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Esterase catalysed enantioselective ring closure

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Porcine liver esterase catalyses the reaction of γ-amino esters in water to give a mixture of the corresponding γ-lactam and the hydrolysis product, deacylation of the acyl enzyme intermediate by ring closure occurs with a high enantioselectivity giving an ee of 90% for the formation of (*S*)-5-phenyl-2-pyrrolidone from racemic ethyl 4-phenyl-4-aminobutanoate.

Enzymes which catalyse reactions in discrete steps by, for example, the formation of a covalently modified enzyme intermediate, have the potential to exhibit their chirality in at least two steps—formation and breakdown of the intermediate. Esterase enzymes catalyse the hydrolysis of esters through the intermediate formation of an acyl enzyme[1] but generally exhibit little significant enantioselectivity with racemic esters with chiral centres in either the alcohol or carboxylic acid residues.[2] Acyl enzyme intermediates potentially can be trapped not only by external nucleophiles but also intramolecularly to form cyclic products. This ring closure may be favoured over hydrolysis either because of the entropy effect[3] and/or because it is enzyme catalysed.

In the absence of enzymes, the intermolecular aminolysis of esters occurs readily only with reactive amines or with activated esters with good leaving groups,[3] or those with electron-withdrawing substituents in the acyl group.[4] Intramolecular lactam formation from amino esters occurs more easily but is normally hydroxide ion catalysed and requires high pH.[5] In aqueous solution, amide or lactam formation occurs in competition with hydrolysis which is also usually hydroxide ion catalysed and so the ratio of aminolysis to hydrolysis is dependent on the pK_a of the amino ester and the pH. By contrast, enzyme catalysed hydrolysis of esters can occur under mild conditions near neutrality. The esterase catalysed reaction of γ-amino esters generates an acyl–enzyme intermediate which could be trapped by the amino group to form a γ-lactam rather than the hydrolysis product (Scheme 1).[6]

Although the stereoselective enzymatic acylation of alcohols is well established, the enzymatic resolution of amines is less common.[7] The ring closure reaction offers a potential method of enantioselective cyclisation as it occurs at the enzyme surface, even though the first acylation step shows little or no selectivity.[2] A problem with such reactions is that it may be expected that the amino group would be protonated at neutral pH and therefore the enzyme reaction would be sensitive to pH and the pK_a of the aminium ion of the amino ester.

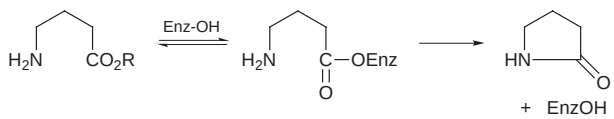
In the absence of enzyme, the reaction of ethyl 4-aminobutanoate in aqueous solution gives both hydrolysis and ring closed products. The ratio of 4-aminobutanoic acid to 2-pyrrolidone depends on the pH, buffer type and buffer concentration. For example, at pH 9.0 and 30 °C, no γ-lactam is formed, whereas at pH 10.0 and 12.0, 16 and 100% of the product is 2-pyrrolidone, respectively. At pHs above the pK_a of 9.9, the amount of lactam formed increases because the rate of lactamisation is given by eqn. (1), where RNH_2 and RN^+H_3 are the unprotonated and protonated forms of the γ-amino ester,

$$\text{Rate} = k_{OH}(RNH_2)(OH^-) = \frac{k_{OH}K_a}{K_w}(RNH_3^+)(OH^-)^2 \quad (1)$$

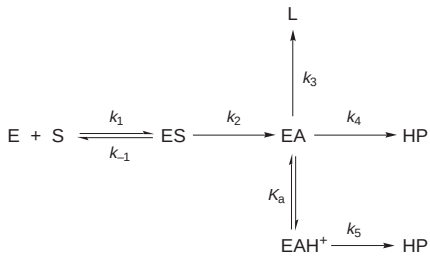
respectively, and K_a is its dissociation constant. Consequently, the rate of lactamisation is second-order in hydroxide ion at pHs below its pK_a and the rate of ring closure falls off rapidly with decreasing pH.

Likewise it was anticipated that the amount of lactam formed in the enzyme catalysed reaction and the extent of catalysis would be critically dependent on the pH and the pK_a of the amino ester. The degradation of ethyl 4-aminobutanoate in water is catalysed very effectively by pig liver esterase, with an apparent first order rate constant which shows a first order dependence on enzyme concentration. At pH 9.0, 30 °C and an enzyme concentration of 1.2×10^{-7} mol dm^{-3}, the rate of reaction is increased 360-fold over the non-enzyme catalysed reaction under the same conditions. Whereas there is no γ-lactam formed at this pH in the absence of enzyme, in its presence 48% of the product is 2-pyrrolidone. The amount of γ-lactam formed is independent of enzyme concentration, as expected for the ring closure reaction taking place within the acyl–enzyme intermediate. The amount of ring closed product formed increases with pH ranging from 20% at pH 7 to 80% at pH 10.5. The logarithm of the second order rate constant for enzyme catalysed reaction (k_{cat}/K_m) has a unit positive slope with respect to pH from pH 7.0 to 9.5, but becomes pH independent above the pK_a of the substrate with a maximal value of k_{cat}/K_m of 1.33×10^6 dm^3 mol^{-1} s^{-1}. This suggests that the substrate for the esterase catalysed reaction of the amino esters is the unprotonated form of the substrate. Below pH 7 the rate of hydrolysis of *neutral* esters falls off very fast because enzyme activity is dependent on an unknown ionising group of pK_a 7 in its basic form.[8] Above pH 7, the rate of the esterase catalysed hydrolysis of *neutral* esters is pH independent.

