Enzyme-catalysed Synthesis of Peptides containing D-Amino Acids

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Practical procedures are described for the preparation of D-amino acid-containing dipeptides using α-chymotrypsin and the $Met(O)_{192}$-modified enzyme as a catalyst.

α-Chymotrypsin was first reported in 1977 to be a 'poor' catalyst in the synthesis of dipeptides using D-Leu-NH_2 as a nucleophile.[1] It was not used for preparative synthesis of peptides containing D-amino acids until 1984 when Petkov and others illustrated the practicality of the system in a controlled kinetic approach.[2—4] Although the reaction was slow (*ca.* 10% as fast as that using L-amino acid derivatives as nucleophiles) and the enzyme was deactivated quickly at room temperature under the alkaline conditions used, it might open a new route to a number of biologically important peptides containing D-amino acids.[5] We have attempted to investigate the generality of this type of unusual catalysis and to optimize the reactions by changing the concentrations of substrates and organic solvents and found that protected dipeptides could be obtained in 75—80% yield in a carbonate buffer (0.2 M, pH 9)–dimethylformamide (DMF) (1 : 1 v/v) solution containing 50 mM Z-Phe-OMe or Z-Tyr-OMe (Z = benzyloxycarbonyl), 200 mM D-amino acid methyl esters or amides, and 0.2 mM α-chymotrypsin. Under these conditions, the concentration of peptides produced reached a maximum in 20 min when all the Z-amino acid ester was consumed as indicated in h.p.l.c. analyses. No precipitate was observed during the reactions. The products were simply isolated by extraction with chloroform and further purified by gel filtration chromatography using Sephadex LH-20 media. As shown in Table 1, several dipeptides containing D-amino acids were prepared and purified. Amino acid and ^1H n.m.r. analyses, and measurement of the optical rotations indicated that the products were identical to those prepared chemically by the azide method.[6] The reaction yield for each of the syntheses was reasonably high except when a sterically hindered D-amino acid derivative was used as a nucleophile. Similar results were observed when sterically hindered L-amino acid derivatives were used, indicating that the low yield was not due to the D-configuration. Interestingly, when the N-terminal L-Phe or L-Tyr derivative was replaced with the corresponding D-derivative, no condensation reaction was observed even when L-amino acid esters were used as nucleophiles. Further, the formation of L,D-dipeptides is virtually kinetically irreversible. The concentration of L,D-peptides remained essentially the same after reaching the highest yield, while that of L,L-peptides produced in reactions using L-amino acid esters as nucleophiles decreased substantially, presumably owing to rehydrolysis of the L,L-peptides catalysed by the enzyme.

The enzyme activity observed at the conclusion of each of the reactions, however, was about 20% of the original. To improve the stability of the enzyme under the working conditions, the Met residue at position 192 was selectively modified to methionine sulphoxide.[7] The modified enzyme [$Met(O)_{192}$-chymotrypsin] was almost as active as the unmodified one in the synthesis of (**1**) and 92% of the original activity was observed at the conclusion of the synthesis. The modified enzyme was then immobilized in poly(acrylamide-*co*-*N*-acryloxysuccinimide) (42% yield)[8] and used for the synthesis of (**1**) under the same conditions as that used in the reactions with soluble enzymes except that the concentration of the immobilized enzyme was about 0.01 mM. After a 25 h reaction with stirring in a 50 ml mixture, the protected dipeptide was isolated in 76% yield and the immobilized enzyme was recovered in 92% yield by centrifugation.

Examination of the active site model[9,10] developed on the basis of the *X*-ray structure[11] provides the rationale of the observed substrate specificity of chymotrypsin (Figure 1). As shown, the ar, h, and am regions can only accept groups with specific configuration such as *N*-acyl-L-amino acids, which must be bound in such a way that the aromatic residue is oriented in the hydrophobic pocket (the ar region) and the acyl amino group in the am region. The n region, however, is quite flexible and will accommodate different groups.[10] It is not surprising that D-amino acid derivatives could enter this region and act as a slow nucleophile.

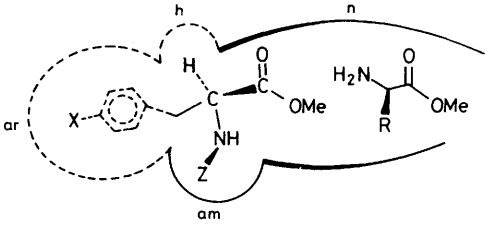

Figure 1. The orientation of substrates in Cohen's active site model.

Table 1. Physical data of the dipeptides prepared in chymotrypsin-catalysed syntheses. Z = Benzyloxycarbonyl.

Product		Yield, %	M.p., °C	$[\alpha]_D^{20}$ (*c* 1 M, $CHCl_3$)
Z-L-Tyr-D-Met-OMe	(**1**)	80	143—144	+18.1
Z-L-Tyr-D-Ser-OMe	(**2**)	72	164—165	− 9.2
Z-L-Tyr-D-Phe-OMe	(**3**)	75	170—173	+ 8.1
Z-L-Tyr-D-Arg-OMe	(**4**)	70	120—124	+10.1
Z-L-Tyr-L-Arg-OMe	(**5**)	71	118—121	− 7.3
Z-L-Tyr-D-Leu-OMe	(**6**)	74	114—116	+12.1
Z-L-Tyr-L-Val-OMe	(**7**)	20	143—145	−11.1
Z-L-Tyr-D-Val-OMe	(**8**)	20	118—119	+ 5.2
Z-L-Phe-D-Met-OMe	(**9**)	76	191—193	+10.0
Z-L-Phe-D-Ala-OMe	(**10**)	57	125—126	+11.3
Z-L-Phe-D-Val-NH_2	(**11**)	20	125—127	+22.9

In summary, the procedures illustrated here extend the scope of synthetic application of proteases.[10,12] The irreversibility of the reactions makes isolation of the products very simple. Further, the starting materials used in the syntheses are readily available and need not be pure; contamination of free amino acids or inorganic salts would not cause problems. The compounds prepared in this study are useful intermediates for a variety of peptides with neural activities (Morley and Manavalan in ref. 5).

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