Active-Site Model for Interpreting and Predicting the Specificity of Pig Liver Esterase[1]

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Abstract: Pig liver esterase (PLE) is one of the most useful enzymes for the preparation of valuable chiral synthons. However, its applications in asymmetric synthesis have been hampered by its seemingly unpredictable specificity. This disadvantage has now been overcome through the development of a simple and easy-to-use active-site model. The model, which is based on cubic-space descriptors, accounts for the structural selectivity and stereoselectivity of PLE-catalyzed hydrolyses reported so far and is also of predictive value for new substrate structures. The development and specification of the model, and its application in analyzing the specificity of the enzyme toward a representative and structurally diverse range of methyl ester substrates, are described.

Enzymes have now been generally accepted as valuable catalysts in organic synthesis for effecting a wide range of transformations.[2] Hydrolytic enzymes, in particular lipases and esterases, are especially attractive since many of the most useful enzymes, which possess broad substrate specificities and are highly stereoselective in their catalyses, are available commercially at a low cost. Furthermore, hydrolytic enzymes do not require expensive and unstable coenzyme systems. One such hydrolytic enzyme that has received much current interest is pig liver esterase (PLE, E.C. 3.1.1.1).[3,4] The first application of PLE in asymmetric synthesis was reported in 1903,[5] but it is only since the enzyme was reintroduced as a chiral catalyst by Sih,[6] during part of his pioneering studies in the enzyme applications field, that its broad potential for the generation of chirons[7] has been widely exploited. PLE is a serine hydrolase that catalyzes the hydrolysis of a wide range of ester structures with considerable specificity, and numerous stereoselective transformations of prochiral substrates[4] and some resolutions of racemates[3] have been reported.

In order for an enzyme to be applied as widely as possible, it is desirable that the factors controlling its specificity be understood and that they be rational. Unfortunately, in the past, PLE has demonstrated some patterns of stereochemical discrimination that were apparently anomalous and that undermined confidence in its logical utilization in asymmetric synthesis. In particular, PLE exhibited reversals in stereoselectivity, such as changing from *R* center to *S* center ester preference within structurally similar series of substrates that were triggered by apparently trivial changes in substrate size or structure.[8]

This seemingly unpredictable and fickle stereochemical behavior represented a major disadvantage in planning the synthetic uses of PLE and prompted efforts toward the development of an active-site model of the enzyme that would permit all of the enzyme's specificity properties to be interpreted and predicted. Several previous approaches to such a model have been reported. The first was that of Tamm's group,[9] which was based on an attempt to formulate an optimum substrate structure. The second approach, by Ohno,[10] proposed a Y-shaped active site and was directed toward interpreting diester hydrolysis only. Neither of these models attempted to account for the stereoselectivity-reversal phenomenon. This aspect was addressed by Lam et al.,[11] whose proposal incorporated a hydrophobic binding region composed of two pockets, one small and one large. Reversal of stereoselectivity was attributed to small substrates binding into the small site until too big to do so, whereupon binding into the large site would be mandatory, thereby inverting the stereoselectivity. The possibility of a two-pocket active site was recognized independently by Norin and co-workers,[8c] who more recently also proposed a further model to explain the stereoselectivity of PLE.[8d] This latter model advocated a single hydrophobic pocket in which the hydrophobic moieties of substrates would bind until they became too large to do so, after which point no binding of the large groups would occur. More recently, Zemlicka and co-workers proposed a schematic single hydrophobic pocket model that also recognized that the hydrophilic binding needs of ester substrates must be accommodated.[12]

While these models provided an excellent beginning toward rationalizing PLE's specificity, they worked well only for the limited structural range of substrates for which they were designed. A need remained for a comprehensive specification of the active site that was capable of interpreting all aspects of the enzyme's specificity and that would be of predictive value for new structures that were potential PLE substrates. This paper reports an active-site model that meets these criteria and is capable of being

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extended to accommodate new specificity data as they become available.

Approach

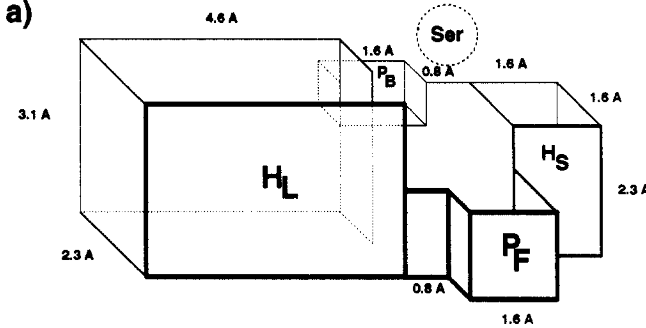
The absence of an X-ray structure of PLE dictated that an empirical approach to model creation be followed. For this first model specification, only stereoselectivity distinctions due to chirality differences in the acyl moieties were considered. The potential complication of alteration of enantiomeric selectivity of PLE in some cases when esters of other alcohol moieties are used[13] was avoided by assessing only methyl ester substrates, for which the achiral methoxyl leaving group is the simplest possible. An additional factor in making this structural restriction was that methyl esters are the most common in preparative applications. Approximately 100 methyl esters with broadly representative structures whose preparative-scale PLE-catalyzed hydrolyses had been reported in the literature were then identified and the results analyzed collectively. First, the active-site location of the catalytically vital serine residue involved in the ester hydrolysis was arbitrarily fixed in an unique location in space. Then, with use of the CHEM-X computer-assisted molecular design package, for each substrate the ester group hydrolyzed was placed in the serine nucleophile region and the remaining parts of the substrate structures were overlaid in order to identify the components that could occupy common volumes. Whether the overlapping moieties were polar or nonpolar was noted, the degree of stereoselectivity was determined experimentally, and the absolute configuration of the product was taken into account. The CHEM-X protocol was supplemented by stick structures such as those created from Framework Molecular Model (FMM) or equivalent kits, which permit easy use of the model at the bench. For such stick structures, a scale of 1 cm = 0.257 Å (1 Å = 3.89 cm, 6 cm = one sp^3–sp^3 C–C bond length of 1.54 Å) is convenient for both the model and substrates. From this analysis, a three-dimensional picture emerged of the spaces available at the active site for accommodating diverse substrate groups, of their sizes, and relative orientations, and of their propensity for polar or hydrophobic groups.

