Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 305–307

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Active Site Geometry of Porcine Pancreatic Lipase: An Interesting Switchover from Jones' to Seebach's Model

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Received 25 August 2000; accepted 10 November 2000

Abstract—Porcine pancreatic lipase (PPL)-catalyzed hydrolysis of *cis* 3-acetoxyethyl-1,4-diphenyl and *cis* 3-acetoxyethyl-1-phenyl-4-(2-furyl) β-lactams **1a** and **1b** proceeded with opposite stereoselectivity while the corresponding thienyl β-lactam **1c** showed no selectivity at all. An explanation based upon the dominance of hydrophobic and polar pockets in governing the stereoselectivity has been proposed.

The active site geometry of porcine pancreatic lipase (PPL), one of the widely used[1] hydrolytic enzyme, is still a matter of controversy. Two different models, one proposed by Seebach[2] and the other by Jones[3] are currently in use to explain the stereochemical outcome of hydrolysis. Although both the models recognize the existence of same four types of pockets, the two stereogenic active sites are enantiomeric (Fig. 1). This implies that substrates following Jones' model will generate products that will have a heterochiral relationship with those produced from substrates binding according to Seebach's model. While studying the hydrolysis of a series of 3-acetoxymethyl 4-substituted β-lactams, we had earlier[4] reported that the *cis* isomers underwent hydrolysis according to Jones' model while the *trans* compounds followed Seebach's model. We had proposed that this type of ambiguity could be explained if the hydrophobic and the polar pockets, are assumed to be the dominant factors in the binding of the substrate, while the catalytic site is somewhat flexible so that it can accept an α or a β acetate. In this paper we provide an interesting example where changing the substituent from a phenyl to a furyl in 3-acetoxyethyl 4-substituted β-lactams caused a shift in the preferential binding mode from Jones' to Seebach's model during PPL-catalyzed hydrolysis. A probable explanation has also been put forward that reinforces the earlier proposition about the chiral nature of the hydrophobic pocket, its dominant role in binding along with the polar pocket and the flexibility of the catalytic site.

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The 3-acetoxyethyl β-lactams **1a**–**1c** were prepared via the modified Kinugasa reaction[5] involving cycloaddition between the nitrones **5a**–**5c** and 3-butyn-1-ol followed by acetylation (Ac_2O/Py). The *cis* relationship between the C-3 and C-4 substituents was established from the coupling constant ($J = 5.5$ Hz) between the hydrogens in those positions.[6] The acetates **1a** and **1b** were then subjected to the PPL-catalyzed hydrolytic conditions. The hydrolysis was allowed to progress up to about 50% (checked by ^{1}H NMR) and the hydrolyzed product and the unreacted acetate were isolated by column chromatography. Chiral shift experiments revealed that the hydrolysis proceeded with moderate enantioselectivity. The enantiomeric excess for the 4-phenyl β-lactam **2a** was 50%. For the 4-furyl β-lactam **2b** the *ee* decreased to about 35%. Surprisingly the sign of specific rotation for the two alcohols obtained after hydrolysis was opposite. Thus it appeared that the two products have opposite configuration (heterochiral) with the β-lactam **2a** having 3*R*,4*R*-configuration while the corresponding furyl β-lactam **3b** has been assigned the 3*S*,4*S*-geometry. Final confirmation regarding the absolute configurations came from the CD spectroscopic measurements[7] (Fig. 2). The positive Cotton effect shown by **2a** is in agreement with its 3*R*,4*R*-configuration while the furyl β-lactam **2b** showed negative Cotton effect thus confirming its 3*S*,4*S*-configuration (the projections in different octants has been shown in Fig. 3). The contribution of the strongly chromophoric phenyl or the furyl group towards the Cotton effect far exceeds that of the hydroxy-ethyl group. From the absolute configurations of the product alcohols, it is clear that the preferential mode of binding for the acetate **1a** with a 4-phenyl

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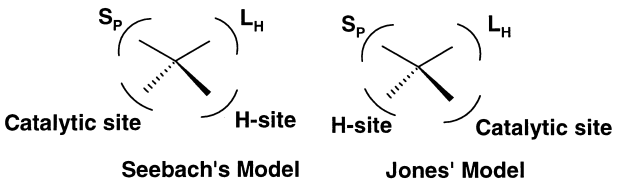
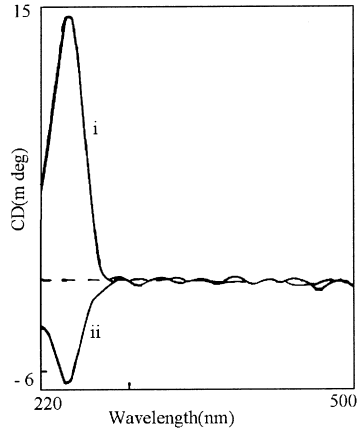


Figure 1.



i for 2a
ii for 2b

Figure 2.

