory reaction.

LTA4H is a member of the M1 family of metalloproteases,[11] characterized by a common Zn binding signature, HEXXH, in which the His residues function as Zn binding ligands and the Glu serves as the general base catalyst in the peptide hydrolysis. The zinc-binding site is completed by a Glu residue located 18 amino acids downstream of the HEXXH motif. All members of the family exhibit the aminopeptidase activity; however, the epoxide hydrolase activity is not conserved despite considerable sequence conservation. This activity can only be detected among vertebrates, including birds, frogs, and fish.[12–16] Thus, although aminopeptidase 1 from *Caenorhabditis elegans* is highly homologous at the amino acid level to human LTA4H, no LTA_4 hydrolysis could be detected[17] (see Table 1 for homology percentages). These observations raise questions on the evolutionary relationship of these enzymes, how the presence of the epoxide hydrolase activity was established in these proteins, and whether conclusions drawn for other moonlighting proteins play a role for LTA4H.

Apart from the structure of human LTA4H,[21] structures of other members of the family have recently become available. The most homologous is the structure of a cold-active aminopeptidase from *Colwellia psychreythraea*,[20] while more distant structures are represented by those of the Tricorn interacting factor F3[19] and the aminopeptidases N from *Escherichia coli*,[18,22] *Neisseria meningitidis*,[23] and *Plasmodium falciparum*.[24] All these enzymes are built from several domains coming together in a hook-like formation with the active site positioned in a deep cavity between the domains. They also exhibit sequential and structural conservation for the two N-terminal domains, while there is considerable

Table 1. Homology of selected related proteins to human LTA4H

Enzyme	Species	Identity (%)	Similarity (%)	Residue overlap
LTA4 hydrolase[18]	*S. cerevisiae*	40.9	68.8	616
Aminopeptidase 1[13]	*C. elegans*	36	63.5	636
Cold-active aminopeptidase[15]	*C. psychreythraea*	33.9	62.3	623
Aminopeptidase N[19]	*P. falciparum*	26.5	53.5	465
Aminopeptidase N[20]	*N. meningtitis*	24.8	50.0	468
Aminopeptidase N[17,21]	*E. coli*	22.9	54.3	494
Tricorn interacting factor F3[16]	*T.acidophilum*	22.0	54.5	451

Homology comparisons were calculated by using FASTA at the EBI with BLOSUM50 as similarity matrix. Sequence for *hum*LTA4H from Haeggström *et al.*, 1990.[3]