Chiral α-substituted α-hydroxy acids

Moorlag, Hendrik

CHAPTER V

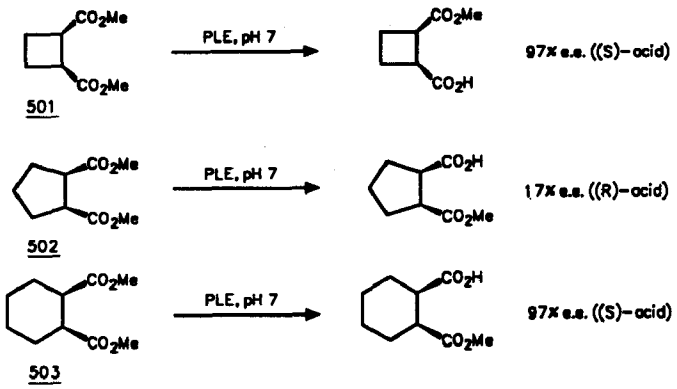
RATIONALIZATION OF PLE STEREOSELECTIVITY USING AN ACTIVE SITE MODEL

5.1 *INTRODUCTION*

In the preceeding Chapter, we reported on the pig liver esterase (PLE) catalyzed hydrolysis of α-substituted α-hydroxy esters (substituted mandelates and lactates). PLE can accept and hydrolyse almost any of these esters independent of the type of substituents. Although a wide variety of esters did react, there were large differences in the enantioselectivity of the reaction with PLE. Only in the series of substituted mandelic esters high enantioselectivity was found in hydrolysis. At first glance it may be hard to find any underlying reason for PLE 's stereoselectivity preference as major changes in stereoselectivity were triggered by apparently minor variations in substrate structure.

The difficulty of understanding and predicting the stereochemistry of an enzymatic reaction is a major drawback for application of enzymes in organic synthesis. PLE occupies a prominent position in this regard because its tertiary structure is unknown. One is forced into "black box" explanations. One special worry that has come up is that PLE is not constant in its stereoselectivity within a homologous series of substrates.[1]

A dramatic example of the fickle nature of PLE is demonstrated within a series of meso 1,2-diesters (Scheme 5.1).[1a] PLE catalyzed hydrolysis is stereoselective for the (S)-center of cyclobutyl diester **501**. On the other hand, hydrolysis of cyclohexyl diester **503** is completely stereoselective for the (R)-center. The cyclopentyl diester **502** represents the "change over" structure: both ester groups are hydrolyzed to similar extents to give the product with only 17% ee.



Scheme 5.1

Knowledge about the enzyme's structure, especially about the catalytically active site, would not only help to rationalize the stereochemical outcome of the PLE catalyzed hydrolysis, but would also greatly facilitate the design of substrates for PLE that could be hydrolyzed with high stereoselectivity. Due to its micro-heterogenity (Section 5.2) PLE has never been crystallized which would allow elucidation of its structure by X-ray diffraction. Therefore, efforts have been made toward the development of an active site model of the enzyme that would permit the unpredictable nature of PLE stereoselectivity to be resolved.

Before entering details about active site models, some features about PLE concerning its physical properties and mechanism of action will be discussed.

PIG LIVER ESTERASE: THE ENZYME

Although the function of PLE and other mammalian carboxylesterases is still obscure, they may play a role in the detoxification system in the body by their ability to metabolize multifarious foreign compounds with ester and amide bonds. PLE is a quite complicated enzyme system. The molecular weight of PLE is about 168 000.[2] The commercially available PLE preparations are a mixture of at least five isozymes[3]. The isozymes have been shown to differ in properties such as pH dependence and

inhibition or activation by organic solvents.[4] However, the isozymes have been reported to react with similar stereoselectivity for some meso diester substrates, which has caused commercial PLE to be treated "as if it were a single protein".[5]

PLE belongs to the class of serine proteases. Proteases operate via formation of an acyl enzyme intermediate depicted in the mechanism of hydrolysis shown in Figure 5.1.[6] The amino acid numberings is that of the representative serine protease, chymotrypsin. The catalytic triad of Ser-195, His-57 and Asp-102 form the heart of the active site. The major steps in the catalytic process of hydrolysis are believed to be the following. After initial formation of a complex (ES) between the enzyme and a substrate (an ester is chosen as an example), wherein the backbone NH groups of Ser-195 and Gly-193 bind the carbonyl oxygen, a covalent tetrahedral intermediate or transition state (TI_1, the question whether there is a discrete intermediate or not is still unanswered) is formed.

