

Chapter – V

Summary and Outlook

CHAPTER V

SUMMARY AND OUTLOOK

Solid phase synthesis of partial sequence of rubber elongation factor (REF) protein on a 2 mol% HDODA-crosslinked polystyrene support forms the main objective of the thesis. The presence of REF on rubber particle is essential for the elongation of rubber. Removal of REF from rubber particles and reconstitution of a rubber biosynthetic system has not been successful to date. Fragments of REF which suffer recovery problems due to its hydrophobic nature were synthesized using 2% HDODA-crosslinked polystyrene support.

The success of solid phase synthesis depends on the swelling and solvation of polymer support and peptide chain. The first requirement for steady progress in solid phase peptide synthesis is the effective solvation of support and peptide in all solvents used for the synthesis. The synthesis of hydrophobic peptides remained to be a challenging problem to peptide chemists. The inherent nature of peptide to aggregate via hydrogen bonded β -sheet structures, makes the synthesis extremely difficult. The concept of optimum hydrophobic-hydrophilic balance serves as a guideline for the development of a support with styrene and 1,6-hexanediol diacrylate. This

polymer support showed better solvation and swelling in solvents used for the synthesis of peptides.

In the first and second chapters, a brief overview of recent advances in solid phase peptide synthesis, including development of new supports, anchoring linkages, novel protecting/deprotecting and coupling agents, and cleavage techniques. The third chapter deals with the preparation of polymer support and synthesis, purification and characterization of peptides. The fourth chapter consists of two parts:

1. Preparation and functionalisation of polymer support
2. Detailed procedure for solid phase peptide synthesis.

Polymer support with uniform size and shape were prepared by suspension polymerization of hydrophobic styrene as monomer and hydrophilic hexanediol diacrylate as crosslinking agent. The support was functionalized with chloromethyl group. The model peptides synthesized are:

1. Ala - Pro - Ala
2. Ala - Ala - Pro - Ala
3. Ala - Ala - Ala - Ala - Ala
4. Ala - Ala - Pro
5. Ala - Ala - Ala - Pro - Ala
6. Ala - Pro - Gly - Pro - Arg.

The following fragments of REF were synthesized on a chloromethyl HDODA-crosslinked polystyrene using the system of benzyl derived side chain blocking group in conjunction with Boc-amino acids with some modifications. For coupling, active esters of amino acids with HOBT were used. NMP was used as solvent for coupling reaction. Each step was monitored by Kaiser test.

1. Gln - Gln - Gly - Gln - Gly (7-11)
2. Val - Gln - Asp - Ala - Ala - Thr - Tyr - Ala (20-27)
3. Pro - Leu - Gln - Pro - Gly - Val - Asp - Ile - Ile-Glu-Gly-Pro (44-55)
4. Val - Lys - Asn - Val - Ala - Val - Pro (56-62)
5. Tyr - Ile - Pro - Asn - Gly - Ala - Leu - Lys - Phe - Val - Asp -Ser-Thr-Val-Val - Ala (69-84)
6. Gln - Thr - Lys - Ile - Leu - Ala - Lys - Val - Phe - Tyr - Gly (125-135).

After the synthesis of desired sequences, peptides were separated from the resin using anhydrous trifluoro acetic acid (TFA), thioanisole and 1,2 ethanedithiol. The purity of these peptides were confirmed by HPLC and characterized by amino acid analysis and ESI-MS.

Fragments of REF protein which are highly hydrophobic, insoluble even in acetonitrile were synthesized in nearly quantitative yield using 2% HDODA-crosslinked polystyrene. This point to the fact that HDODA-PS is an efficient support for the synthesis of peptides especially hydrophobic peptides, the synthesis of which is still a challenge to peptide chemists. The

first attempt to synthesize partial sequences of rubber elongation factor protein by solid phase peptide synthesis is successful. The work described in the thesis and its future refinements may contribute to the synthesis of complete sequences of REF protein and its introduction to synthetic polymer chemistry.