The active-site model that emerged is shown in Figure 1. The model was constructed in cubic-space form for several reasons. First, this approach has been used successfully in the development of active-site models for other enzymes.[14] Second, for an empirically derived model, a simple geometrical representation of the space is easiest to visualize and use. Furthermore, by creating the model in cubic space, any biochemical statement regarding the presence or absence of specific amino acid residues is avoided. Also, there is no temptation to align the bonds of the substrate along the lines of the framework adopted, as is the case for diamond lattice[15] models, for example.

Many compounds that are substrates of PLE possess ring structures that allow axial or equatorial orientations of the hydrolyzed ester group. Tamm's original model suggested that such ester functions were preferentially hydrolyzed when in the equatorial position,[9] and work by Lam et al. confirmed this.[16] Thus, when a substrate is oriented in the model, the ester group to be hydrolyzed is aligned in an orientation corresponding to the equatorial direction in cyclohexane–ester substrates.

The PLE available commercially is a mixture of isozymes. It was initially thought that the stereoselectivity reversals might be attributable to differences in the specificities of these isozymes. However, it has now been demonstrated that all the isozymes act in a more or less equivalent manner and that for preparative purposes PLE can be used as though it were a single species.[17]

a)



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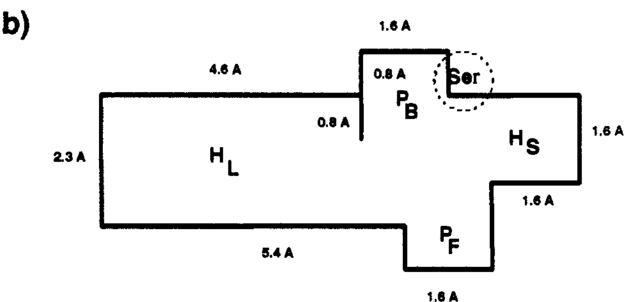

Figure 1. (a) Active-site model of PLE. The boundaries of the model represent the physical constraints placed on the available substrate-binding volume by the amino acid residues of the enzyme. The important binding regions for specificity determinations are two hydrophobic pockets, H_L and H_S, and two pockets of more polar character, P_F and P_B. The best fit, if any, of a substrate is determined by locating the ester group to be hydrolyzed within the serine sphere and then placing, if possible, the remaining substrate moieties in the H and P pockets according to the simple set of rules listed in the text. For working at the bench, stick models with 6 cm for the C–C bond length of 1.54 Å (1 Å = 3.89 cm, 1 cm = 0.257 Å) are convenient for both the model and substrates. In the application of this model, the substrate atoms are considered in point form and neither hydrogen atoms nor van der Waals radii are included. Thus, the actual volumes of the active-site binding regions are somewhat larger than shown. However, since the additions of hydrogen atoms and van der Waals atomic radii add essentially the same volume to both the model and substrate, their inclusion represents an unnecessary complication. Leaving them out makes the model much easier to use. For clarity, only the top perspective of the model shown in b is used in the following model–application examples. In this projection, the restricted "gate" to the H_L pocket, adjacent to the P_B and P_F regions, is clearly seen.

Specification of the Model

The model depicted in Figure 1 is comprised of five binding loci. The boundaries of the binding pockets represent the physical restrictions that amino acids of the enzyme place on substrates binding in the active site, and with the exceptions noted below, substrates may not penetrate them. The catalytically essential region is that encircling the serine residue that initiates hydrolysis by its attack of the carbonyl group of the susceptible ester function. Because dissimilar substrates will orient somewhat differently when bound at the active site, this nucleophilic site is best represented as a zone, rather than in point form. This has been provided for by specifying the serine region as a sphere with a diameter of 1 Å.

The binding regions controlling specificity are composed of two hydrophobic pockets and two others that are more polar in character. The two hydrophobic zones, which interact with the aliphatic or aromatic hydrocarbon portions of a substrate, are designated H_L and H_S (L = large, S = small). The larger of the

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two (H_L) has a volume of approximately 33 Å^3, while the smaller H_S pocket has a volume of roughly 5.5 Å^3. Polar groups such as hydroxy, amino, carbonyl, nitro, etc., are excluded from these areas. The hydrophobic pockets can, however, accommodate less polar heteroatom functions such as halogen and ether or ketal oxygen atoms, if necessary.

The remaining two sectors accept groups that are more polar (P) or hydrophilic. They are located at the front (P_F) and back (P_B) of the active site depicted in Figure 1. P_F's most common role to date has been to bind the second nonhydrolyzed ester function of diester substrates of PLE. However, it can accept nonpolar groups also. The P_B site interacts well with hydrogen-bond donors, and a variety of alcohol, ether, and carbonyl functions can locate comfortably here. Importantly, in contrast to P_F, P_B is too polar to accept hydrophobic moieties. Also, unlike the other binding regions, the rear boundary of the P_B pocket is open and the hydrogen-bonding or similar groups may extend out beyond the back of this region. The area above the model is also open and is completely accessible to any substrate moiety that needs to locate there. Such groups may extend in this direction without restriction.