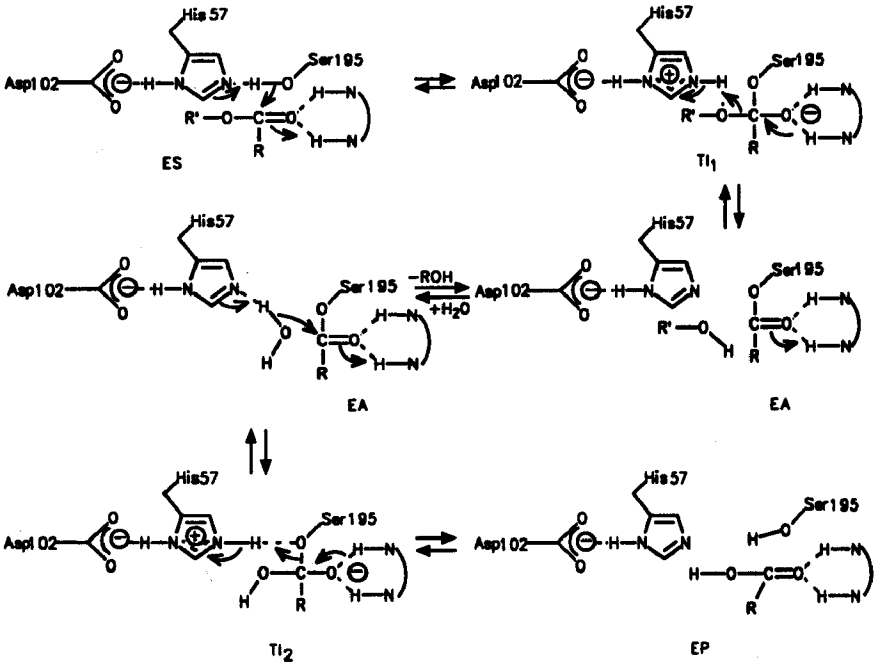


Figure 5.1

This species collapses to give by imidazolium catalyzed protonation of the alcohol leaving group an acyl enzyme (EA). Subsequently, the reverse series of events takes place. Deacylation of the acyl enzyme compound is accomplished by an activated water molecule to form another tetrahedral intermediate (TI_2). This species falls apart again by imidazolium catalyzed protonation, this time of the serine oxygen, to give the acid product. The Ser-His-Asp triad has been directly observed in the catalytic sites of various lipases, whose three dimensional structures have been determined by X-ray crystallography.[7]

5.3 *THE JONES' MODEL FOR THE ACTIVE SITE OF PLE*

As already stated, more insight in the factors that govern the stereoselective behavior of PLE would be of great importance to all users of this enzyme. As direct information about the catalytically active site of PLE is not yet at reach, several groups have proposed a model for the active site. Especially a model that would allow prediction of PLE catalyzed hydrolyses would be of great value to all users of PLE. Initially models were formulated in terms of a generalized substrate structure[1c], or were directed at interpretation of very limited data on diester hydrolysis.[8] A rather undetailed model which could, however, account for the observed reversals of PLE stereoselectivity was proposed by Jones and coworkers.[9] This model contained a hydrophobic binding region in which two pockets can bind a small (up to *n*-propyl in size) and a larger portion of a substrate molecule. The idea of a hydrophobic pocket in the active site was also recognized by Norin and coworkers[10] and later by Zemlicka et al.[11], who also came up with a hydrophilic site to accommodate more polar portions of a substrate.

Very recently Jones and coworkers proposed a rather specific active site model for PLE.[12] This model, which reflects the topography of the enzyme pocket, was based on analysis of the reactions of more than a hundred esters, reported in the literature. Using computer graphic analysis, the five site binding model depicted in Figure 5.2 emerged.

