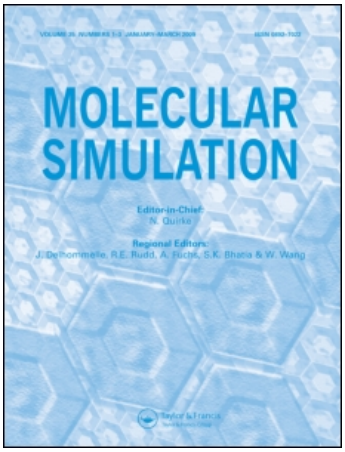

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The Mechanisms of Action and Inhibition of Pancreatic Lipase and Acetylcholinesterase: A Comparative Modeling Study

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THE MECHANISMS OF ACTION AND INHIBITION OF PANCREATIC LIPASE AND ACETYLCHOLINESTERASE: A COMPARATIVE MODELING STUDY

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Pancreatic lipase and acetylcholinesterase are both serine esterases. Their X-ray structures reveal a similar overall fold, but no sequence homology can be detected. A catalytic triad like in the trypsin family of serine proteases consisting of serine, histidine and aspartate (glutamate in acetylcholinesterase) suggests mechanistic similarities. Models of the transition states of the substrate cleavage have been built and possible catalytic pathways were examined. The model that could produce a consistent pathway throughout the reactions had a transition state of the opposite handedness compared to trypsin. These models could be used to rationalise binding modes of inhibitors of both enzymes. The lipase inhibitor tetrahydrolipstatin (THL) contains a gamma-lactone which is opened by the catalytic serine; the alcohol leaving group prohibits deacylation by locking the pathway for incoming water and thus inactivates the enzyme. Carbamate inhibitors of acetylcholinesterase transfer a carbamoyl group to the serine-OH which deacylates slowly. These observations can be used as a starting point for the discovery of new classes of inhibitors.

KEY WORDS: Lipase, acetylcholinesterase, inhibitors, modelling, crystallography

INTRODUCTION

Serine hydrolases are enzymes that play a key role in diverse physiological systems. Examples are: trypsin which digests peptides and proteins, thrombin which initiates blood coagulation by cleaving fibrinogen to fibrin, betalactamases that mediate bacterial resistance to antibiotics by cleaving betalactams, lipases that digest nutrition fat triglycerides and acetylcholinesterase that degrades the synaptical neurotransmitter acetylcholine [1-5].

The hydrolytic reaction of these enzymes follows a two-step mechanism: After the formation of the Michaelis complex, the catalytic serine OH attacks the carbonyl group of the amide or ester and forms an acyl-enzyme intermediate. This serine ester is then cleaved by a water molecule. Proteases and esterases differ by their preference for amide and ester groups and release amines and alcohols, respectively, as the leaving group.

They all use a serine sidechain hydroxy group as a nucleophile in their enzymatic reaction. These serine enzymes belong to several families that share no sequence homology or structural similarity.

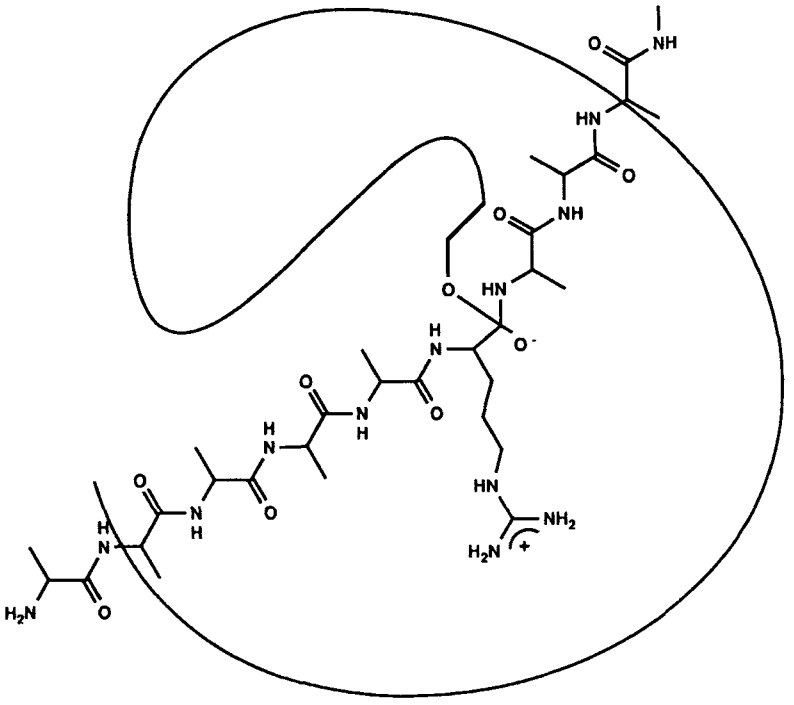


Figure 1 Schematic representation of the cleavage of a peptide by trypsin at the amide bond after an arginine residue.

