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STEREOCHEMICAL APPROACH FOR ENZYME INHIBITOR DESIGN

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Abstract : Inhibitors of α-chymotrypsin have been designed on the basis of the recently proposed three dimensional active site model of the enzyme. Thus, (2S, 3R)-2-benzyl-3,4-epoxybutanoic acid (BEBA) methyl ester inactivated the enzyme irreversibly, while (2S, 3S)-BEBA methyl ester inhibited the enzyme competitively. The other two stereoisomers are substrates for the enzyme, forming respective BEBA upon the treatment with the enzyme.

Enzyme inhibitors are important not only as therapeutic agents but also as tools for studying catalytic mechanism and active site structure of enzymes, and are receiving ever increasing attention[1]. Numerous rational designing approaches for enzyme inhibitors have been developed over the years, generating various types of inhibitors which may be classified into the following four categories[2]: substrate analog inhibitors (antimetabolites),[2a,3] affinity labels (active site directed irreversible inhibitors)[4], transition state analog inhibitors,[4a,5] and mechanism-based inactivators.[4a,6] In this communication we wish to report on a novel design approach which differs conceptually from the existing methods. The present approach makes use of the stereospecificity property of the enzyme as the primary ground for the inhibitor design.

Active sites of enzymes are three dimensional entities having binding and catalytic groups with unique and characteristic spatial arrangements. Recently, a three dimensional schematic representation for the active site of α-chymotrypsin has been proposed.[7] In examining the active site model, it occurred to us that esters of 2-benzyl-3,4-epoxybutanoic acid (BEBA) having S-stereochemistry at the 2-position would bind the active site in a nonproductive fashion, placing the oxirane ring at the catalytic site (Figure 1). It was further thought that if such a mode of binding takes, then there may occur a nucleophilic ring cleavage of the oxirane by the attack of the

hydroxyl of Ser^{195}, leading to an irreversible inhibition of the enzyme with a covalent modification (Figure 1). Indeed, this has been the case with methyl ester of (2S, 3R)-BEBA.

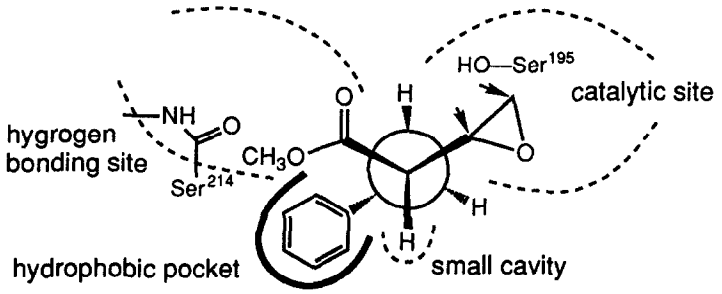

Figure 1. The diastereomers of (2S)-BEBA methyl ester bind the active site of α-chymotrypsin in a nonproductive fashion.

All four possible stereoisomers of BEBA methyl ester were synthesized conveniently by combined usages of an enzymic resolution and chemical transformations starting with vinyl acetic acid. The starting material was converted into 2-vinyl-3-phenylpropanoic acid methyl ester by the benzylation in the presence of 2 molar equivalent amount of LDA, followed by an acid catalyzed esterification with methanol. The racemic methyl ester thus obtained was treated with α-chymotrypsin in an aqueous solution of pH 7.8 to hydrolyze selectively the R-isomer (90% ee), leaving its enantiomer intact. This acid was converted back to the methyl ester, and subjected to epoxidation using m-chloroperoxybenzoic acid in methylene chloride at room temperature to give a mixture of methyl esters of (2R, 3S)- and (2R, 3R)-BEBA, which was readily separated and purified by a silica gel column chromatography. Similarly, there were obtained (2S, 3R)- and (2S, 3S)-BEBA methyl ester from (S)-2-vinyl-3-phenylpropanoic acid methyl ester obtained from the enzymic resolution. The stereochemical assignments of these products were confirmed by an independent asymmetric synthesis starting with optically pure L-malic acid.[8]