In addition to a nucleophilic serine site, the model consists of two hydrophobic binding zones, of which one, H_L, is much larger than the other, H_S.

There are two more hydrophilic zones located at the front (P_F) and the back (P_B) of the active site.

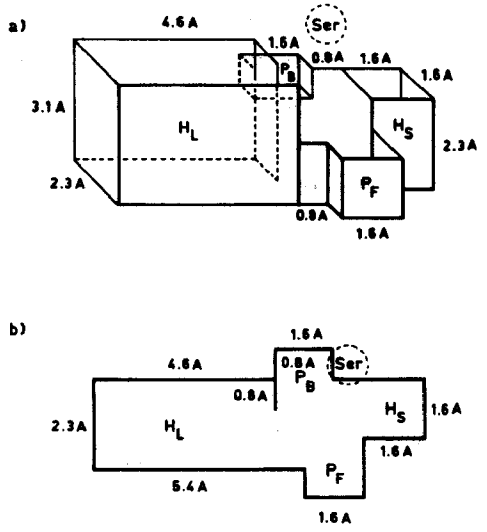


Figure 5.2: a: active site model of PLE; b: top perspective view of the model

The small hydrophobic pocket (H_S) has a volume of about 5.5 Å^3 and can accommodate groups up to four carbons in size, whereas the larger pocket (H_L) has a volume of approximately 33 Å^3. In order for an ester to be a substrate it must be able to fit into these regions. A good fit of a group in one of the hydrophobic pockets will result in a strong hydrophobic interaction[13] between the entering moiety and the active site and this interaction will contribute strongly in binding the substrate. If more than one binding mode for the substrate may be possible, the one that will give the best fit (which means the one that will maximize its binding potential) reflects the preferred enzyme-substrate orientation and will control the stereoselective behavior of PLE in hydrolysis.

The size-induced reversal in stereoselectivity observed for the meso 1,2-diesters mentioned in Section 5.1. can be rationalized on the basis of this model. The top perspective view of the active site model in which the orientations of meso 1,2-diesters **501**, **502** and **503** leading to hydrolysis either at the (R) or the (S)-center are shown in Figure 5.3. The ester group to be hydrolyzed is located in the serine region (represen-

ted by the dotted circle). The small hydrophobic, cyclobutane moiety of diester **501** (Figure 5.3a) will bind preferentially into H_S to give hydrolysis at the (S)-center, since this gives better hydrophobic binding than when this group is located in the larger H_L site, in which case hydrolysis at the (R)-center would occur. Filling the small pocket is far more preferred and hydrophobic groups will do so (Circe effect)[13a] until they become too large to be accommodated in H_S. This is clearly the case for the cyclohexyl substrate **503** (Figure 5.3b) where binding in H_L will dominate, leading to hydrolysis at the (R)-center. Hydrolysis of cyclopentane diester **502** represents the borderline case (Figure 5.3c): although the cyclopentyl group is slightly too large to bind in H_S, binding of this moiety in H_L is not favourable because of the failure of the five membered ring to fill this pocket. A clear preference for one of the two binding modes cannot be made, which is reflected by the low stereoselectivity in hydrolysis (a slight preference for binding of the cyclopentyl moiety in H_L is observed resulting in 17% ee of the 2R acid product of **502**).