Similar catalytic behaviour is seen with the degradation of racemic ethyl 4-phenyl-4-aminobutanoate **1** (Scheme 3, Nu = NH) but because the pK_a is reduced to 8.90 maximal catalytic activity is reached at a lower pH and very effective catalysis occurs with an enzyme concentration of 1.0×10^{-8} mol dm^{-3}. The second order rate constant, k_{cat}/K_m, is 1.78×10^6 dm^3 mol^{-1} s^{-1} at 30 °C. Not only is the γ-lactam formed in the presence of the enzyme but it is predominantly (> 95%) the *S* enantiomer as determined by GC on a β-cyclodextrin column. The enantiomeric excess for 5-phenyl-2-pyrrolidone is independent of pH and enzyme concentration. The enantiomeric excess of the hydrolysis product agrees with that predicted from the assumption that hydrolysis is not stereoselective, as found for the hydrolysis of substituted acyclic esters.[2] For example, at pH 9.0, 38% of the γ-lactam is formed from racemic ethyl 4-phenyl-4-aminobutanoate with 90% ee for the *S* enantiomer, which gives a calculated ee for the *R* amino acid of 56%. This agrees perfectly with the observed ratio of 22% (*S*) and 78% (*R*) for 4-phenyl-4-aminobutanoic acid as determined by chiral



Scheme 1



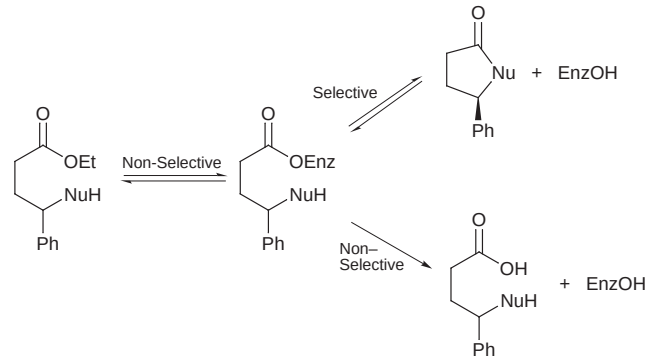
Scheme 2

HPLC (CHIROBIOTIC T250X 4.6 mm, teichoplanin coating eluting with 60 : 40 v/v EtOH–H_2O, t_R 11.5 and 13.2 min).

It is interesting to note that hydrolysis of the acyl enzyme appears to be non-selective whereas the intramolecular aminolysis to give the lactam is enantioselective. Deacylation of the acyl–enzyme is rate-limiting[2] and although the substrate for acylation of the esterase by amino esters is the neutral unprotonated amine, the same may not hold for deacylation. The dominant species at pHs below the pK_a of the amino acyl enzyme should be the protonated amine if the system is at thermodynamic equilibrium. However, the rate of hydrolysis–lactamisation could be faster than proton transfer to allow equilibrium with the pH environment. The unprotonated amino acyl enzyme (EA) must be the substrate for ring closure but not necessarily for hydrolysis (Scheme 2, where L and HP are the lactam and hydrolysis products, respectively). If the mechanism for hydrolysis and lactamisation occur through the same intermediate using the same catalytic apparatus of the enzyme then the ratio of lactam to amino acid product would be pH independent. If hydrolysis can occur through the protonated amine then the ratio of products should change with pH according to eqn. (2). The amount of lactam formed does increase with pH, as predicted by eqn. (2).

$$\frac{[\text{lactam}]}{[\text{hydrolysis}]} = \frac{k_3}{k_4 + k_5(\text{H}^+/K_a)} \qquad (2)$$

Previous work from our laboratory[2,9,10] has shown that the hydrolysis of γ-lactones catalysed by pig liver esterase occurs enantioselectively and the ring opening acylation step is selective (Scheme 3, Nu = O) whereas acylation of the acyclic hydroxy ester does not show selectivity during the hydrolysis mechanism.[9] The present results show that ring closure of γ-amino esters occurs enantioselectively (Scheme 3, Nu = NH)



Scheme 3

and is therefore compatible with intramolecular attack of the amino group occurring from only one face of the acyl enzyme with some form of recognition for the amino group—perhaps involving the enzyme catalytic machinery used for deacylation—and a binding pocket for the phenyl group.

Chiral ring closure is less effective for other γ-substituted amino esters but pig liver esterase does also catalyse the formation of δ-lactams but not β- or ε-lactams, from their respective amino esters.

Notes and references

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