Kinetic studies made with these BEBA methyl esters by a literature method,[9] indeed, showed that the two diastereomers of BEBA methyl ester having S-configuration at the 2-position possess an inhibitory activity against α-chymotrypsin. On the other hand, the other two stereomers having 2R configuration behaved expectedly as substrates for the enzyme, giving BEBAs of respective stereochemistry upon the treatment with α-chymotrypsin. These BEBA products were characterized by comparing them with authentic samples. Further examination of the inhibitory action revealed that (2S, 3S)-BEBA methyl ester is a competitive inhibitor having a K_i value of 9.95 mM when analyzed by the plots of Lineweaver-Burk and Dixon, while (2S, 3R)-BEBA methyl ester showed a time dependent inactivation for α-chymotrypsin (Figure 2), suggesting that

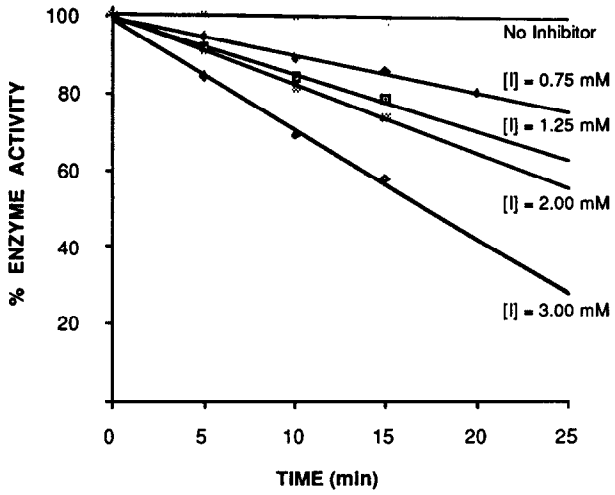

Figure 2. Plot that demonstrates a time–dependent inactivation of α-chymotrypsin by (2S, 3R)-BEBA methyl ester. The buffer solution (0.04 M Tris, pH 7.8, 0.05 M in $CaCl_2$) of α-chymotrypsin and (2S, 3R)-BEBA methyl ester (8.28×10^{-7} M α-chymotrypsin for any given (2S, 3R)-BEBA methyl ester) was incubated at room temperature for 3 min, then at 5 min intervals 100 μl samples were removed and added to 1900 μl of the assay mixture (2.5×10^{-5} M, Suc-AAPF-*p*-NA). Absorbances at 400 nm were measured immediately.

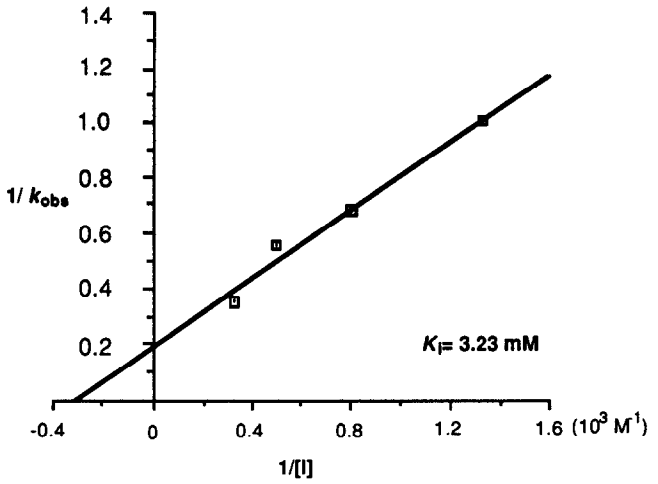

Figure 3. Double reciprocal plot of the observed first-order α-chymotrypsin inactivation rate constants *vs* concentrations of (2S, 3R)-BEBA methyl ester, [I].

there occurs a covalent modification. K_i and k_{inact} for the inactivation were determined as described by Kitz and Wilson[10] using the simplified kinetic scheme of equation (1) to give the respective value of 3.23 mM and 5.24 min^{-1} (Figure 3).

$$E + I \overset{K_i}{\rightleftharpoons} E \cdot I \xrightarrow{k_{inact}} E{-}I \qquad (1)$$

The irreversible inhibition becomes slower by increasing the concentrations of the substrate, indicating that the active site is involved in the inhibition. The covalent modification was further supported by the dialysis experiment (24h) of the incubation mixture of α-chymotrypsin and (2S, 3R)-BEBA methyl ester, which showed no return of the enzymic activity. The plotting of the logarithm of the observed inactivation rate constants of α-chymotrypsin *vs* the logarithm of concentrations of (2S, 3R)-BEBA methyl ester by the method of Levy et al.[11] gave a slope of 0.8, which suggests the binding stoichiometry to be 1:1.

