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Enzymatic peptide synthesis by new supported biocatalysts

Short Communication

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Summary. It is well known that proteases can be successfully used for peptide synthesis in aqueous medium or in water-containing organic solvents. Copolymerized of acrylated derivatives of α-chymotrypsin and polyethylene glycol (PEG) have been prepared and used as biocatalysts for the synthesis of model peptides in organic solvents containing a very low quantity of water. Trypsin has been similarly treated and used as a new biocatalyst particularly for the coupling of non-specific substrates.

Keywords: Amino acids – Segments coupling – α-Chymotrypsin – Trypsin – (non) specific substrates

Introduction

If stepwise chemical peptide synthesis is now a well-established procedure, there is room for the development of new methods in the convergent synthesis of large peptides which is based on segment coupling. Enzymatic catalysis, avoiding both the risk of racemization and the need of lateral chain protection during the chemical activation, appears to be a good alternative strategy.

Reverse action of proteases is a popular procedure for peptide bond formation, either under kinetic or thermodynamic control, but due to the presence of water, undesired hydrolysis may take place on the new formed bond or on any sensitive site(s) already contained in the fragment peptide sequence(s) (Schellenberger et al., 1991).

In order to avoid this effect, water content has been minimized and the enzyme (α-chymotrypsin) subjected to modification by an amphiphilic reagent (Polyethylene glycol).

The resulting adduct, soluble in organic solvent, has been shown active in different peptide couplings in t-amyl alcohol/toluene containing 0.5% water (w/w) (Babonneau et al., 1989).

For recycling the biocatalyst, a copolymerization of acrylic derivatives of both enzyme and PEG has been performed and the resulting gel shown to be an efficient re-usable catalyst of peptide synthesis mediated in t-amyl alcohol (containing 1% water) (Fulcrand et al., 1990, 1991).

A drastic reaction rate increase has been found by applying ultra-sonic irradiation through the reaction vessel which probably results from better substrate and product diffusion across the gel. In contrary to the native enzyme, the supported-biocatalyst activity is preserved (Rolland-Fulcrand et al., 1991).

In this communication, preliminary results concerning a new supported biocatalyst made of TPCK-trypsin instead of α-chymotrypsin are also presented.

Results, discussion

The ability of the immobilized PEG-α-chymotrypsin to introduce D amino acids at the P′1 position in peptides [relatively to the enzyme active site as noted by Schechter and Berger (1967)] is very convenient for the preparation of peptides containing D-residues (Table 1-entries 2 and 3).

The neuropentapeptide [BocLeu]-enkephalinamide, is prepared in a very good yield when an excess of H-Leu-NH2 is used (Table 1-entries 8 vs 7) without any secondary hydrolysis of the other specific site (Tyr-Gly) because of the very low quantity of water (1%) present in the medium.

According to a better solubilization and diffusion of both substrates and products through the gel, the coupling rate can be increased by ultrasonic irradiations. For example the reaction time in the entry 1 is decreased 6 times compare to the normal coupling (12 h) when ultrasonic irradiations are applied (Table 1-entries 1 vs 9). The enzyme activity is preserved, and this is not the case with native enzyme (Data not shown).

Trypsin has been immobilized like α-chymotrypsin and has given an efficient supported biocatalyst which, however, for correct peptide coupling, needs a

Table 1. On the use of immobilized α-chymotrypsin in segment coupling

Acyl donor	Nucleophile	% Conversion	Ester aminolysis, %	Ester hydrolysis, %	Reaction time, h
1-Ac-Tyr-OEt	H-Leu-NH$_2$	100	**98**	2	12
2-Ac-Tyr-OEt	H-(D)Leu-NH$_2$	99.5	**85**	14.5	15
3-Z-Phe-OCam	H-(D)Leu-NH$_2$	95	65	30	24
4-Z-Phe-OCam	H-Phe-Leu-NH$_2$	86.5	54	32.5	24
5-Ac-Tyr-OEt	H-Phe-Leu-NH$_2$	90	**70**	20	36
6-Boc-Leu-Tyr-OEt	H-Gly-Gly-OMe	53	33	20	60
7-Boc-Y-G-G-F-OCam	H-Leu-NH$_2$	90	65	25	60
8-Boc-Y-G-G-F-OCam	H-Leu-NH$_2$*	100	**92**	8	48
9-Ac-Tyr-OEt**	H-Leu-NH$_2$	100	98	2	**2**

[Acyl donor] = [Nucleophile] = 45 mM; Solvent: tert-amyl alcohol (or acetonitrile) + 1% H_2O.

* [Acyl donor]/[Nucleophile] = 1/3

** Using ultasonic irradiation in a thermostated vessel (30°C).

Table 2. On the use of immobilized trypsin in peptide coupling with non specific substrates

Acyl donor	% Conversion	Ester aminolysis, %	Ester hydrolysis, %	Reaction time, h	*Experimental conditions + US
1-Z-Phe-OCam	84	63	21	2	ATA + 10% H_2O
2-Z-Phe-OEt	0	0	0	48	DMF + 25% H_2O
3-Z-Phe-OCH_2-CN	0	0	0	48	DMF + 25% H_2O
4-Boc-Ser(OBzl)-OCam	54	24	30	10	100% H_2O
5-Boc-Ala-OCam	65	25	40	3	ATA + 15% H_2O
6-Boc-Tyr-OCam	10	0	10	48	DMF + 25% H_2O
7-Ac-Tyr-OCam	100	40	60	2	ATA + 15% H_2O
8-Boc-Leu-Tyr-OCam	0	0	0	48	DMF + 25% H_2O
9-Boc-Y-G-G-F-OCam	78	33	45	2	DMF + 25% H_2O

[Nucleophile] = [H-Leu-NH_2] = 22.5 mM.
Biocatalyst/Acyl donor/Nucleophile
1 / 1000 / 500
* Using ultrasonic irradiation in a thermostated vessel (30°C).

higher water content in the medium (15%) (Table 2). In these conditions, interesting results have been obtained specially with O-carboxamidomethyl esters as acyl donors: the enzyme primary specificity for basic residues is not required for coupling catalysis whereas the resulting peptide bond cannot be subjected to the reverse hydrolysis. These carboxamidomethyl esters are often used in enzymatic peptide synthesis with specific substrates in order to improve the coupling rate by increasing the acyl-enzyme intermediate formation as compared to methyl or ethyl esters (Kuhl et al., 1986). OCam esters of hydrophobic amino acids (or derivatives): Phe, Ala, Ser(O-Bzl)) become the best acyl donor residues. However, the Tyr-OCam esters except Ac-Tyr-OCam (Table 2-entry 7) are not substrates in contrary to the results of Mitin et al. (1992) concerning the coupling between Boc-Ala-Glu-Tyr-OCam and H-Ile-Ala-Glu-Phe-OH catalysed by native trypsin in water buffer.

By this method BocLeuEnkephalinamide has been also prepared (Table 2, entry 9) yet in moderate yield resulting from an unavoidable ester hydrolysis.

Conclusion

Combining endopeptidase-PEG copolymerization and ultrasonic treatment leads to excellent results on the enzymatic peptide synthesis in low water content medium.

It would be highly valuable to apply this methodology to hardly available enzymes as they can be easily recovered and reused.

The modification of the enzyme specificity (for example by using OCam esters as acyl donors for P1 specificity) will be applied to other proteases (α-chymotrypsin, papain) as the resulting peptide bond can not be hydrolysed even in high water content.

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