Application of the Model

The model is used to make predictions of the outcome of PLE-catalyzed hydrolyses by applying the following criteria:

(i) When a substrate is fit in the active-site model, the carbon atom of the carbonyl group of the methyl ester being hydrolyzed must be placed within an sp^3 carbon–oxygen bond length of the serine sphere, so that it may proceed readily to the tetrahedral intermediate required in serine protease-catalyzed hydrolyses.[18]

(ii) The remaining groups of the substrate are then matched with the most appropriate of the other areas of the model, according to the following guidelines:

(a) Hydrophobic portions of the substrate will prefer to bind into one of the two hydrophobic pockets, with the best hydrophobic interaction resulting when the entering species completely fills a hydrophobic pocket.[19] The most important consequence of this concept is that the smaller nonpolar, usually aliphatic, groups will always bind more efficiently into the H_S pocket, unless they are too large to do so. Only then will bulkier, such as aromatic, residues be induced to bind in the larger H_L binding site. This requirement is the basis for the size-induced reversals of stereoselectivity that are observed.

(b) If the substrate is a 1,2, or structurally similar, dimethyl ester, the unhydrolyzed ester group locates in the P_F binding site.

(c) Any functions capable of forming hydrogen bonds with the P_B region will do so, if possible.

For a prochiral substrate, the possible binding modes leading to the individual enantiomeric products are analyzed as outlined above. With racemic methyl esters, the fit of each enantiomer is assessed separately in the same fashion. If it *is not* possible to fit the potential substrate structure into the model according to the above criteria, the compound will not be a substrate for PLE. For structures that are substrates, one or more binding orientations may be possible. The binding mode that, according to the criteria specified above, gives the "best" fit in the model will represent the form preferentially hydrolyzed, and the stereoselectivity will reflect this enzyme–substrate orientation. When multiple binding interactions are involved, they are considered additively. For example, if two different binding modes seem identical with respect to the contributing H_L, H_S, and P_F interactions, but one mode possesses an additional hydrogen bond with the P_B site, the latter will predominate and will control the stereoselectivity outcome of the catalysis. The binding mode with the most energetically favorable overall combination of steric and polar interactions will always be the preferred one.

If one of two binding modes of opposite stereoselectivity is greatly favored over the other, the model predicts that a high level of enantiomeric excess (>75%) will be observed. If one such binding mode is judged to be merely superior to the other, with the less dominant orientation still representing an acceptable fit, the model predicts that the level of enantiomeric excess will be moderate (45–75%). Also, if the binding modes of two competing stereochemical pathways appear essentially equivalent, the model predicts a product of low (0–45%) enantiomeric excess. In the latter situations, the absolute configuration of a product enantiomer deemed to be marginally preferred cannot be predicted with any confidence. At present, this structural and stereochemical forecasting ability does not extend to rate of hydrolysis projections, and the model cannot yet make predictions regarding any kinetic features of substrate hydrolyses.

Application of the Model to PLE Specificity Analyses

The following analyses illustrate how the model is applied to interpret the stereoselectivities of the PLE-catalyzed hydrolyses of a representative range of structurally different methyl ester substrates. Since prochiral substrates are of the greatest asymmetric synthetic interest, they will be discussed first.

1. Prochiral Methyl Diesters. A. Monocyclic Diesters. This group of substrates, in which is observed the most dramatic of the size-induced reversals of stereoselectivity of the enzyme, provided our initial stimulus for the development of an active-site model capable of interpreting PLE specificity. As shown in Table I, PLE catalyzes the hydrolysis of the cyclobutyl diester **1** with *S* center stereoselectivity to give the acid ester **1A**. In contrast, hydrolysis of the cyclohexyl diester **3** gives exclusively the *R* acid product **3A**. The cyclopentyl substrate **2** represents the stereoselectivity-reversal structure, with PLE-catalyzed hydrolysis occurring with almost equal facility at both the *R* and *S* ester centers. These results are in accord with the model's projections, as summarized in Figure 2.

Figure 2a shows dimethyl cyclobutane-1,2-dicarboxylate (**1**) bound into the active site with its *S* center ester in the serine sphere and, thus, being hydrolyzed. In this orientation, the *R* center ester can locate acceptably within the P_F pocket, and the cyclobutyl group is directed into the H_S site, where it fits well. This is clearly a favorable ES complex. The alternative binding mode that would lead to hydrolysis of the *R* center ester of **1** is shown in Figure 2b. In this potential ES complex, the *S* center ester must now locate in the P_F binding site and the cyclobutyl residue is in the H_L pocket. There is certainly plenty of room in H_L for cyclobutyl in this orientation. However, the "Circe effect" criteria[19] in the binding rules specified above require that hydrophobic groups fit into the smaller H_S pocket until they are too large to do so. The binding mode shown in Figure 2b, while allowed, is therefore less favored than that of Figure 2a, and the model predicts hyrolysis via this latter ES complex to give the 2*S* acid product **1A** of high ee, as is observed experimentally.[8a]

Parts c and d of Figure 2 depict the projected binding modes for *R* and *S* center ester hydrolyses, respectively, of dimethyl cyclohexane-1,2-dicarboxylate (**3**). In Figure 2c, the *R* center ester is positioned in the serine sphere and the *S* center ester in the P_F pocket, where it cannot undergo hydrolysis. The hydrophobic cyclohexyl fits into the H_L pocket with considerable room to spare. This substrate orientation leaves empty space in H_L and, thus, does not maximize the hydrophobic binding potential of the site.[19] Nevertheless, it is the favored ES complex that directs the hydrolysis toward the experimentally observed[8a] 2*R* acid **3A** because the Figure 2d alternative binding mode required for 2*S* acid formation is sterically precluded by the inability of H_S to accommodate a group as large as cyclohexyl. Because the choices are so clear-cut, the model again forecasts the high ee level of the 2*R* acid product **3A**.[8a]

