Incorporation of unnatural amino acid derivatives into a peptide bond *via* an oxime ester catalysed by papain or lipase

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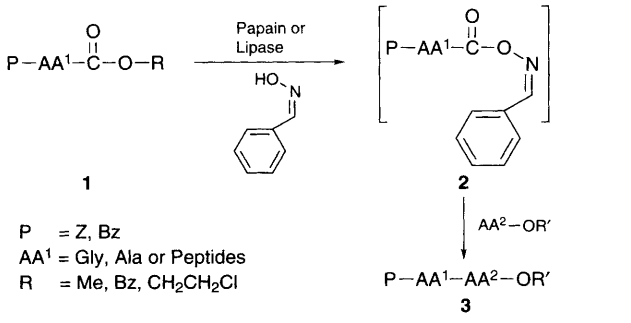
In the presence of an oxime in the reaction solution, papain and lipase P (*Pseudomonas* from Amano) catalysed the stereoselective transesterification of an *N*-protected amino acid or peptide ester to form an active (oxime) ester which in turn underwent peptide bond formation with several natural and unnatural amino acid derivatives (proline, *N*-methylglycine, *N*-methylalanine, α-methylphenylalanine).

Proteases can be used as catalysts for peptide synthesis in organic solvents.[1,2] Use of oxime compounds as the acyl donor in reversible acylation catalysed by lipase is documented,[3] and oxime esters of amino acids and peptides are widely used as active esters to form amide bonds in peptide synthesis.[4] We have found that, in the presence of oximes, unnatural amino acid derivatives can form a peptide bond *via* the oxime active ester, catalysed by papain or lipase. Scheme 1 shows the consecutive reactions of this synthesis. Papain or lipase P-catalysed transesterification of an *N*-protected amino acid or peptide with an oxime leads to the stereoselective formation of an active (oxime) ester which, in turn, reacts with an unnatural amino acid in a non-selective fashion to yield peptide **3**. The second step of amide bond formation is non-specific; thus unnatural amino acid derivatives {proline, *N*-methylglycine (Me-Gly), *N*-methylalanine (Me-Ala) and α-methylphenylalanine [Phe(2-Me)]} formed the peptide bond with the oxime ester.



Scheme 1

In a typical reaction, to Z-Gly-OBzl (300 mg, 1.0 mmol), Pro-OBzl·HCl (3 equiv., 774 mg, 3.0 mmol), triethylamine (0.42 cm^3 3.0 mmol), dithiothreitol (10 mg) and *syn*-benzaldehyde oxime (2.5 equiv., 302 mg, 2.5 mmol) dissolved in toluene (5.0 cm^3) was added papain (50 mg, Merck 5 × UPS, 30 000 USP-U mg^{-1}). The mixture was shaken at 35 °C until all the acyl donor was consumed [*ca.* 2.5 d, monitored by thin layer chromatography (TLC)]. The resulting mixture was diluted with ethyl acetate (200 cm^3), washed with citric acid (5%, 3 × 25 cm^3), water (3 × 25 cm^3), sodium hydrogen carbonate (5%, 3 × 25 cm^3); then the solution was dried over anhydrous sodium sulfate, and evaporated to afford crude Z-Gly-Pro-OBzl that contained a trace of *syn*-benzaldehyde oxime. The crude product was purified *via* silica gel flash column chromatography eluted with MeOH–CH_2Cl_2 (1:9, *v*/*v*) to yield pure Z-Gly-Pro-OBzl (0.211 g, 51%). In a similar manner, peptides containing unnatural amino acid derivatives were synthesized using either papain or lipase† as catalyst. Results are shown in Table 1. The physical properties were confirmed by optical rotation, amino acid analysis of the peptide hydrolysate, and FAB-MS.