The serine hydrolases that are best studied belong to the trypsin family of proteases. Because these enzymes will be used as a reference in the discussion of the mechanism of the esterases, two prominent examples of this class and their mechanism will be briefly reviewed in the following.

Trypsin and Thrombin

The mechanism of action of this class of enzymes is well understood based on a large number of structural studies [1]. These include crystallographic work with substrates and derivatives, with mechanism based inhibitors and with noncovalent inhibitors, as well as NMR studies with substrate peptides [6–9]. To summarise, the schema of the substrate cleavage by trypsin is shown in Figure 1. A model of the transition state of the acylation reaction is shown in overview in stereo picture 1 and a close-up is shown on stereo picture 2.* The transition state is characterised by a tetrahedral carbon covalently linked to the catalytic serine Oγ, a negatively charged oxy-anion stabilised by two hydrogen bonds to two backbone NH groups, and a protonated histidine which has received the proton from the serine-OH. In the next step, the leaving group will be protonated by the histidine to an amine, the former amide bond will break and a serine ester will be formed. The

*The stereo pictures referred to in this paper are colour plates 7–20 and can be found at the end of this issue.

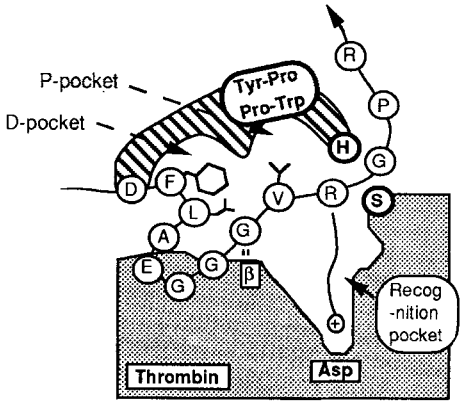


Figure 2 Cartoon of the binding mode of fibrinogen in thrombin with the arginine side chain in the recognition pocket, the valine side chain in the P-pocket and the leucine and phenylalanine side chains in the D-pocket.

deacylation is initiated by the attack of a water molecule. It approaches the ester carbonyl from the same direction that the amine group left. The water molecule is activated by the same basic histidine that activated the serine. A second tetrahedral transition state forms and it decays to the acid; the protease is restored in the active form.

The three-dimensional shape of the transition state and the protein environment exhibit ideal complementarity to each other, which is the prerequisite for the observed rate enhancement of the hydrolytic reaction.

The specificity for cleavage after arginine or lysine is mediated by a recognition pocket with an aspartate side chain at the bottom. This pocket accommodates the positively charged side chain and forms a salt bridge with the carboxylate of the aspartic acid.

In thrombin, the specificity extends far beyond the residue before the cleavage site. NMR [6, 7] and X-ray studies [2, 9] revealed that three hydrophobic residues two, eight and nine residues before the cleavage site occupy unique hydrophobic pockets (Figure 2 and stereo picture 3). These hydrophobic pockets are formed by a unique additional loop Tyr-Pro-Pro-Trp above the active site.

MD805 and NAPAP in Thrombin

The binding mode of these two inhibitors (Figure 3) as revealed in the X-ray structure of their complexes with thrombin [2, 10] has some unexpected features: The basic moiety binding in the specificity pocket is directly connected to hydrophobic portions occupying the two hydrophobic pockets (stereo picture 4); there is no interaction with the catalytic serine. The inhibitors thus do not bind substrate-like, but bypass the catalytic site and preferrentially interact with the hydrophobic pockets. In the complex with fibrinogen, the same pockets are occupied by the hydrophobic sidechains before the cleavage site. Based on these findings, new potent thrombin inhibitors have been discovered [11, 12] and are currently evaluated as possible antithrombotic drugs.