Hydrolysis of dimethyl cyclopentane-1,2-dicarboxylate (**2**) is intermediate between the two cases above, giving only a small enantiomeric excess of the 2*R* acid ester **2A**. This low selectivity is observed because the cyclopentane ring is only marginally bigger than H_S, and both *R* and *S* center ester locations in the serine sphere give acceptable active-site fits, as shown in Figure 2 parts e and f, respectively. The fact that H_S is not quite large enough to accept cyclopentyl optimally is offset by the failure of the

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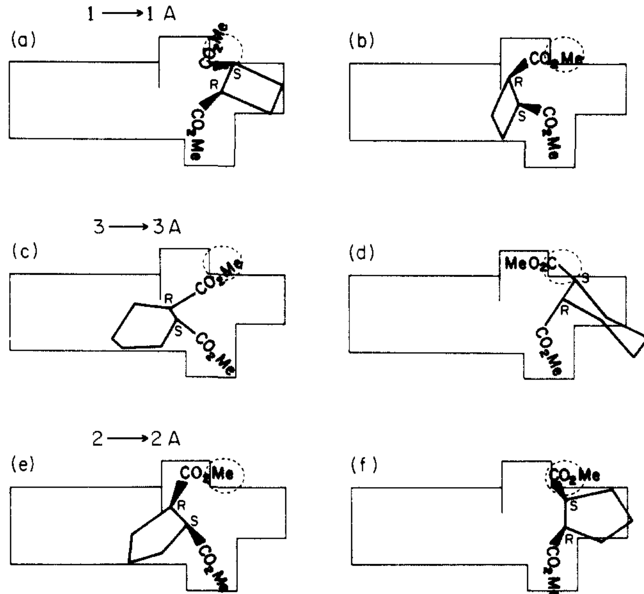

Figure 2. Top perspective view of the active-site model used to illustrate the binding mode selections for diesters **1**–**3**. Hydrolysis of an ester group can only occur when it locates within the spherical locus of the catalytically active serine function. Dimethyl cyclobutane-1,2-dicarboxylate (**1**): The *S* center ester hydrolysis orientation shown in a permits all substrate groups to be accommodated in the active-site binding pockets, with the cyclobutane ring fitting cleanly into, and filling, the H_S pocket. The alternative binding mode required for hydrolysis of the *R* center ester would place a portion of the cyclobutyl ring in the H_L pocket (Figure 2b). Since binding of hydrophobic groups must occur in the H_S rather than the H_L site if sterically possible, substrate binding as in a is preferred, in accord with the observed formation of the 2*S* acid product **1A**. Dimethyl cyclohexane-1,2-dicarboxylate (**3**): The binding depicted in c shows the preferred ES complex for hydrolysis of the *R* center ester to the 2*R* acid **3A**, with the cyclohexane ring bound in the large hydrophobic pocket. Hydrolysis of the *S* ester would require the orientation shown in d. This is clearly precluded since the H_S pocket is clearly too small to accept the six-membered ring. Dimethyl cyclopentane-1,2-dicarboxylate (**2**): In this instance, both *R* and *S* center ester functions can be acceptably located in the serine sphere, as shown in e and f, respectively. The cyclopentyl group is marginally too large for optimum fit into H_S, ensuing in a slight preference for hydrolysis via e that results in a 17% ee of the corresponding 2*R* acid **2A**.

five-membered ring to fill H_L. In such situations, where an unequivocal choice of the preferred binding mode is not possible, the model forecasts that the level of discrimination will be low. For dimethyl 1,2-cyclopentanedicarboxylate, the lack of clear-cut choices is reflected by the 17% ee[8a] of the 2*R* acid product **2A** of the PLE-catalyzed hydrolysis of **2** via slight favoring of the Figure 2e orientation. In effect, this borderline case defines the size of the H_S pocket.

cis-1,3-Cycloalkane diester hydrolyses are analyzed in the same way. This is illustrated in Figure 3 for dimethyl cyclopentanedicarboxylate (**4**). Now, the cycloalkyl moiety can be partly accommodated by the H_S site to give acceptable ES complexes when either the *R* or *S* center ester is in the serine sphere. Thus, hydrolysis via both modes is expected, and found,[20] with the 3*S*

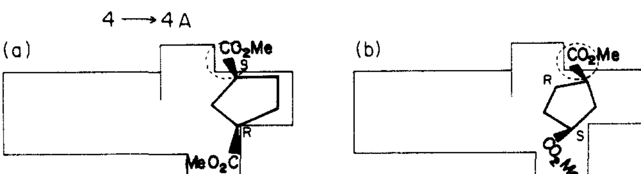

Figure 3. With dimethyl cyclopentane-1,3-dicarboxylate (**4**) as substrate, substrate orientations placing *S* (a) and *R* (b) center esters in the hydrolytic sphere. However, hydrolysis of the *S* ester via the ES complex depicted in a is somewhat preferred because it allows the cyclopentyl ring to extend more fully into the H_S pocket. With two nearly equivalent binding modes, the model predicts a low level of enantiomeric excess. Although in such situations it is not always possible to predict unequivocally the absolute configuration of the preferred product, an excess of the 3*S* acid ester product **4A** corresponding to the better complex fit (a) is observed experimentally.