The acyl-oxime **2** was unstable, but Bz-Gly-oxime and Z-Ala-oxime could be isolated using silica gel flash column chromatography. In the absence of papain or lipase, the isolated Bz-Gly-oxime reacted rapidly with D-Pro-OBzl. This may be the reason that the concentration of the active intermediate was very low during the reaction. Z-Ala-oxime was converted to Z-Ala-OMe rapidly when catalysed by papain in methanol. We

Table 1 Papain or lipase P-catalysed synthesis of peptides containing unnatural amino acids

Acyl donor	Additive	Nucleophile	Enzyme	Reaction period/d[a]	Yield (%)[a]	$[\alpha]_D^{25}$ (*c* 4, MeOH)	mp/°C	FAB-MS
Z-Gly-OBzl	oxime	Pro-OBzl	papain	2.5(3)	51(64)	−51.3	oil	396
Z-Gly-OBzl	none	Pro-OBzl	papain	2	0	—	—	—
Z-Gly-OBzl	oxime	D-Pro-OBzl	papain	1.5	46	54.0	oil	396
Bz-Gly-OBzl	oxime	Pro-OBzl	papain	1.5	54	−83.5	oil	366
Z-Ala-OBzl	oxime	Pro-OBzl	papain	2	61	−69.3	oil	410
Z-Ala-OCH_2CH_2Cl	oxime	Pro-OBzl	lipase[b]	2	71	−69.3	oil	410
Z-D,L-Ala-OBzl	oxime	Pro-OBzl	papain	(2.5)	(75)[c]	−69.1	oil	410
Z-Phe-Gly-OBzl	oxime	Pro-NH_2	papain	1.5	61	−11.4	192–194	452
Bz-Gly-OBzl	oxime	D-Pro-NH_2	papain	(3)	(65)	50.7	174–176	275
Z-Ala-OBzl	oxime	Me-Ala-OBzl	papain	2	59	21.5	131–134	398
Z-Ala-OMe	oxime	Me-Gly-OBzl	papain	2	60	−2.84	oil	384
Z-Ala-OCH_2CH_2Cl	oxime	Me-Gly-OBzl	lipase[b]	2	80	−2.84	oil	384
Z-Ala-OCH_2CH_2Cl	oxime	Me-Gly-OBzl	lipase[b]	3	85	−2.84	oil	384
Z-Ala-OMe	oxime	Phe(2-Me)-OMe	papain	2	79	2.93	94–96	398

[a] The figure in parentheses is for the reaction using immobilized papain (2.0 g, 500–550 U g^{-1}) instead of free papain in toluene; the reaction period in this case was Bd. [b] See footnote†. [c] Yield calculated was based on Z-L-Ala-OBzl.

tried subtilisin and chymotrypsin as catalysts for the reaction, but no product was observed. It is possible that the intermediate acyl-oxime **2** is an inhibitor for serine-type protease enzymes.[5] Using immobilized papain on XAD-7 as the biocatalyst,[6] the yield was increased but not significantly. The papain catalysed reaction was slow but in this reaction the enzyme proved to be stable for several weeks. Using the chloroethyl ester of Z-Gly as a substrate in DMF–phosphate buffer (8:2) we found that papain maintained 75% of its activity after incubation in the reaction conditions for 3 d and 45% activity after 7 d relative to the initial rate of reaction.

Without *syn*-benzaldehyde oxime in the reaction no product was found, while using acetaldoxime and pyridine-3-aldoxime as additives caused varied yields. The yield of the reaction generally depended on the structure of the acyl donor; using peptides as acyl donors, the yield was greater than that using amino acid derivatives. The reaction was not affected by the structure of the nucleophiles; using derivatives of L-proline or D-proline as nucleophiles in the reaction, the yields were similar. Using a nucleophile with a benzoyl protecting group gave a yield greater than that obtained using a nucleophile with an amide at the C-terminal. The small yield of the latter may reflect the solubility of the products in aqueous solution. Using Z-D,L-Ala-OMe as a donor, Z-Ala-Pro-OBzl was obtained (92% ee as determined by established methods).[7] With lipase as catalyst in diisopropyl ether, both the rate and yield of the peptide formation were greater than with papain as catalyst, but in this case chloroethyl esters of *N*-protected amino acids must be used instead of a methyl or benzyl ester.

