Inhibition of Carboxypeptidase by Cyclopropane-containing Peptides

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Benzamido-1-aminocyclopropylcarbonyl-phenylalanine and -proline are shown to be irreversible inhibitors of carboxypeptidase A by a mechanism probably involving glutamate-270 as a nucleophile.

The rational design of drugs based upon enzyme inhibitors has been greatly advanced by taking advantage of the mechanism of action of the enzyme to generate a locally reactive group.[1] The reactivity of the cyclopropane group has been harnessed for enzyme inhibition by radical ring opening,[2] nucleophilic addition to cyclopropanones,[3] and in our studies, nucleophilic ring opening of cyclopropanes substituted with electron withdrawing groups.[4] Small groups such as cyclopropanes can be incorporated into substrate analogues for many enzymes and the transposability of such latent reactive groups is an attractive strategy for the design of enzyme inhibitors. Having established that cyclopropane derivatives are latent inhibitors of alcohol dehydrogenase and lactate dehydrogenase[4,5] through oxidative activation of a cyclopropylmethanol, we decided to investigate the transposability of the cyclopropane ring to the inhibition of a peptidase that uses a zinc cation as a Lewis acid catalyst in an analogous manner to alcohol dehydrogenase. Carboxypeptidase A is the prototype of many such enzymes,[6] and, bearing in mind the established substrate preferences of this enzyme, we planned to inhibit it by the strategy shown in Scheme 1.

Peptide derivatives (**1**) and (**2**) were synthesised from the benzamides of 1-aminocyclopropanecarboxylic acid by carbodiimide coupling with phenylalanine methyl ester and proline methyl ester respectively and subsequent alkaline hydrolysis of the ester. Both peptides were characterised by spectroscopy (i.r., high field n.m.r.) and had satisfactory microanalyses. The peptides were tested as inhibitors of carboxypeptidase A using hippurylphenylalanine as the substrate for spectro-

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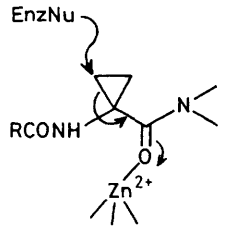
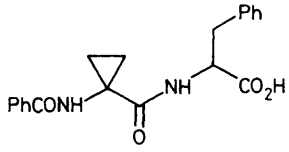


Scheme 1



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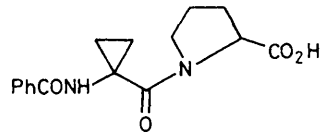
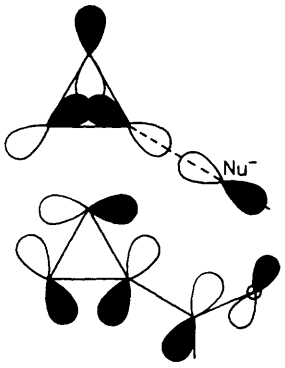
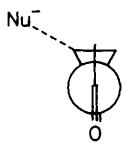


(2)





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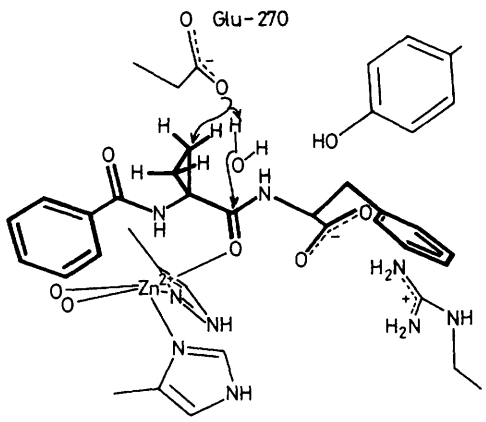