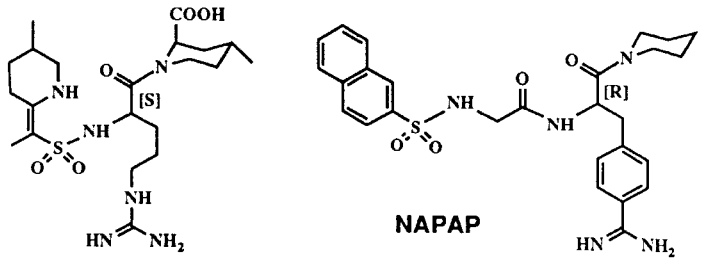


Figure 3 Formulae of the MD805 and NAPAP thrombin inhibitors.

Human Pancreatic Lipase (hPL)

Human pancreatic lipase is a protein of 445 amino acids. It folds in two domains, the larger N-terminal of which contains the catalytic site. The overall folding is represented in a ribbon-diagram in stereo picture 5. This crystallographic structure of the human pancreatic lipase represents an inactive form of the enzyme [4]. The active site is totally burried by a loop and the catalytic serine is inaccessible to solvent and substrate (stereo picture 6). The enzyme has to undergo interfacial activation [13, 14] at the surface of a fat droplet and thereby exposes the hydrophobic interior of this loop to the fat; the active site becomes accessible.

Model of the trilaurin triglyceride substrate binding

Since the structure observed in the crystals represents an inactive form of the enzyme, several structural modifications were required to accommodate a substrate molecule in the active site. These modifications were performed such that the resulting model remained as similar as possible to the experimental structure. First, the loop covering the active site was removed between Cys237 and Cys261 which is the disulfide bridge that holds the loop in place. Before that, it was determined that this loop can adopt a conformation that does not interfere with substrate binding and at the same time contains no strained conformation. Second, the side chain orientations of three aromatic residues near the active site had to be changed to an alternative low energy conformation ($\chi_1 = 60°$ or $180°$) (Stereo picture 7). Third, the segment around Phe77 had to be shifted away from the catalytic serine by about 1.5 Å; this segment has no distinct interaction with the rest of the protein so this shift seems to be acceptable. The model created by these three steps was then used to dock a substrate trilaurin triglyceride molecule starting from the X-ray structure of the molecule alone [15] with the following requirements in mind: the trilaurin ester carbonyl comes in contact with the $O\gamma$ of the catalytic serine; the ester carbonyl oxygen sits in the oxy-anion hole formed by backbone NH groups of Leu 153 and Phe 77; the ester is in the trans conformation and the fatty acid tails protrude to where the fat droplet is assumed to be located. Starting from this model of the Michaelis complex, the consecutive catalytic steps (Figure 4) were built (the hemiacetal model is shown in stereo picture 7). These models represent a reaction trajectory with essentially strainless intermediates. The backprotonation of the leaving alcohol group and the activation of the incoming water could be

