Enzyme-assisted Preparation of Pure Alkanedicarboxylic Acid Monoesters: Chain-length Dependence of Porcine Liver Esterase (PLE)-catalysed Hydrolyses

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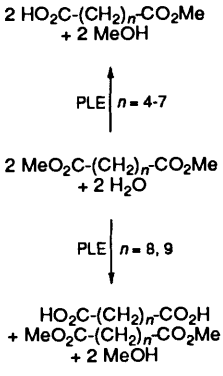
Porcine liver esterase (PLE)-catalysed hydrolyses of alkanedicarboxylic esters $MeO_2C-(CH_2)_n-CO_2Me$ lead exclusively to pure monoesters if $n \leq 7$. Surprisingly, the yields are dramatically dependent on the chain length and drop sharply to zero for $n \geq 8$. Using the active site model for PLE an interpretation of these surprising observations is attempted.

Frequently, in synthetic organic protocols, monoesters of alkanedicarboxylic acids are used as starting materials for further transformations,[1] *e.g.* dimerisations by Kolbe synthesis.[2,3] The preparation of such molecules is often a challenging task, in particular if the corresponding cyclic anhydrides are not readily available. Hydrolyses of the corresponding dicarboxylic esters under classical chemical conditions using stoichiometric amounts of base notoriously result in mixtures of diacids, monoesters and unchanged diesters, which have to be separated. Consequently, these chemical routes are only of limited synthetic value.

In contrast, it is well known that porcine liver esterase (PLE)-catalysed hydrolyses of certain 1,2-, 1,3-, 1,4- and 1,5-dicarboxylic acid esters lead almost exclusively to the corresponding monoesters.[4-6] It was of interest, therefore, to discover whether these reactions could also be used for the preparation of monoesters derived from longer chain alkanedicarboxylic acids on a synthetically useful scale. We, therefore, decided to study systematically the PLE-catalysed hydrolyses of a series of such dicarboxylic acid esters (Scheme 1, n = 4–9).



Scheme 1

In typical experiments, 15 mmol of the corresponding dicarboxylic acid ester was suspended in phosphate buffer (pH 7.0, 0.05 mol dm^{-3}; 20 cm^3). With vigorous stirring 150 mm^3 of PLE suspension † was added while the pH was kept constant at pH 7 by continuous addition of 1 mol dm^{-3} aqueous NaOH from an autoburette. After consumption of 1 equiv. of NaOH the reaction mixtures were filtered through Celite and unchanged starting material removed by extraction with ether. After acidification of the aqueous phases with HCl to pH 2 the reaction products of the enzymatic hydrolyses were isolated again by extraction with ether. The obtained yields of products and their proportions are summarized in Table 1.

† Pig Liver Esterase from Boehringer Mannheim (Art.-Nr.: 104698), Suspension in 3.2 mol dm^{-3} aqueous NH_4SO_4, content: 399 mg ml^{-1}, specific activity: 176 U mg^{-1} (25 °C, ethyl butyrate as substrate).

Table 1 Enzymatic hydrolyses of alkanedicarboxylic acid esters in the presence of PLE. Products and their proportions after hydrolysis of one ester equivalent

	Yield (%)[a]		
n	diester	monoester	diacid
4		96	
5		97	
6		98	
7		97	
8	49		49
9	48		49

[a] Isolated, prior to distillation.

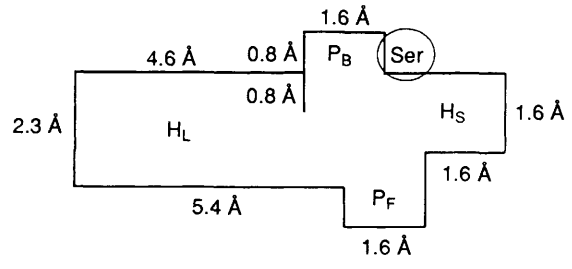


Fig. 1 Top view of the three-dimensional active site model proposed for PLE by J. B. Jones.[5] H_L = large hydrophobic binding pocket, H_S = small hydrophobic binding pocket, P_F = more hydrophilic binding pocket in the front, P_B = strong hydrophilic binding pocket in the back; Ser = Serine of the catalyst.

Obviously, the selective production of monoesters is strongly dependent on the chain length of the dicarboxylic acid esters employed. While for diesters up to n = 7 (azelaic acid dimethyl ester) monoesters are produced exclusively, their yields essentially drop to zero for n = 8 (sebacic acid dimethyl ester). Most surprising is the dramatic change of behaviour in going from azelaic acid dimethyl ester to sebacic acid dimethyl ester, two molecules which are different by only one methylene group. An explanation for the surprising observations can be attempted using the active site model proposed for PLE by J. B. Jones[5] (Fig. 1).

Using a scaled three-dimensional representation of this active site model and of the dicarboxylic acid esters one may envisage that for these molecules with $n \geq 8$ one carboxylic acid ester function would be positioned in the vicinity of the chemical operator serine, while the other ester function is comfortably docked at the more polar pocket P_F. The hydrocarbon chain should always occupy the large hydrophobic pocket H_L (Fig. 2).