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Footnote

† *General procedure* for the lipase catalysed reaction is as follows; Me-Gly-OBzl·Tos (880 mg, 2.5 mmol) was dissolved in water (20 cm^3) and the pH of the mixture was adjusted to 7.5 by the addition of NaOH (0.5 mol dm^{-3}); the resulting solution was lyophilized to dryness. To the lyophilized Me-Gly-OBzl, Z-Ala-OCH_2CH_2Cl (287 mg, 1.0 mmol) and *syn*-benzaldehyde oxime (121 mg, 1.0 mmol) dissolved in diisopropyl ether (25.0 cm^3) was added lipase (Amano P, 2.0 g). The mixture was shaken at 25 °C until all acyl donor had been consumed (*ca.* 2.5 d, monitored by TLC). The resulting mixture was diluted with ethyl acetate (200 cm^3) and washed with citric acid (5%, 3 × 25 cm^3), water (3 × 25 cm^3), sodium hydrogen carbonate (5%, 3 × 25 cm^3); then the solution was dried over anhydrous sodium sulfate, and evaporated to afford crude Z-Ala-Me-Gly-OBzl (0.308 g, 80%).

References

1 C. H. Wong and K. T. Wang, *Experientia*, 1991, **47**, 1123; V. Schellenberger and H. D. Jakubke, *Angew. Chem., Int. Ed. Engl.*, 1991, **30**, 1437; A. M. Klibanov, *Chemtech.*, 1986, **16**, 354.
2 P. Kuhl, P. J. Halling and H. D. Jakubke, *Tetrahedron Lett.*, 1990, **31**, 5213; H. Kise and Y. Tomiuchi, *Biotechnol. Lett.*, 1991, **13**, 317; S. T. Chen, S. Y. Chen and K. T. Wang, *J. Org. Chem.*, 1992, **57**, 6960; A. M. Klibanov, *TIBS*, 1989, April, 141.
3 M. Mischitz, U. Poschl and K. Faber, *Biotechnol. Lett.*, 1991, **13**, 657; A. Ghogare and G. S. Kumar, *J. Chem. Soc., Chem. Commun.*, 1989, 1533; V. Gotor and E. Menendez, *Synlett*, 1990, 699; R. Pulido and V. Gotor, *Carbohydr. Res.*, 1994, **252**, 55; M. Murakata, M. Imai, M. Tamura and O. Hoshino, *Tetrahedron: Asymmetry*, 1994, **5**, 2019; F. Moris and V. Gotor, *Tetrahedron*, 1994, **50**, 6927.
4 S. H. Nakagawa, H. S. H. Lau, F. J. Kezdy and E. T. Kaiser, *J. Am. Chem. Soc.*, 1985, **107**, 7087; S. M. Beaumout, B. O. Handford, J. H. Jones and G. T. Yang, *J. Chem. Soc., Chem. Commun.*, 1965, **4**, 53; S. Bittner, Y. Knobler and M. Frankel, *Tetrahedron Lett.*, 1965, **2**, 95.
5 H. U. Demuth, C. Schonlein and A. Barth, *Biochem. Biophys. Acta*, 1989, **996**, 19; R. A. Smith, P. J. Coles, R. W. Spencer, L. J. Copp, C. S. Jones and A. Krantz, *Biochem. Biophys. Res. Commun.*, 1988, **155**, 1201.
6 C. F. Barbas, III and C. H. Wong, *J. Chem. Soc., Chem. Commun.*, 1987, 533; S. T. Chen and K. T. Wang, *J. Chem. Soc., Chem. Commun.*, 1988, 327.
7 L. C. Lo, S. T. Chen, S. H. Wu and K. T. Wang, *J. Chromatogr.*, 1989, **472**, 336.

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