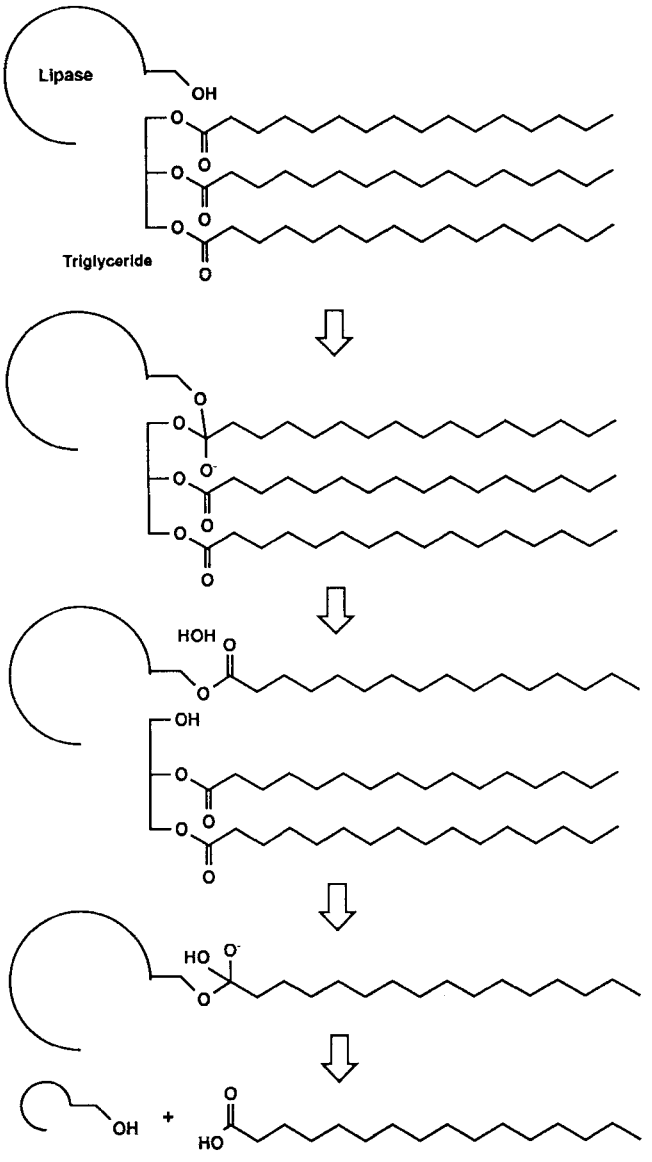


Figure 4 Reaction scheme of the triglyceride cleavage by pancreatic lipase.

performed by the neighbouring histidine Nε. Alternative conformations and orientations of the trilaurin substrate have been tried and did not produce acceptable models. All models discussed here have been subjected to Moloc [16–18] energy minimisation and have been inspected for collisions of nonbonded atoms and for deviations from standard bond lengths, valance angles and torsional angles.

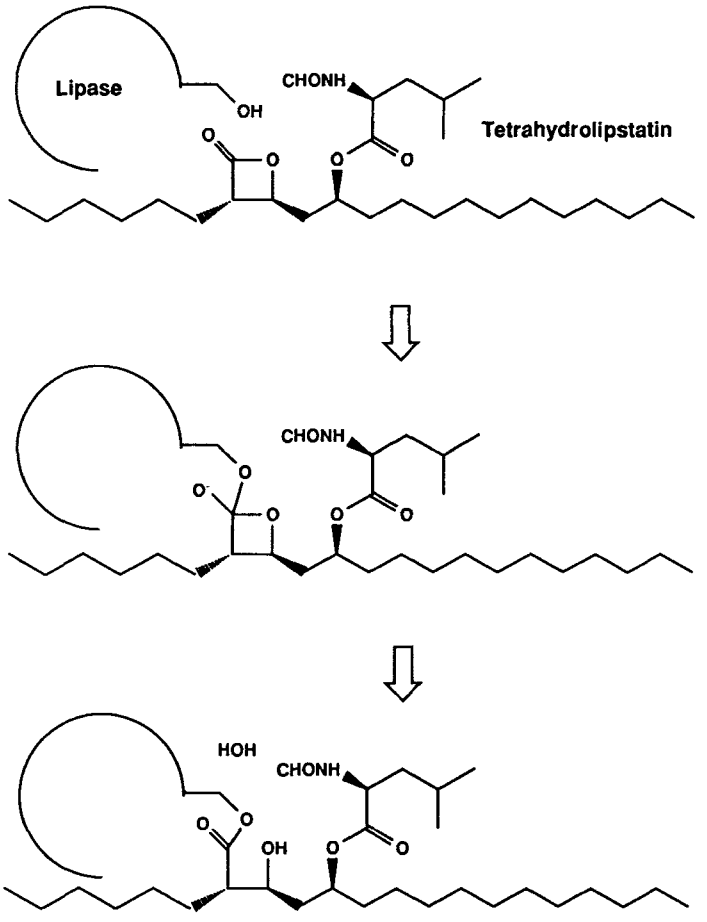


Figure 5 Reaction scheme of the inhibition of pancreatic lipase by THL. The reaction stops at the acyl-enzyme intermediate because the alcohol group prevents the attack by a deacylating water molecule.

Similar results could be obtained with other appropriately parametrised force fields (like AMBER [19], CHARMM [20] etc.).

Tetrahydrolipstatin (THL)

THL, a hydrogenated derivative of lipstatin originally isolated from Streptomyces toxytricini, is a potent inhibitor of hPL [21, 22] and is currently evaluated in clinical trials. The model (Figure 5, stereo picture 8) derived from the previously discussed substrate model suggests that the tetrahedral hemiacetal intermediate of the betalactone decays though ring opening. The hydroxy ester formed is conformationally restricted by the protein environment in the narrow cleft below the catalytic serine. The hydroxy group thus protects the ester group from incoming deacylating water. The enzyme remains acylated and therefore inactivated. The hydrophobic moieties

of THL, the two hydrocarbon chains on the lactone ring and the leucine sidechain coincide with the three fatty acid chains in the trilaurin model which are thought to protrude into the fat droplet.