After hydrolysis of the first ester function, the thus produced carboxylate anion could position itself at the polar pocket P_B,

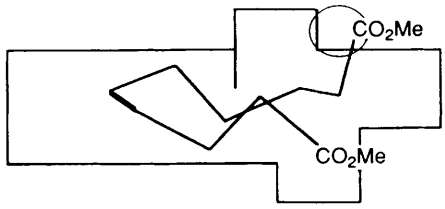


Fig. 2 Binding orientation of MeO_2C–$(CH_2)_8$–CO_2Me in the active site model (top view)

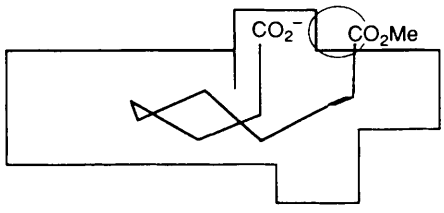


Fig. 3 Binding orientation of MeO_2C–$(CH_2)_8$–CO_2^- in the active site model (top view)

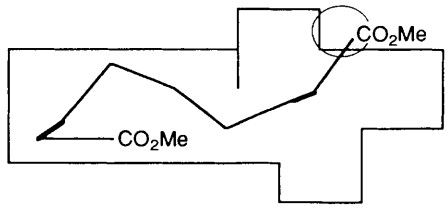


Fig. 4 Binding orientation of MeO_2C–$(CH_2)_7$–CO_2Me in the active site model (top view)

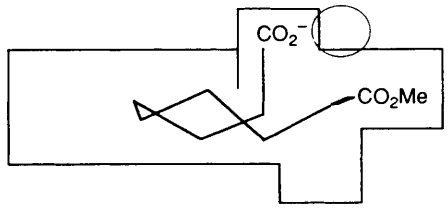


Fig. 5 Binding orientation of MeO_2C–$(CH_2)_7$–CO_2^- in the active site model (top view)

while the rest of the molecule could easily refold to position the second ester function again at the serine moiety (Fig. 3).

This may also explain the somewhat surprising observation that not even a trace of monoester can be isolated from these reactions. It is tempting, therefore, to assume that these molecules never leave the binding site before complete hydrolysis has occurred.

While for diesters with $n \leq 7$ one carboxylic ester function can be positioned at the serine moiety for hydrolysis (Fig. 4), the thus produced carboxylate anion, if located at the polar pocket P_B, due to the shorter chain length, does not allow an orientation of the second ester group at the serine site (Fig. 5). Consequently, the produced monoester is not accepted as substrate by the enzyme and is released into the medium.

In an attempt to verify these explanations experimentally we studied the PLE-catalysed hydrolysis of the two 'borderline'-monoesters derived from azelaic acid ($n = 7$) and sebacic acid ($n = 8$) in comparison with the corresponding diesters. For this, 15 mmol each of chemically prepared sebacic acid monomethyl ester,[7] enzymatically prepared azelaic acid monomethyl ester (see above) and the corresponding diesters were hydrolysed in presence of PLE using the experimental procedure described above. From the initial rates of transformation the specific enzyme activities were determined in all cases.

The results clearly support our tentative interpretation. While, as expected, the specific activity observed with the monoester of azelaic acid (1.0 U mg^{-1} PLE) is about one order of magnitude smaller than for the corresponding diester (10.4 U mg PLE), the respective rates for the monoester of sebacic acid (4.2 U mg^{-1} PLE) and of the diester (4.4 U mg^{-1} PLE) are comparable. Thus, the monoester of azelaic acid clearly is a much less preferred substrate for the enzyme for the reasons given above and will, therefore, accumulate as the major product during the PLE-catalysed hydrolysis of the diester.

As a consequence of the results described above, for $n \leq 7$ synthetically useful quantities of pure monoesters can be obtained in high isolated yields as demonstrated in the preparative procedure described below.

Suberic acid dimethyl ester ($n = 6$) (113.3 g, 0.56 mol) was suspended in phosphate buffer (pH 7.0, 0.05 mol dm^{-3}; 560 cm^3). With vigorous stirring a PLE suspension (5.6 cm^3) was added while the pH was kept constant at pH 7 by continuous addition of 1 mol dm^{-3} aqueous NaOH from an autoburette. After consumption of 560 cm^3 of NaOH (6 h) the reaction mixture was filtered through Celite. The filtrate was washed with ether (110 cm^3). The buffer layer was acidified to pH 2 by addition of half concentrated HCl and extracted with ether (3 × 170 cm^3). The combined ether layers were dried ($MgSO_4$) and evaporated under reduced pressure. The residue was purified by vacuum distillation, using a Vigreux column (30 cm long) to yield suberic acid monomethyl ester (91.4 g, 0.486 mol, 87%), b.p. 135 °C at 0.02 mbar.

Acknowledgements

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