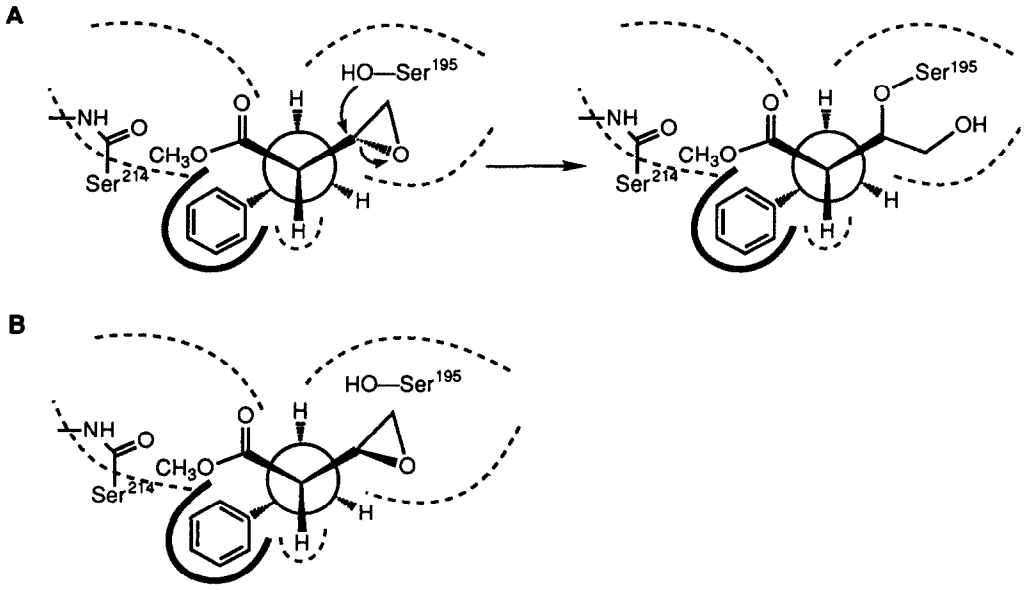

Figure 4. Methyl ester of (2S, 3R)-BEBA inactivates the enzyme with a covalent modification at the hydroxyl of Ser^{195} (A), while (2S, 3S)-BEBA methyl ester inhibits α-chymotrypsin competitively by reversible binding (B).

The inactivation of α-chymotrypsin only by (2S, 3R)-BEBA methyl ester strongly suggests that the nucleophilic attack of the hydroxyl occurs exclusively at the 3-position of the inhibitor: Its oxirane C_3–O bond is apparently oriented periplanar to the hydroxyl when it binds the active site, enabling the nucleophile to attack at the 3-position with a resultant oxirane ring cleavage. Thus, the covalent attachment of the inhibitor to the enzyme follows[12](Figure 4A). On the other hand, in the case of (2S, 3S)-BEBA methyl ester the unfavorable stereochemical orientation of the C_3–O bond for the hydroxyl to attack at the 3-position prevents the inhibitor from covalently linking to the enzyme but renders to function as a competitive inhibitor (Figure 4B).

In summary, although the potency of the inhibitory activities of these compounds are relatively low[13], the present study clearly demonstrates that inhibitors of an enzyme can be designed rationally on the basis of the stereospecificity of the target enzyme. In this approach the topology of the active site including the catalytic moiety, which is responsible for the stereospecificity of the enzyme serves as the basis for the inhibitor design. This novel approach is potentially applicable to other enzymes whose active sites have been characterized, and thus constitutes an additional avenue to the inhibitor designing methodologies.[2-6] We are in the process of examining the generality of the present approach. Finally, the present study bears an important implication in reference to the different biological effects, sometimes of opposite properties, exhibited by stereoisomers of chiral therapeutic agents.[14]

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12. Accordingly, it is expected that in the α-chymotrypsin catalyzed proteolysis reaction, the nucleophilic attack of the Ser^{195} hydroxyl at the scissile carbonyl carbon occurs in the *si* fashion.
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