Synthesis of adhesive protein from the vitellaria of the liver fluke
_Fasciola hepatica_

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Summary. The polynonapeptide (Gly-Gly-Gly-Tyr-Gly-Gly-Tyr-Gly-Lys)_n_, which is a precursor sequence of adhesive protein from the vitellaria of the liver fluke _Fasciola hepatica_ has been synthesized by the fragment coupling, followed by polycondensation, and by cleavage of the protecting groups by hydrogen bromide. The synthetic adhesive protein was estimated to have the molecular weight of 10,100 (12 repeating units as nonapeptide) and was found to have satisfactory amino acid compositions. The Tyr residues of the synthesized precursor polynonapeptides can be converted to the Dopa residues by tyrosinase, giving the synthetic adhesive protein of the liver fluke.

Keywords: Amino acids – Adhesive protein – Polynonapeptide – Liver fluke

Introduction

Since 1983 the primary structures of some adhesive proteins secreted from the marine invertebrate have been determined by Waite (1990, 1985) as “consensus peptide sequences”. The adhesive proteins are simple proteins and have been identified as L-β-3,4-dihydroxyphenyl-α-alanine (Dopa) and Lys containing proteins. The adhesive proteins have been investigated with the intention of applying the adhesive properties to medical and dental purposes (Waite, 1986; Benedict and Picciano, 1989). Two different approaches, one is polymer chemical and the other is gene technological, are competitive strategies to prepare these adhesive proteins. In fact, our group first synthesized a polydecapeptide (Ala-Lys-Pro-Ser-Tyr-Hyp-Hyp-Thr-Dopa-Lys)_n_ from mussel _Mytilus edulis_ (Yamamoto, 1987) and later polyheptapeptide (Ala-Gly-Dopa-Gly-Gly-X-Lys)_m_ from Chilean mussel _Aulacomya ater_ (Yamamoto et al., 1991) by polycondensation, and in the same time two groups prepared a precursor form Ala-Lys-Pro-Ser-Tyr-Pro-Pro-Thr-Tyr-Lys by genetic engineering technology (Maugh,
1984; Strasberg et al., 1989). The latter genetic products were converted to the final adhesive proteins by a modification reaction by tyrosinase.

Among the primary structures, the adhesive proteins from the egg shell hardening protein sclerotin have also been reported. The adhesive protein of the vitellaria of the liver fluke *Fasciola hepatica* has the simplest repeating sequences consisting of only three amino acids (Gly, Tyr, Lys) among the adhesive proteins and therefore may be the most promising bioadhesive formulation (Waite and Rice-Ficht, 1987). The adhesive protein of the vitellaria of the liver fluke has been analyzed to have a sequence of (Gly-Gly-Gly-Dopa-Gly-Gly-Dopa-Gly-Lys) and a molecular weight of about 31,000 (about 35 nonapeptide units). In this article we report the synthesis of the precursor polynonapeptides (Gly-Gly-Tyr-Gly-Gly-Tyr-Gly-Lys) and the modification of the precursor polynonapeptides to final protein by the treatment of tyrosinase.

**Experimental**

**Strategy**

The synthesis of the liver fluke adhesive protein is outlined in Scheme 1. The combination of the $\beta$-benzylxycarbonyl (Z) and $\alpha$-$\alpha$-nitrophenylsulfenyl (Nps) groups was chosen to protect the amino groups of Lys, since the Z and Nps amino protecting groups can be selectively removable by a mild acidolysis, and ethyl (Et) and $p$-nitrophenyl (Np) carboxyl protecting groups were used. To protect hydroxyl groups of Tyr, the benzyl (Bzl) groups

![Scheme 1. Preparation of poly(Gly-Gly-Tyr-Gly-Gly-Tyr-Gly-Lys). Z Benzylxycarbonyl; OEt ethyl ester; ONp p-nitrophenyl ester; Nps $\alpha$-nitrophenylsulfenyl; Bzl benzyl ether](image-url)