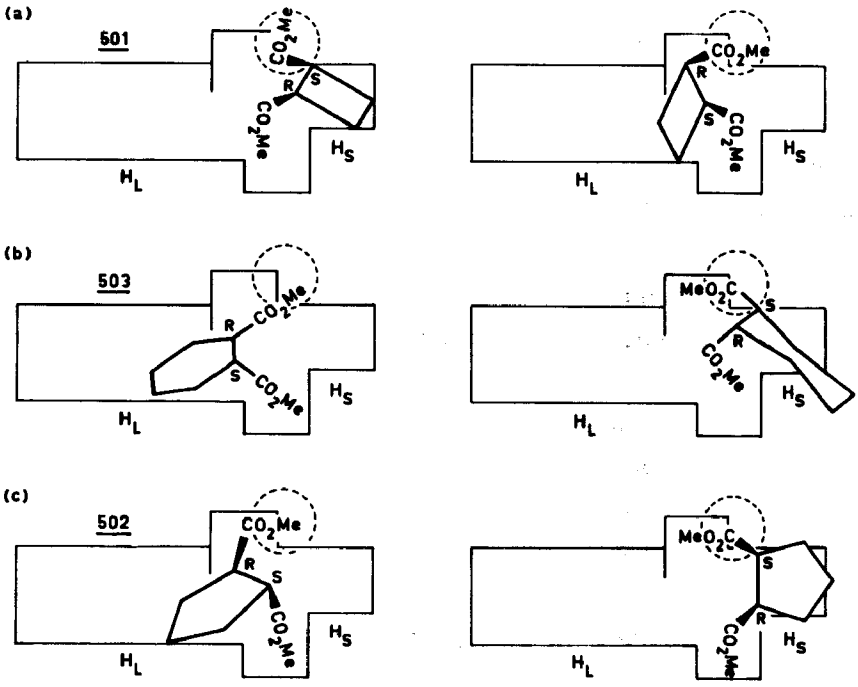


Figure 5.3

It should be noted that the function of P_F for these substrates is primarily to bind the non-hydrolyzed ester group of these meso diester substrates.

When we try in the next Section to rationalize the stereochemical outcome of PLE catalyzed hydrolyses of α-substituted α-hydroxy esters on the basis of this active site model, one should bear in mind that this probably is much more difficult than for the above discussed cyclic meso diesters due to the increased comformational flexibilities of our substrate molecules. Therefore high stereoselectivity is only to be expected if the conformational mobility of the substrates is greatly reduced by strong enzyme-substrate interactions.

5.4 *RATIONALIZATION OF PLE CATALYZED HYDROLYSIS OF α-SUBSTITUTED α-HYDROXY ESTERS USING AN ACTIVE SITE MODEL*

From the results of the PLE catalyzed hydrolysis of substituted mandelates and lactates presented in Chapter IV (Sections 4.4.4 and 4.4.5), it can be deduced that there is a very subtle balance between reactivity and enantioselectivity of PLE. However, some factors responsible for PLE stereoselectivity may be delineated using Jones' active site model.

The observed stereoselective hydrolysis of α-allyl mandelate (**414a**, E = 30)[14] by PLE-A and PLE-S may be explained as follows (see Figure 5.4): in order to be hydrolyzed, the carboxylic ester group should be located at the serine site of the enzyme. The allyl group will fit comfortably in H_S and interacts well with this pocket. The phenyl group can be positioned in H_L and may interact there with the active site (Fig. 5.4a). In this favoured enzyme-substrate complex where the hydrophobic interactions are maximized, the enzyme catalyzed hydrolysis of compound **414a** should lead to highly enantioselective formation of (S)-**416a** and (R)-**414a**, as observed experimentally. Hydrolysis of the other enantiomer requires in this model that the phenyl group be located in H_S, which is difficult for steric reasons (Fig. 5.4b).

A similar enzyme-substrate complex can account for the high enantioselectivity, found for α-methallyl mandelate (**414b**, E = 25).

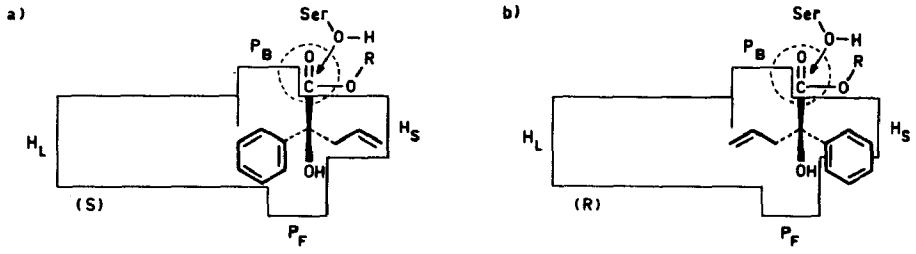


Figure 5.4: Enzyme-substrate complexes for substrate 414a

We observed also a reasonable enantioselectivity for hydrolysis of α-crotyl mandelate (**414c**, E = 13), although lower than for the cases discussed above. This may be explained by assuming that the crotyl substituent can bind in the small hydrophobic pocket (H_S), which will result in hydrolysis of the (S)-enantiomer, but the four carbon atom chain length of this fragment seems to be marginally larger than the size of the small pocket (Figure 5.5) and so there has to be another binding mode of the substrate, leading to the hydrolysis of the other (R)-enantiomer. There is, however, a prevalent binding of the crotyl moiety in the small hydrophobic pocket resulting in a reasonable enantiomeric excess of the (S)-product.