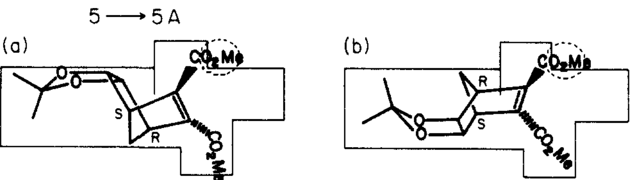

Figure 4. Binding orientations. For PLE-catalyzed hydrolysis of the tricyclic substrate **5**, the binding orientation in a, with the ester adjacent to the *S* center in the serine sphere, does not violate the boundaries of any binding site. It is cearly preferred over that required for hydrolysis of the ester next to the *R* center, depicted in b, for which some adverse interaction with the "gate" to H_L cannot be avoided. The model therefore predicts a high enantiomeric excess of acid **5A** from the ES complex of a, as is observed experimentally (Table I).[21]

acid product **4A** from the Figure 3a orientation being somewhat favored because H_S is more completely filled in this case. The reversal of stereoselectivity that occurs in the PLE-catalyzed hydrolysis[20] of the tetrahydrofuranyl and tetrahydrothiophenyl analogues of **4** is due to the Figure 3b complex becoming preferred due to the positive interaction of the ring–heteroatom with the polar P_B site.

B. Bicyclic Diesters. As these diesters, and their derivatives, are all analyzed similarly, one example only is given. In their syntheses of *C*-nucleoside and related antibiotics, Ohno and coworkers[8b] have exploited PLE-catalyzed hydrolyses of functionalized bridged-bicyclic diesters such as **5** to **5A** of high ee. The ability of the model to interpret the stereoselectivities of these types of substrates is demonstrated in Figure 4.

C. Acyclic Diesters. (i) Substituted Dimethyl Malonates. Predicting the stereoselectivities of enzyme-catalyzed transformations of acyclic substrates is much more difficult than for cyclic derivatives due to the increased conformational mobilities of open-chain compounds. Malonates are good structures for initial evaluations of the viability of the active-site model for acyclic substrates since their three-carbon backbone significantly restricts the number of conformational possibilities that must be considered when the best substrate fit is identified. The stereoselectivity of PLE-catalyzed hydrolysis of a large number of malonate substrates has been documented, with those recorded in Table I being representative. Norin and co-workers reported a size-induced change in stereoselectivity within an homologous series of dimethyl 2-alkyl-2-methylmalonates.[8c] With a small 2-ethyl substituent, as in **6**, the hydrolysis proceeds with pro-*R* ester preference to give acid ester **6A**, but when the larger 2-*n*-heptyl substituent of **7** is present, for pro-*S* ester is cleaved almost exclusively to afford **7A**. The pro-*R* to pro-*S* changeover point in the series occurs between the substrate analogues of **6** and **7** that have 3-*n*-butyl and 3-*n*-pentyl substituents. This corresponds to the limitation, noted previously in the cycloalkane 1,2-diester series, of the inability of H_S to accept a group of greater than four carbon atoms. The full rationalization of these results with the use of the model is summarized in Figure 5.

Keese and his group have also examined malonate hydrolyses, to develop strategies for improving ee levels.[22] They found that

(20) Jones, J. B.; Hinks, R. S.; Hultin, P. G. *Can. J. Chem.* **1985**, *63*, 452.
(21) *exo*- and *endo*-diester bicyclic analogues of **5** are reported to be, at best, very poor PLE substrates,[8b] and we have confirmed this independently for both the carbocyclic and 7-oxa series. Toone, E. J. Ph.D. Thesis, University of Toronto, 1988. Jones, J. B.; Francis, C. F. *Can. J. Chem.* **1984**, *62*, 2578. The cubic-model analyses are in accord with these observations. Toone, E. J. *Ibid.* Guibe-Jampel and co-workers also record such compounds as poor substrates or nonsubstrates but report that methyl diesters in the 7-oxa series undergo slow but stereoselective hydrolysis when abnormally high levels of enzyme are used. Bloch, R.; Guibe-Jampel, E.; Girard, C. *Tetrahedron Lett.* **1985**, *26*, 4087. While reinvestigation of all the substrates of this type would help to clarify the apparent contradictions, in any event the model is not advocated for analysis of hydrolyses that are severely disfavored, since in such cases the combinations of weak enzyme–substrate interactions responsible for stereoselectivity distinctions cannot be identified with any certainty.

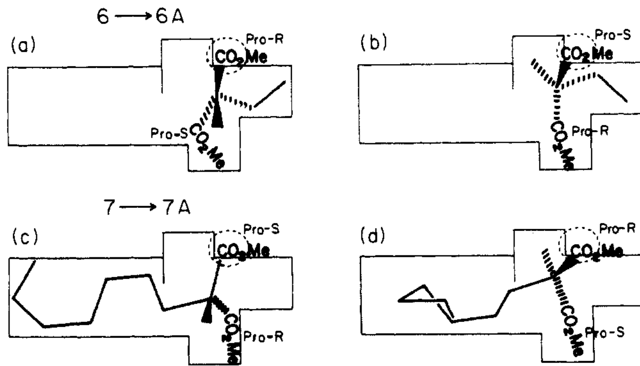

Figure 5. ES complexes. Dimethyl 2-ethyl-2-methylmalonate (6): Because small hydrophobic groups must bind in H_S if they can, the 2-ethyl group must be placed in this pocket. The ES complexes for pro-*R* or pro-*S* ester hydrolysis are then a and b, respectively, for both of which the ester at the other stereocenter can locate acceptably in P_F space. However, the orientation in a enables the 2-methyl function to place itself in the empty space above P_F. As a consequence, a is the only allowed complex since P_B is a polar site that will not tolerate hydrophobic groups, as would be the case in b, where the 2-methyl group would be obliged to be directed into P_B. The latter is, therefore, strongly disfavored, and the formation of the 2*S* acid product **6A** of 73% ee reflects this situation. Dimethyl 2-heptyl-2-methylmalonate (**7**): In this case, the hydrophobic *n*-heptyl chain is too large to bind in the H_S pocket, and binding modes of the a and b types are both impossible. With the heptyl chain required to bind in the H_L pocket, the *ES* complexes leading to pro-*S* and pro-*R* ester hydrolysis, respectively, are depicted in c and d, respectively. In both, the other enantiotopic ester group can locate well in P_F. However, the orientation in c is the operative one as its 2-methyl group can locate in the empty region above P_F, while in d it would have to go into the hostile P_B environment. As a result, a is highly favored, and the product is 2*R* acid ester **7A** of 88% ee.