Acetylcholinesterase

The X-ray structure of AChE [5] of the electric eel Torpedo californica reveals a single domain fold with a characteristic central eleven strand β-sheet. Although no detectable homology is present between hPL and AChE, the folding in the core of the catalytic domain of the lipase is very similar to that of AChE (Stereo picture 9). The central β-sheet can be superimposed such that the long helix which carries the catalytic serine at its N-terminus aligns. The geometry of the active site functional groups is also similar, but there are distinct differences. The active site of AChE is located at the bottom of a deep gorge open to solvent. This gorge is coated with aromatic side chains. The catalytic triad Ser-His-Glu is similar in geometry, but the His and Glu side chains are attached to different secondary structure elements than in hPL [23]. The oxy-anion hole consists of two equivalent NH-groups (Ala201 and Gly118), but a third NH of Gly119 is located at a position suitable to form an additional stabilising H-bond to the transition state carbonyl oxy anion (stereo picture 10). In contrast to the hPL structure, the AChE structure appears to represent the active form of the enzyme with appropriate geometry of the active site residues. Gratifyingly, the geometry of the active site in the hypothetical model of the active form of the lipase (which had been built prior to the availability of the AChE structure) is very similar to AChE.

Model of the Acetylcholine Substrate binding

Starting from the hypothetical model of the active hPL, it was straightforward to build models of the AChE reaction mechanism (Figure 6 and stereo picture 11). In the hemiacetal transition state, the choline leaving group is at hydrogen bonding distance from the Nε of His440. The choline molecule is accommodated in the fully extended conformation and positions the trimethylammonium group towards the face of the indol system of Trp84. The methyl group of the acetyl moiety is placed in a small pocket which could not accommodate larger or branched acid components. This is in agreement with the observed reduced hydrolysis rates [24]. The acetylated serine can be attacked by a water molecule which follows the reverse trajectory of the leaving alcohol group. The water molecule can be activated by transfer of a proton to the Nε of the catalytic histidine. A superposition of the transition state models of hPL with trilaurin and AChE and ACh is shown in stereo picture 11. The functional groups of the enzyme involved in the catalysis occupy similar positions. The acid moiety of the triglyceride extends through a narrow channel in the lipase while there is no such channel in AChE. The leaving diglyceride in the lipase extends to the protein surface while in the AChE the choline fills the bottom of the gorge.

Physostigmine

The alkaloid physostigmine from calabar bean [25] inactivates AChE through transfer of a carbamoyl group to the catalytic serine; this serine carbamate is only

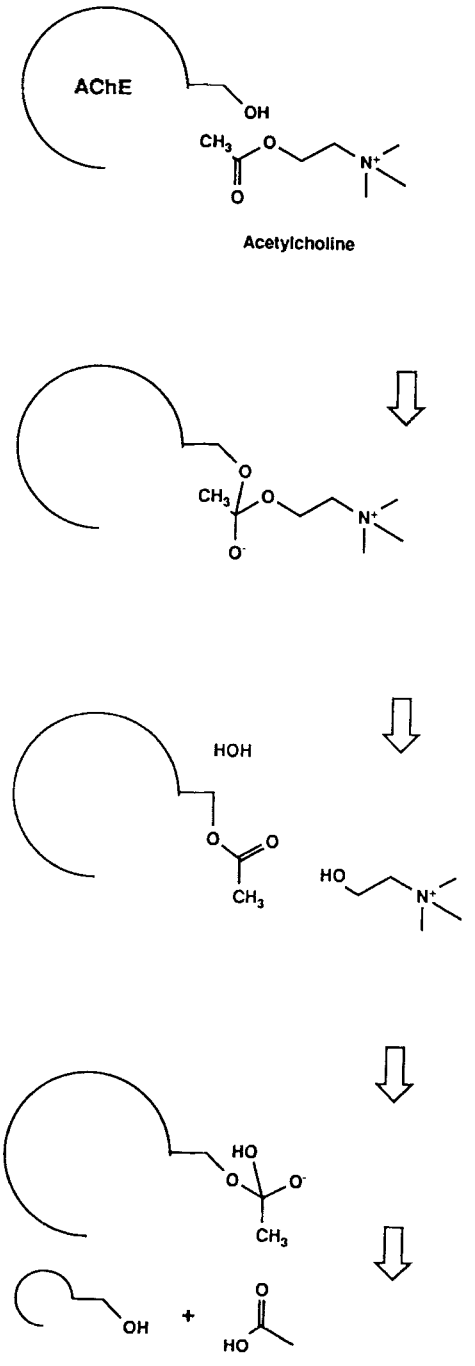


Figure 6 Reaction scheme of the cleavage of acetylcholine by acetylcholinesterase.