Figure 1

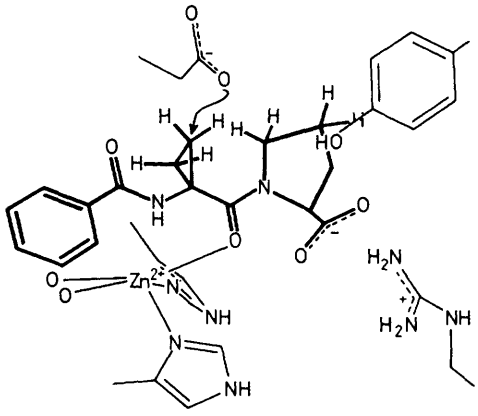

Figure 2

photometric assay at 254 nm. The phenylalanine derivative (**1**) caused time dependent inactivation of the enzyme and inhibition could not be reversed by gel filtration. Thus incubation of carboxypeptidase A (9.8×10^{-8} M) in Tris–HCl buffer pH 7.5 at 25 °C with hippurylphenylalanine (0.7 mM) and the cyclopropylphenylalanyl peptide (**1**) (0.7 mM) led to total inactivation of the enzyme ($t_{1/2}$, 4.5 min) following first order kinetics with an apparent $K_i = 8.4 \times 10^{-4}$ M. The rate constant for this inhibition reaction was determined to be 5.6×10^{-3} s^{-1} which compares with 2.5×10^{-3} s^{-1} for the peptide (**1**) acting as a substrate of carboxypeptidase. Thus inactivation occurs approximately 2.3 times faster than hydrolysis for this inhibitor.

It is, however, desirable that the inhibitor should not be hydrolysed by the enzyme. We found surprisingly that the proline derivative (**2**) is a potent inhibitor of carboxypeptidase A without showing any reactivity as a substrate. Under identical conditions and concentrations to those noted above for (**1**), the proline derivative (**2**) inhibited carboxypeptidase A with $t_{1/2}$ 3 min, and the corresponding rate constant for inhibition was determined to be 6.9×10^{-3} s^{-1} with an apparent K_i of 5.5×10^{-4} M.

The reasons for the activity of (**2**) are of interest and they can be investigated by considering the stereoelectronic requirements for nucleophilic attack on a cyclopropane activated by a carbonyl group in combination with the expected binding mode of the peptides at the active site as revealed by computer graphics. It would be expected that the preferred orientation of a carbonyl substituent on a cyclopropane ring would be in a bisected conformation illustrated by (**3**) in which the antibonding acceptor orbital of the carbonyl group can interact strongly with the HOMO of the cyclopropane.[5,7] Nucleophilic attack of a cyclopropane ring has been shown to occur preferentially with inversion of configuration at the site of attack[8] and this result can be rationalised by an interaction of the donor orbital of the nucleophile with the LUMO of the cyclopropane.[5] The preferred direction of attack is then seen to be at 150° to the cyclopropane ring in the plane of the ring carbons (**3**).

These requirements were brought into the context of the enzyme's active site using the Strathclyde molecular graphics system INTERCHEM.[9] Incorporation of the inhibitors in place of the substrate glycyltyrosine as determined by *X*-ray crystallography[10] and manipulation of the torsion angles of the peptide inhibitors to allow for *trans* amide bonds and the bisected conformation described above led to the arrays shown in Figures 1 and 2. It was immediately apparent that the carboxylate of Glu-270 could attack the cyclopropane in the stereoelectronically required manner. This result offers a hypothesis to account for inhibition and hydrolysis in terms of

a competition between nucleophilic attack at the cyclopropane ring or at the carbonyl group as follows.

The substrate, hippurylphenylalanine, and the two inhibitors make up an interesting series and the accessibility of water to attack the carbonyl group can be investigated for each case by examining the solvent accessible surface.[11] For the substrate and the phenylalanine derivative, it appeared that a water molecule could approach the carbonyl group aided by hydrogen bonding with the glutamate thereby leading to hydrolysis (Figure 1). In contrast, the five membered ring of proline butts closely up to the cyclopropane ring in the optimal conformation for binding and inhibition and may thereby prevent the access of water (Figure 2). This hypothesis accounts for the ability of (**2**) to act as an inhibitor but not as a substrate.

It can be concluded that cyclopropane-containing peptides can act as effective enzyme inhibitors. The results described in this communication suggest a wide range of potential applications of the transposable cyclopropane group.

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