the low stereoselectivity of PLE-catalyzed hydrolysis of dimethyl 2-(hydroxymethyl)-2-methylmalonate (**8**) could be improved dramatically by protecting the small, polar, hydroxymethyl group with a bulky, hydrophobic, (*tert*-butyldimethylsilyl)oxy function, as in **9** (Table I). Hydrolysis of the latter malonate yields the 2*R* acid product **9A** of 95% ee.[22] This approach works well because, in the case of the small, polar, substrate **8**, several equally favorable ES complexes can be created. The model then predicts little or no selectivity. In contrast, only one preferred orientation can be identified for **9**. The model analyses are given in Figure 6. This series of substrates is of additional significance since it demonstrates that relatively nonpolar heteroatoms such as ether oxygen and silicon can be accommodated in the H_L site.

(ii) 3-Substituted Dimethyl Glutarates. The stereoselectivities of PLE-catalyzed hydrolyses of a broad range of 3-substituted dimethyl glutarates have been reported.[6,8b,9,11,23] The behavior of the gluatarate diesters **10**–**13** illustrates the applicability of the model to this series. Dimethyl 3-hydroxyglutarate (**10**) and dimethyl 3-hydroxy-3-methylgultarate (**11**) are an interesting pair of substrates in that while both possess a polar hydroxyl group at C-3, the addition of the methyl group at C-3 results in the enantiomeric excess of the PLE-derived acid esters being raised from 12% for **10A** to >99% for **11A**, as shown by Tamm and his group.[9] Rationalization of these results by the model is shown in Figure 7.

Ohno and co-workers observed a reversal in stereoselectivity in the hydrolysis of N-acylated 3-aminoglutarates.[8b] As in the case of the cyclic diesters **1**–**3** of Figure 2, this specificity reversal is also size-induced. Interpretation of the specificity reversals within this glutarate series is depicted in Figure 8, with the analyses of the representative *N*-acetyl and *N*-carbobenzyloxy (C bz) substrates **12** and **13**. Hydrolysis of **12** proceeds with pro-*S* selectivity to give predominantly **12A**[8b] due to controlling *N*-acetyl to H_S binding (Figure 8a). In contrast, the larger Cbz group of **13** is obliged to bind in the larger H_L site, thereby placing the pro-*R* ester at the serine locus (Figure 8b) and resulting in the formation of **13A** of high ee. Dimethyl 3-aminoglutarate substrates of the **12** and **13** types with intermediate-size amino-

(22) Luyten, M.; Muller, S.; Herzog, B.; Keese, R. *Helv. Chim. Acta* **1987**, *70*, 1250.

(23) (a) Brooks, D. W.; Palmer, J. T. *Tetrahedron Lett.* **1983**, *24*, 3059. (b) Mohr, P.; Tori, M.; Grossen, P.; Herold, P.; Tamm, C. *Helv. Chim. Acta* **1982**, *65*, 1412.

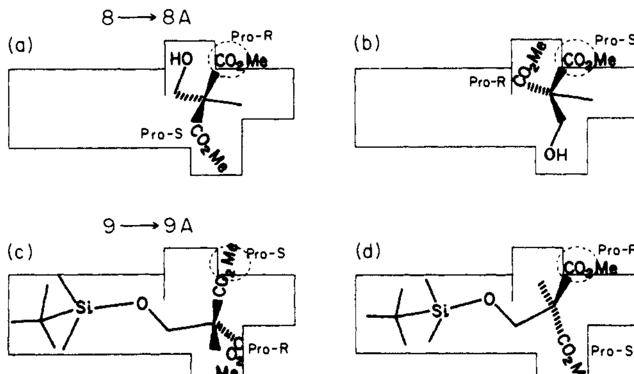

Figure 6. ES Complexes. Dimethyl 2-(hydroxymethyl)-2-methylmalonate (**8**): The small, hydrophobic, 2-methyl group will prefer to be in the H_S pocket. This then allows two acceptable and virtually equivalent ES complexes a and b to be drawn. In a, with the pro-*R* ester adjacent to serine, the other ester and hydroxymethyl are above and in P_B, respectively. For b, the situation is similar, with the pro-*S* carboxymethyl in the serine sphere and the locations of the –COOMe and –CH_2OH functions reversed. With a and b being similarly allowed, racemic or low-ee product acid is forecast, in accord with the 6% ee level observed (Table I). Dimethyl *O*-(*tert*-butyldimethylsilyl)-2-(hydroxymethyl)-2-methylmalonate (**9**): Binding of this substrate, with its large, hydrophobic, C-3 substituent, takes place according to the criteria outlined for substrate **7** in Figure 5. Complex c is thus much preferred over d. The stereoselectivity of the PLE-catalyzed hydrolysis supports this conclusion, with the product acid **9A** of 2*R* configuration being of 95% ee.