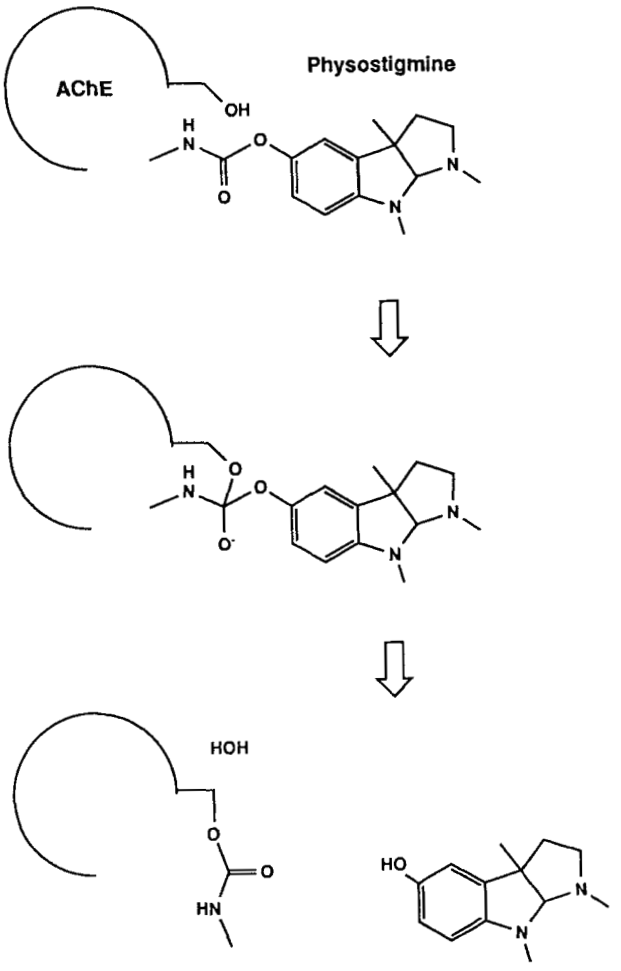
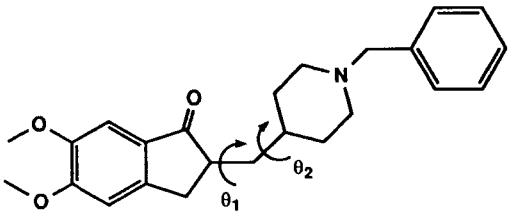


Figure 7 Reaction scheme of the carbamoylation reaction of physostigmine with acetylcholinesterase.

slowly hydrolysed. The molecule (Figure 7) has very little flexibility. Starting from the X-ray structure of the molecule alone [26], physostigmine was docked to the AChE active site such that the catalytic serine could be carbamoylated. The only conformation fitting the active site has the basic nitrogen near the trimethylammonium group in the substrate model. The tricyclic system essentially fills the bottom of the gorge (stereo picture 12). The N-methyl group of the carbamate protrudes into the restricted pocket which accommodates the acetyl group in the substrate model; larger substituents at this position would require a different, more strained conformation. This is in agreement with the structure-activity relationship found for such a series of compounds [27].



Eisai E2020

Figure 8 Formula of the E2020 AChE inhibitor with two torsional angles indicated.