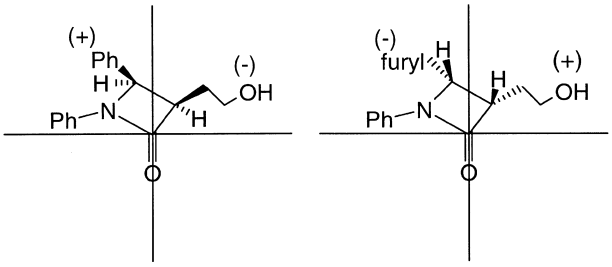


Figure 3.

substituent was in accordance with Jones' model thus leading to a 3*R*,4*R*-alcohol while the Seebach model was the preferred mode of binding for the acetate **1b** with a 4-furyl group. Based on our earlier proposition[4] that the hydrophobic pocket recognizes only a β-substituent, in the case of the 4-phenyl β-lactam, the strongly hydrophobic β-phenyl group occupies the large hydrophobic pocket while the carbonyl binds to the polar pocket. The β-acetoxyethyl group now sits in the catalytic site (Jones' model) and thus undergoes hydrolysis (Fig. 4). In the case of the furyl analogue, the absolute configuration of the product indicates that the preferred mode of binding is the one that puts the 4-furyl substituent α to the L_H pocket thus weakening the stabilizing interaction. In this case it is probable that the *N*-phenyl group because of the partial pyramidal character[8] of the β-lactam nitrogen would assume a predominantly *trans* relation with the furyl substituent at C-4 and hence take part in binding when it is β (Fig. 5). The β-lactam carbonyl suitably places itself in the polar pocket and the α-acetoxyethyl undergoes hydrolysis in the catalytic-site

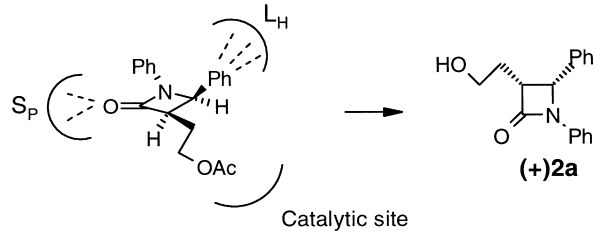


Figure 4. Binding according to Jones' model.

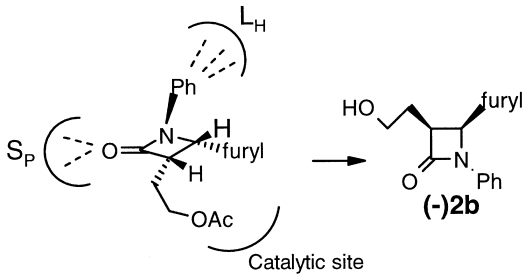


Figure 5. Binding according to Seebach's model.