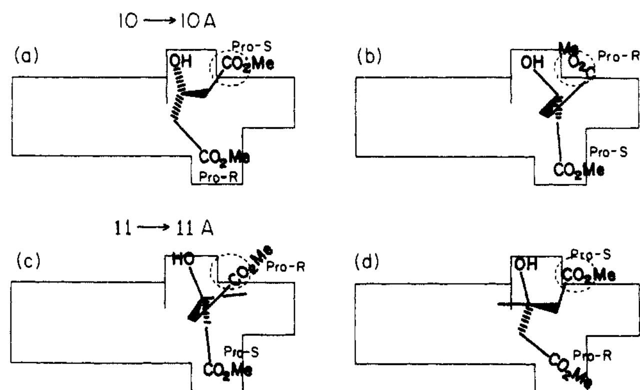

Figure 7. ES complexes. Dimethyl 3-hydroxyglutarate (**10**): For this substrate, hydrolysis of the pro-*R* and pro-*S* ester groups occurs with almost equal facility. For both competing complexes, represented by a and b, the fits are fully allowed and include a hydrogen-bonding interaction between the 3-hydroxyl substituent and the P_B site. Since these binding modes appear virtually equivalent, the model predicts no or low stereoselectivity. The 12% ee of product acid (3*S*)-**10A** is in accord with this analysis. Dimethyl 3-hydroxy-3-methylgultarate (**11**): In this case, the addition of a methyl group at C-3 precludes one of the two binding modes available to **10**. Binding leading to hydrolysis of the pro-*R* ester, as in c, places the methyl group favorably in the H_S site and is thus allowed. In contrast, hydrolysis of the pro-*S* ester would require the C-3 methyl group to penetrate the restriction to the entrance to the H_L pocket, which is prohibited. Any attempt to alleviate this interaction with another substrate orientation disrupts the positive C-3-hydroxyl to P_B interaction and also moves the ester group away from the serine site. Furthermore, even if binding of the methyl group in the H_L pocket could be arranged, it would not take preference over locating it in the H_S pocket, as in c. The pro-*S* ester stereoselectivity of PLE-catalyzed hydrolysis of **11** predicted is observed experimentally, with 3*R* acid ester **11A** of >99% ee isolated.

Table I. PLE-Catalyzed Hydrolysis of Methyl Ester Substrates

no.	substrate	major product	no., (%ee, ref)
1			**1A** (>97%, 8a)
2			**2A** (17%, 8a)
3			**3A** (>97%, 8a)
4			**4A** (34%, 20)
5			**5A** (85%, 8b)
6			**6A** (73%, 8c)
7			**7A** (88%, 8c)
8			**8A** (6%, 21)
9			**9A** (95%, 21)
10			**10A** (12%, 9)
11			**11A** (99%, 9)
12			**12A** (93%, 8b)
13			**13A** (93%, 8b)
14			**14A** (>95%, 23)
15			**15A**, (0%, 24)
16			**16A** (93%, 24)

protecting groups, such as 3-*N*-propanoyl, are hydrolyzed in low enantiomeric excess.[8b] This is due to the fact that, for the *N*-propanoyl substrate, while the four-atom chain C-3 group can fill H_S, some binding in H_L competes successfully. The pro-*S* to pro-*R* reversal in stereoselectivity occurs between the 3-*N*-butanoyl and -pentanoyl substrates.[8b]

Great care must be taken in analyzing acyclic substrates, such as glutarate diesters, because of the number of conformational possibilities whose binding at the active site must be evaluated. When no unambiguous distinctions can be perceived in the binding of two or more conformations of opposite stereochemical directions, unequivocal predictions cannot be made. In such cases, the model indicates that low stereoselectivity should be anticipated. For example, for C-3-substituted glutarate diesters, it is noteworthy that high stereoselectivity is unusual unless the substrate's conformational flexibility is reduced, such as by interaction of a polar C-3 function with P_B as in the case of **12** and **13** (Figure 8).[8b,23]

2. Racemic Methyl Esters. Pig liver esterase has been widely explored, but with mixed success, in racemate ester resolution.[3] It seems to exert its greatest degree of enantiomeric selectivity in its catalysis of the hydrolysis of acetate esters of chiral alcohols, the stereoselectivity interpretations of which are outside the scope of the present model. In contrast, with racemic methyl esters of chiral acids as substrates, few examples are recorded in which PLE manifests complete enantiomer discrimination. However, the behavior of PLE toward esters of racemic chiral acids is fully rationalized by the model. For low to moderate ee partial resolutions, binding of both enantiomers is competitive to a greater or lesser degree. Accordingly, in this paper the illustrations of the ability of the model to interpret the enantiomeric specificity of PLE have been restricted to the representative examples recorded in Table I for which PLE-catalyzed resolutions are effective. Tamm and co-workers subjected dimethyl *trans*-3,4-epoxyadipate ((±)-**14**) to PLE-catalyzed resolution and obtained

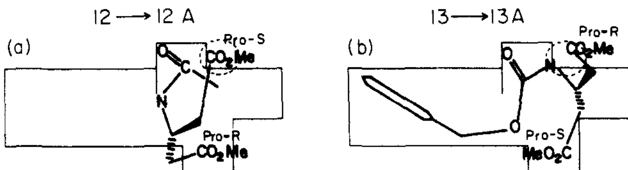

Figure 8. (a) Dimethyl 3-(*N*-acetylamino)glutarate (**12**). Only the favored binding orientation is shown, with the methyl group of the *N*-acetyl function in H_S. With this controlling interaction in place, only the pro-*S* ester can locate in the serine region, leading to the 3*R* acid ester **12A** of 93% ee. The placement of the proester in the serine sphere would require the less favored positioning of the small *N*-acetylmethyl in H_L. (b) Dimethyl 3-[*N*-(carbobenzyloxy)amino]glutarate (**13**). In this case, the only binding possible of the large Cbz-protecting group is in H_L. This causes a reversal of stereoselectivity from that in the hydrolysis of **12** as above since it dictates that a favorable ES complex can only form with the pro-*R* ester at the serine site. Enantiomerically pure 3*S* acid ester **13A** results. It should be noted that, for both substrates **12** and **13**, the amide–carbonyl portion of the C-3 function interacts in a hydrogen-bonding manner with the polar P_B site. This helps to restrict the conformational mobility of these acyclic substrates.