Eisai E2020

The AChE inhibitor E2020 developed at Eisai is a representative of a large series of very potent compounds [28–30]. Initial attempts to position the indanone carbonyl group of E2020 close to the catalytic serine were unsuccessful; the compound had to be folded and could then not fit the gorge. Positioning the basic nitrogen close to the corresponding position in the other models allows only one orientation of the molecule: The indanone portion fills the gorge towards the solvent while the benzyl group fills a cavity at the bottom of the gorge. The model shown on stereo picture 13 has exactly the same conformation with respect to θ_1 and θ_2 (Figure 8) that had been deduced from an extensive QSAR study [29]. The conformation of the benzyl group is different and adopts an endo orientation in order to reach the cavity. The QSAR data seem to confirm this model: a wide variety of substituents is tolerated at different positions of the indanone aromatic ring; in the model this portion is directed toward the exterior. In contrast, substitution at the para postion of the benzyl group reduces activity dramatically; also benzoyl instead of benzyl is not tolerated. This can be rationalised from the model showing that the benzyl group essentially fills up the cavity in the conformation shown.

These results suggest that E2020 is a noncovalent AChE inhibitor which binds to the hydrophobic gorge but does not interact with the catalytic residues.

Opposite handedness of the transition state

Pancreatic lipase and acetylcholinesterase belong to a family of enzymes that share what has been termed the α/β hydrolase fold [31]. The members of that family have a similar core and a similar catalytical triad. It has been proposed that they also share a similar catalytic mechanism and a similar geometry of the transition state.

Comparing the stereochemistry of the transition state in trypsin and thrombin on one side and in lipase and AChE on the other, they are of opposite handedness (Figure 9). At the same time, the functional groups constituting the catalytic machinery of the hydrolases are similar to the mirror image of the ones in trypsin [13].

Interestingly, crystallographic studies on a phosphonate ester complex of a fungal lipase from Rhizomucor Miehei [32] which also belongs to the α/β hydrolase family, support the postulated opposite handedness of the transition state. The phosphonate ester can be considered to be a transition state isoster; the stereochemistry observed in the complex is exactly the one proposed for the lipase transition state of the ester cleavage.

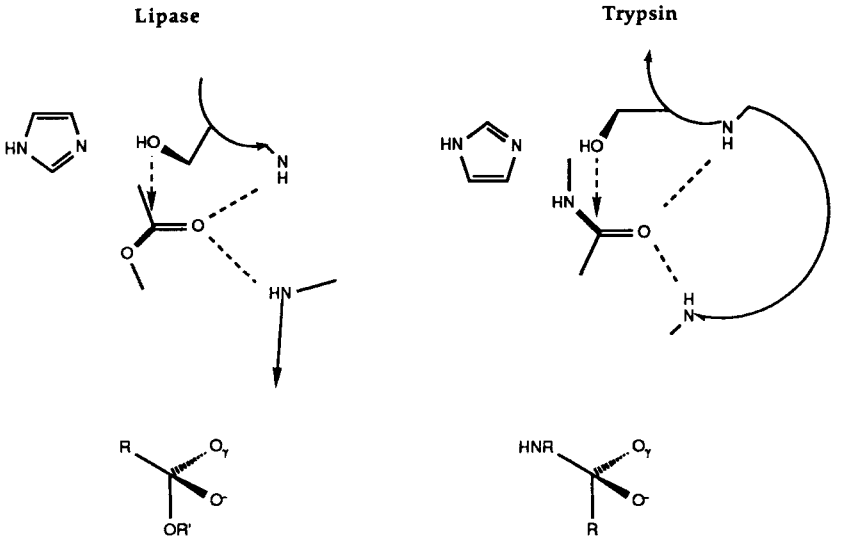


Figure 9 Comparison of the reaction stereochemistry between lipase and trypsin. The transition state formed by the attack of the serine Oγ has the opposite handedness.

CONCLUSIONS

Proteases and esterases catalyse similar hydrolytic reactions. They use similar functional groups in a distinct spatial arrangement to achieve this catalysis. Since in contrast to substrate and product the transistion state is chiral, there should correspondingly be two possible classes of hydrolytic enzymes utilising each of the two possible enantiomeric transition states. It appears that trypsin-like serine proteases belong to one stereochemical class and that the esterases of the hydrolase family belong to the other. Together with the totally different folding topology, this is indicative of a convergent evolution of enzymes catalysing hydrolytic reactions using a catalytic triad Ser-His-Asp(or Glu) and an oxy-anion hole.

Some noncovalent inhibitors appear to preferrentially occupy hydrophobic pockets rather than interact with the catalytic functionalities. This indicates that exclusion of water is the main driving force for intermolecular association in this case.

The detailed knowledge of macromolecular target structures and the mechanisms of action provide a firm basis for the rationalisation of structure affinity relationships and the design of novel potent inhibitors, which may open the door to new therapeutic opportunities.

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