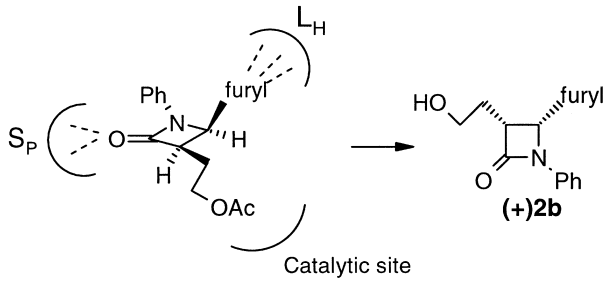
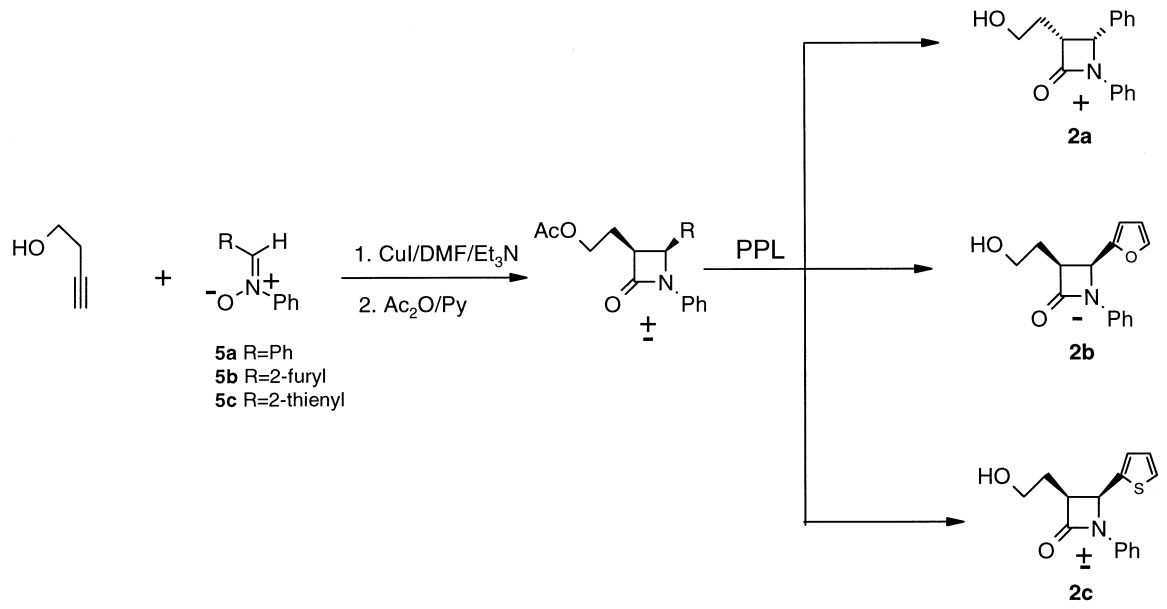


Figure 6. Binding according to Jones' model.

(according to Seebach's model) that may be regarded as having sufficient flexibility to also hydrolyze an α-acetate. The alternate binding mode shown in Figure 6, with a β-furyl interacting with the L_H pocket, obviously competes with the previous one, thus minimizing the *ee* to a significant extent as compared to the case of 4-phenyl β-lactam (Scheme 1).

Judging from the relative hydrophobicities [9] of furan (DM 0.71 D) and thiophene (DM 0.52 D) it can be predicted that in the PPL-catalyzed hydrolysis of 3-acetoxyethyl-4-thienyl β-lactam **1c**, the two possible modes of binding, one in which the β-thienyl interacting with the L_H (Fig. 7) and in the other (Fig. 8) it is the β-*N*-phenyl, may compete favourably with one another. This should lower the *ee* to a greater extent as compared to the furyl β-lactam. This prediction indeed turned out to be true. As a matter of fact, the hydrolysis of the thienyl β-lactam **1c** proceeded with no selectivity at all! The above results enabled us to propose the following relative order of hydrophobic interaction with the L_H pocket in the case of hydrolysis with β-lactam substrates by PPL: 4-β-phenyl > *N*-β-phenyl ≈ 4-β-thienyl > 4-β-furyl. Thus our studies emphasized the importance of L_H and polar pockets in the active site of PPL. The



Scheme 1.

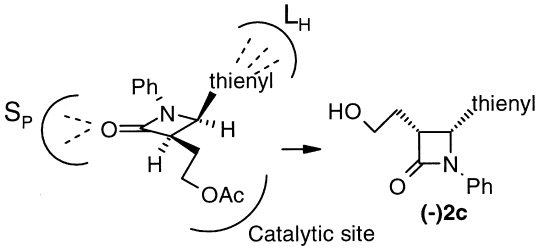


Figure 7. Binding according to Jones' model.

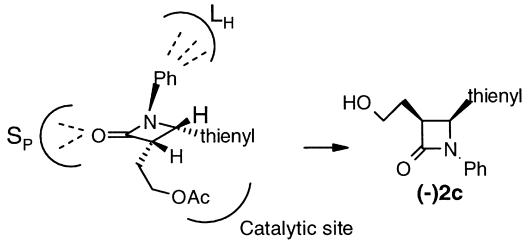


Figure 8. Binding according to Seebach's model.

proposition that the L_H pocket recognizes only a β-hydrophobic group, at least in the case of binding with β-lactams, needs to be validated by studying the hydrolysis of a larger number of substrates. Current studies are aimed towards that end.

Acknowledgements

The author (A.B.) wished to thank Department of Biotechnology, Government of India for funding the project.

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