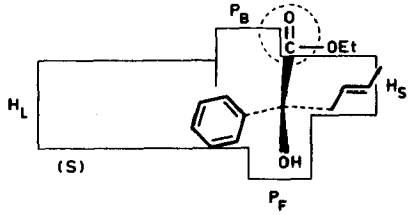


Figure 5.5: Binding orientation of substrate 414c, leading to hydrolysis of the (S)-enantiomer.

These findings help to define the size of the H_S pocket, as there is a significant interaction of an allyl or methallyl group with the active site and the crotyl group seems to be the border line structural moiety that can be accommodated in the small hydrophobic pocket. This is in good agreement with the size of H_S reported by Jones.[12]

In order to rationalize the hydrolysis of the (R)-ester of α-crotyl mandelate (**414c**), one must conclude that as there is no room for the phenyl group in H_S, there has to be some other space within the active site where groups that do not significantly interact with the active site, can be located. We suggest that such groups may extend in the direction of P_F,[15] thereby directing the hydroxyl group of the ester substrate into the polar P_B pocket (Figure 5.6).

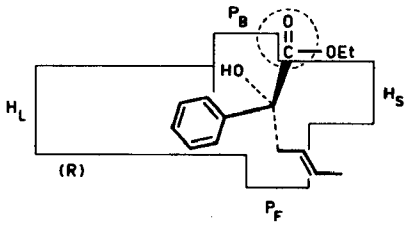


*Figure 5.6: Enzyme-substrate complex for **414c**, leading to hydrolysis of the (R)-enantiomer.*

Extending groups into the P_F binding site will, however, little affect the overall binding of the substrate as there is very low stereoselectivity in hydrolysis of, for example, α-benzyl mandelate (**414e**). H_S cannot contribute in binding this sterically bulky substrate.

For α-methyl mandelate (**414f**, E = 5), H_S may be involved in binding of the substrate as the methyl group can be accommodated in this pocket (Fig. 5.7a). Clearly there is not much interaction of this substituent with the small hydrophobic pocket as reflected in the low enantioselectivity observed in hydrolysis of ester **414f**. In this case the methyl group may be located as well in or above the P_F pocket (Figure 5.7b).

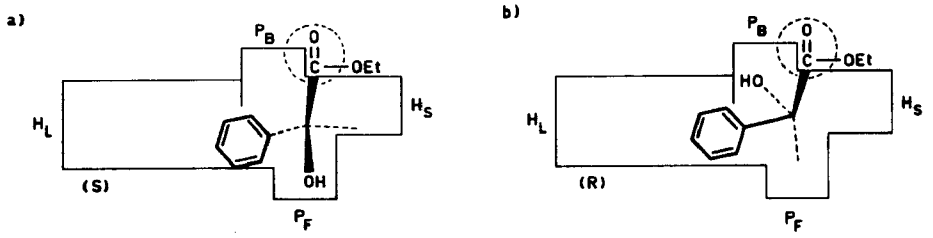


*Figure 5.7: Binding orientations of substrate **414f**.*

A tentative conclusion is that there is a significant interaction of an allyl or a methallyl group within H_S because these moieties in their fit maximize the hydrophobic binding potential.

