PEPTIDE BOND SYNTHESIS BY CLOSTRIDIOPEPTIDASE B.*


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SUMMARY

Clostridiopeptidase B was used for peptide bond synthesis with Cbz-ARG-OMe as substrate and 17 amino acid amides as nucleophiles. Synthetic yields ranging from 75 to 98% were obtained for most of the amides.

INTRODUCTION

The synthesis of peptide bonds using proteases as catalysts was first reported in 1938 (Bergmann and Fraenkel-Conrat, 1938). During the last decade, interest in this synthetic method has been renewed (Kullman, 1985; Whitesides & Wang, 1985). Today, it can be used as a complementary tool for stepwise peptide elongation and peptide fragment condensation.

In solid or liquid phase peptide synthesis, the incorporations of some amino acids, e.g. arginine, remain problematical. Arginyl peptide bond formation using ARG as substrate in the presence of nucleophilic amino acid amides has been reported to be catalyzed by trypsin (Oka & Morihara, 1977; Homandberg et al., 1978; Widmer et al., 1985), carboxypeptidase Y (Breddam et al., 1980), carboxypeptidase M (Breddam & Ottesen, 1984) and papain (Tsuzuki et al., 1980). In these studies, only trypsin was specific for arginyl or lysyl residues.

Clostridiopeptidase (Clostripain, E.C. 3.4.22.8) is highly specific for the hydrolysis of arginyl residues (Ogle & Tytell, 1953; Gros & Labouesse, 1960). Contrary to trypsin,
Clostripain has a minor hydrolytic action toward lysine (Mitchell & Harrington, 1968).

There have been few reports of the use of clostripain for peptide bond synthesis. These include the condensation of ribonuclease peptide fragments in the presence of a miscible cosolvent (Homandberg et al., 1982) and synthesis of the arginyl-proline bond to form di- or tri-peptides (Andersen, 1986). This work describes an investigation of the usefulness of clostripain in synthesizing 17 arginyl dipeptides.

**MATERIALS AND METHODS**

Reagents: L-Amino acid derivatives were synthesized following classical procedures (Greenstein and Winitz, 1961) or purchased from Sigma. All reagents were of analytical grade.

Peptides synthesis: The reaction system consisted of 10 mMoles of carbobenzyloxyarginyl methyl ester (Cbz-ARG-OMe) dissolved in MeOH, 100 mMoles of a amino acid amide and 0.2 M Tris-HCl to a total volume of 1 ml. Clostripain (0.5 unit; Sigma, 38 units/mg) was added and the mixture was incubated at 25°C for times and pHs described in the text. The reaction mixture was analyzed by HPLC (Vista 5500, Varian), using a Ultrasphere XL-ODS (3.0 u, 4.6 mm X 7.0 cm, Beckman), a gradient of H₂O/CH₃CN + 0.05% TFA and monitoring at 215 nm. Quantitations were done using a Varian DS 601 data system.

Product identification: The Cbz-dipeptides were deblocked using Palladium-PEI beads (Pierce) in 4.5% formic acid in MeOH for 30 minutes at 25°C (Coleman & Royer, 1980) and hydrolyzed with 6N HCl at 110°C overnight. The N(O,S)-heptfluorobutyryl isobutyl ester of the hydrolysate was prepared (MacKenzie and Tenaschuk, 1979) and the amino acid content determined by GC using an HP 5710A equipped with a Megabore DB-1 column (15m X 0.54mm), using helium as the carrier gas (3.6 ml/min). Peaks were detected with a flame ionization detector and quantitated using an HP 3354 Lab Data System.

**RESULTS AND DISCUSSION.**

The effect of pH on the yield of Cbz-ARG-GLY-NH₂ and Cbz-ARG-PRO-NH₂ was studied to determine optimal conditions for arginyl bond synthesis by clostripain. Reaction times were chosen to reflect the effect of pH on the rate of dipeptide synthesis. The pH optima for synthesis of these dipeptides are respectively 7.3 and 7.5 (Fig.1). Both values are lower than the pH 7.8 reported as optimal for the hydrolytic activity of clostripain (Mitchell & Harrington, 1968). Furthermore, the different pH optima obtained in these reac-