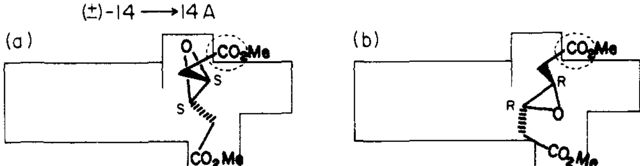

Figure 9. Resolution of racemic dimethyl *trans*-3,4-epoxyadipate ((±)-**14**) by favored hydrolysis of an ester group of (3*S*,4*S*)-**14** via ES complex a in which a lone pair of the oxirane oxygen interacts with the polar P_B site. This positive oxirane–P_B association is not possible when an *R* center ester is placed at the serine locus because the ring oxygen is then oriented away from P_B as shown in b. The ≥95% ee of the product acid ester **14A** and recovered diester (2*R*,3*R*)-**14** are as forecast by this picture.

the 3*S*,4*S* acid ester **14A** of ≥95% ee.[3a,24] The recovered diester (3*R*,4*R*)-**14** was also of ≥95% ee. These results are as foreseen by the model, as shown in Figure 9.

Prompted by the paucity of routes to stereoisomerically pure allenes, previous work in our laboratories had explored the enantiomeric specificity of PLE toward a series of racemic allenic methyl esters.[25] Although such structures are unusual PLE substrates in that their stereogenic centers are not tetrahedral, the model handles them without difficulty. The model analyses of the differences in enantiomeric seletivity of PLE toward the enantiomers of the di- and tetrasubstituted allenic esters (±)-**15** and (±)-**16** are summarized in Figure 10.

Summary and Conclusions

The model as presently formulated was based on analyses of the specificity of PLE toward over 100 structurally varied methyl

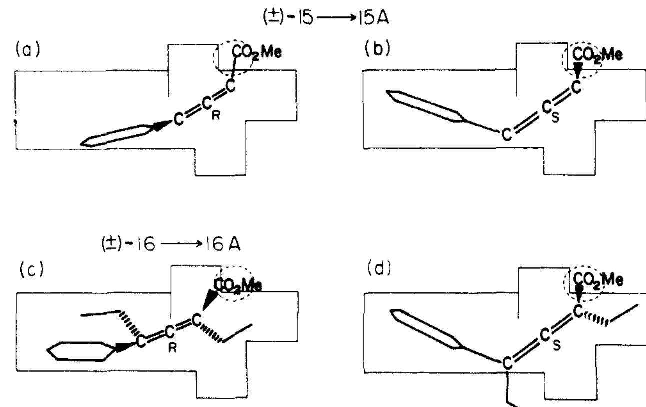

Figure 10. Methyl 4-phenylbuta-2,3-dienoate ((±)-**15**). Both enantiomers of this substrate can bind equally effectively with both the *R* stereoisomer of a and the *S* enantiomer of b giving ES complexes in which the substrate can locate in fully allowed space within the pockets and with the large hydrophobic moiety in H_L. The model, therefore, predicts little or no enantiomeric selectivity, and the acid **15A** and recovered ester **15** products of the PLE-catalyzed hydrolysis of (±)-**15** are in fact racemic. Methyl 5-phenylhepta-3,4-dienoate ((±)-**16**). With this substrate, when the ester group is placed in the serine sphere, only the *R* enantiomer can bind to give a fully allowed fit, as represented in c, with the 2-ethyl group directed toward H_S and the 4-ethyl group and phenyl group in H_L. For the other enantiomer, (*S*)-**16**, with the ester group in the prerequisite for hydrolysis serine location, the 2-ethyl group is still accommodated by H_S as shown in d. However, in this case, no orientation can be found that avoids penetration of the boundary of the H_L pocket by the 4-ethyl function. ES complexes of the d type are therefore precluded. The 93% ee of the product acid (*R*)-**16A** reflects the dominance of PLE-catalyzed hydrolysis via complex c.

ester substrates. It can be used with confidence for a very broad range of methyl ester substrate structures. It is also of predictive value and, as such, adds significantly to the potential of PLE when new asymmetric synthetic applications are being considered. As additional specificity data appear, it will be necessary to fine tune the model's specifications, particularly with respect to the maximum dimensions of the H_L, P_F, and P_B pockets. So far, these have been created with the minimum space needed to accommodate the substrate–structure base employed. Experiments designed to delineate completely the pocket sizes and properties are now in progress. As new results emerge, from our own and other laboratories, the sizes and shapes of the pockets will be refined as necessary on a continuing basis, with the exception of H_S. Because the constraints on this site have been so well established by the stereoselectivity reversal studies, the Figure 1 specification of H_S is considered to be precise.

Acknowledgment. Support from the Natural Sciences and Engineering Research Council of Canada and the award (to E.J.T.) of University of Toronto Open Scholarships are gratefully acknowledged. We also thank Louis Provencher for his assistance in creating Figure 1.

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(25) Ramaswamy, S.; Hui, R. A. H. F.; Jones, J. B. *J. Chem. Soc., Chem. Commun.* **1986**, 1545.