However, there has to be another effective interaction between a portion of the substrate molecule and the active site in order to control the stereochemical outcome of the hydrolysis. This can be a polar interaction as shown to occur in the stereoselective hydrolysis of diester substrates. For our substrates, however, a polar interaction seems to be less dominant. This can be concluded from the large difference in stereospecificity of PLE for α-allyl- or α-crotyl mandelate (**414a** and **414c**) as compared with α-allyl- or α-crotyl lactate (**415a** and **415b**). As there have to be at least three significant interactions of a substrate molecule with the active site in order to obtain a high preference for a single enzyme-substrate complex, we conclude that a phenyl group seems to interact far better than a methyl group, which may be indicative for a π-interaction from the aromatic group with the active site. It has been suggested that the presence of unsaturation near the chiral center may contribute strongly in the factors that determine the enantioselectivity of PLE in the hydrolysis.[16] Such an interaction indeed may be responsible for the better recognition of the substituted mandelic esters compared to the substituted lactic esters.

Although we observe a small preference (E = 2) for location of the allyl group of α-allyl lactate (**415a**) in the small hydrophobic pocket (resulting in (R)-ester hydrolysis), this interaction alone is not sufficient for a distinct discrimination of possible enzyme-substrate complexes. On the other hand, an aromatic interaction is insufficient also, as we observed, in agreement with Dakin,[17] very low enantioselectivity in PLE catalyzed hydrolysis of ethyl mandelate (E = 1).

The lack of stereoselectivity in PLE catalyzed hydrolysis of α-substituted lactates can be ascribed to the lack of substituents suitable to undergo a significant interaction with the enzyme's active site. This will make the enzyme unable to make a clear difference between the many possible binding modes of these conformationally flexible substrates. Since the enantiomeric ratio for our substrates is greatly influenced by the size of the substituent, one must conclude that the interactions that determine the enantioselectivity of PLE are extremely subtle for compounds with a great degree of conformational flexibility. From these experiments it is also clear that a substrate

with a substituent that will not maximize the hydrophobic binding potential, because it is too large to fit in the pocket or too small to interact well, will give low enantioselectivity in PLE catalyzed hydrolysis.

Although Jones' active site model seems to work well in rationalizing the outcome of PLE catalyzed hydrolysis of mainly diester substrates, it is less adequate to interpret fully the stereoselectivity observed in the hydrolysis of racemic monoesters. Furthermore the enzyme can accept substrates that will not bind effectively in the active site but still can be located near the active hydrolytic region. Some of the hydrolyzed esters (for example silylated α-cinnamyl lactate **415g**) are of a size that these substrates exceed the dimensions of the proposed pockets. This has recently been observed by another group also as the PLE catalyzed hydrolysis of a benzylpenicilloate diester was reported to proceed selectively, although a large portion of this ester substrate, when fit in the active site model of Jones, lies outside the proposed cavities.[18]

Finally, the possibility that the different isozymes, present in PLE, respond differently in hydrolysis of these substrates, cannot completely be ruled out.[19] One could speculate that some isozymes can accept a wide range of substrates but because of their flexibility to hydrolyze a variety of esters with broad structural range, some of their activity to hydrolyze stereoselectively may be lost. In the literature there still is controversy about the way in which the isozymes of PLE behave: in contrast to findings reported by Jones et al.[5], Norin and coworkers[20] stated that different isozymes have different enantioselectivities.

5.5 *CONCLUDING REMARKS*

In summary it is clear that although some aspects of the catalytic active site of PLE have become clear there still is a question of optimal fit for optimal enantioselectivity of hydrolysis. The lactic acid family seems to be characterized by an improper set of substituents about the stereogenic carbon; the compounds are accepted by PLE but the interplay of substituents with the enzyme is too weak, too aselective, to allow good enantiorecognition. On the other hand, the mandelic acid family has members that are hydrolyzed with good to excellent enantioselectivities. However, likely because

of much tighter and more selective interplay of substituents with the enzyme, the risk of total loss of reactivity becomes higher (which may be concluded from the lower reactivity of PLE towards these substrates when compared to most of the substituted lactates).

Such a conclusion seems inherently logical - a "fit" can be too small, about right, or too large. The present work provides some definition of what "too small", "about right", and "too large" may be.

5.6 *REFERENCES*

1. a) Sabbioni, G.; Jones, J.B. *J. Org. Chem.* **1987**, *52*, 4565.
   b) Ohno, M.; Kobayashi, S.; Iimori, T.; Wang, Y.F.; Izawa, T. *J. Am. Chem. Soc.* **1981**, *103*, 2405.
   c) Mohr, P.; Waespe-Sarcevic, N.; Tamm, C.; Gawronska, K.; Gawronski, J.K. *Helv. Chim. Acta* **1983**, *66*, 2501.
   d) Wang, Y.F.; Wong, C.H. *J. Org. Chem.* **1988**, *53*, 3127.
   e) Bjorkling, F.; Boutelje, J.; Gatenbeck, S.; Hult, K.; Norin, T.; Szmulik, P. *Tetrahedron* **1985**, *41*, 1347.
   f) Gais, H.J.; Bulow, G.; Zatorski, A.; Jentsch, M.; Maidonis, P.; Hemmerle, H. *J. Org. Chem.* **1989**, *54*, 5115, and references therein.
2. Barker, D.L.; Jencks, W.P. *Biochem*, **1969**, *8*, 3879.
3. Farb, D.; Jencks, W.P. *Arch. Biochem. Biophys.* **1980**, *203*, 214.
4. a) Junge, W.; Heymann, E. *Eur. J. Biochem.* **1979**, *95*, 519.
   b) Farb, D.; Jencks, W.P. *Arch. Biochem. Biophys.* **1980**, *203*, 227.
5. Lam. L.K.P.; Brown, C.M.; De Jeso, B.; Lym, L.; Toone, E.J.; Jones, J.B. *J. Am. Chem. Soc.* **1988**, *110*, 4409.
6. Fink, A.L. In *Enzyme Mechanisms. 1 Enzymes*; Page, M.I; Williams, A. Eds.; The Royal Society of Chemistry: London, 1987.
7. a) Schrag, J.D.; Li, Y.; Shan, W.; Cygler, M. *Nature* **1991**, *351*, 761.
   b) Brady, L.; Brzozowski, A.M.; Derewenda, Z.S.; Dodson, E.; Dodson, G.; Tolly, S.; Turkenburg, J.P; Christiansen, L.; Huge-Jensen, B.; Norskov, L.; Thim. L.; Menge, U. *Nature* **1990**, *343*, 767.
   c) Winkler, F.K.; D'Arcy, A.; Hunziker, W. *Nature* **1990**, *343*, 771.
8. Ohno, M. In *Enzymes in Organic Synthesis*; Ciba Foundation Symposium 111; Porter, R.; Clark, S., Eds.; Pitman: London, 1985, p. 171.
9. Lam, L.K.P.; Hui, R.A.H.F.; Jones, J.B. *J. Org. Chem.* **1986**, *51*, 2047.
10. Boutelje, J.; Hjalmarsson, M.; Szmulik, P.; Norin, T.; Hult, K. In *Biocatalysis in Organic Media*; Laane, C.; Tramper, J.; Lilly, M.D. Eds.; Elsevier: Amsterdam, 1987.
11. Zemlicka, J.; Craine, L.E.; Heeg, M.J.; Oliver, J.P. *J. Org. Chem.* **1988**, *53*, 937.
12. Toone, E.J.; Werth, M.J.; Jones, J.B. *J. Am. Chem. Soc.* **1990**, *112*, 4946.
13. a) Jencks, W.P. *Adv. Enzymol.*, **1975**, *43*, 219.
    b) Hansch, C. *Acc. Chem. Res.* **1969**, 232.

14. E = enantiomeric ratio, see Section 4.3.
15. Accomodation of hydrophobic moieties in or above P_F has very recently been proposed by Jones too: Toone, E.J.; Jones, J.B. *Tetrahedron: Asymmetry* **1991**, *3*, 207.
16. Nakada, M.; Kobayashi, S.; Ohno, M.; Iwasaki, S.; Okuda, S. *Tetrahedron Lett.* **1988**, *29*, 3951.
17. Dakin C. J. Physiol. **1904**, *30*, 253.
18. Jones, M.; Page, M.I. *J. Chem. Soc., Chem. Commun.* **1991**, 316.
19. Schneider, M.P, University of Wuppertal, Germany, personal communication.
20. Boutelje, J.; Hjalmarsson, M.; Hult, K.; Lindback, M.; Norin, T. *Bioorg. Chem* **1988**, *16*, 364.