Chapter 5

Summary and Observations
5.1 Summary

Hydrophobic peptides play a major role in biology and related fields. The synthesis of these peptides is a challenging problem to peptide chemists. The inherent nature of peptide to aggregate, via hydrogen bonded secondary structures, makes the synthesis extremely difficult. Therefore, the aggregation propensity of peptides should be suppressed for the easy synthesis. With this view in mind the peptide synthesis group of Mahatma Gandhi University developed and optimized a new polystyrene support with 1,4-butanediol dimethacrylate as cross-linking agent (BDDMA-PS). Various short and medium sized hydrophobic peptides could be synthesized using the new support in appreciable yield.

In the present study 2% 1,4-butanediol dimethacrylate cross-linked polystyrene (BDDMA-PS) has been used as the polymer matrix. The experiences with the synthesis of target peptides are summarized below. The experiences show that it is an efficient polymer matrix for the synthesis of short and medium sized hydrophobic peptides.

PELLETFL (Yield= 93%)
LLETFL (Yield=94.5%)
FLSEWIG (Yield= 95%)
FSASCVPG (Yield=93%) &
AVGEQELRGCNQWSGL (Yield= 92%)

[Green- Single coupling with 3 molar excess of the reagents, Blue- Double coupling with 3 molar excess of the reagents, Brown- Three times coupling with 3 molar excess of the reagent, Red- Four times coupling with 3 molar excess of the reagents].
Several reasons exist for the maintained interest in the conformational features of peptides. In drug design, knowledge of the preferred conformation of active peptides can assist in developing compounds with enhanced activity. A deeper understanding of peptide conformational stability also provides insights into the early events in protein folding and local stability. The importance of the study of conformation and interactions of peptides in solution has grown in recent years thanks to progress in NMR spectroscopy and peptide synthesis methodologies. The factors dictating their conformational stability are still poorly understood due to the inherent difficulty in examining these flexible molecules. More often than not, they are in quick exchange between conformations and the overall population of any preferred fold is usually small. Still, the amount of data available for the solution conformation of peptides is growing steadily and the most detailed informations stem from NMR experiments. The chemical shift of the proton and the carbon contains valuable information about the secondary structure of a peptide, and this information can be complemented by other data obtained by NMR (nOe connectivities, the $^{3}J_{NNH}$, coupling constant, H/D exchange data,...). Secondary structure analysis does appear to help in identifying short peptides possessing biological activity.

There are a number of peptide/protein secondary structure prediction methods. The Advanced Protein Secondary Structure Prediction (APSSP) method is a handy tool for secondary structure prediction. The results obtained with APSSP for our target sequences are given below.
<table>
<thead>
<tr>
<th>Sequence</th>
<th>Secondary structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>PELLETFL</td>
<td>Residues L4, E5 and T6 occupy ( \alpha )-helix. The rest form random coil.</td>
</tr>
<tr>
<td>LLETFL</td>
<td>Random coil structure</td>
</tr>
<tr>
<td>FLSEWIG</td>
<td>Random coil structure</td>
</tr>
<tr>
<td>FSASCVPG</td>
<td>Random coil structure</td>
</tr>
<tr>
<td>AVGEQELRGCNQWSGL</td>
<td>Residues from E4 to R8 occupy ( \alpha )-helix. The rest form random coil.</td>
</tr>
</tbody>
</table>

The above peptides are derived from two biologically important proteins—bovine seminalplasmin (SPLN) and human lactoferrin (HLf) (Chapter 2).

Both CD and NMR studies show that SPLN maintains a random coil structure in aqueous solution. However, upon binding to a hydrophobic/hydrophilic interface, an increase in the content of regular secondary structure is observed. In water/dodecylphosphocholine, SPLN adopts a secondary structure by formation of two helical segments. Segment 1 begins at Leu21 and extends to Asn27. The second segment begins with Leu31 and, with an interruption at Phe34, extends to the C terminus. We have synthesized and analyzed three analogues of SPF, the 28-40 fragment of SPLN. The 31-40 stretch forms an \( \alpha \)-helix in the presence of detergent micelles.

We have concentrated on the 153-160 and residue substituted 349-364 fragments of human lactoferrin. Crystallographic studies show that the sequence stretch 153-158 is found to adopt a \( \beta \)-strand
and the stretch 158-161 a β-turn. The sequence stretch 349-364 is found to adopt an α-helical conformation.

The dominating solution conformation of the five peptides has been solved by using high resolution NMR and restrained molecular dynamics in DMSO-d6 at 25°C. The results are summarized below.

<table>
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<tr>
<th>Sequence</th>
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</tr>
</thead>
<tbody>
<tr>
<td>PELLETFL</td>
<td>Extended backbone conformation</td>
</tr>
<tr>
<td>LLETFL</td>
<td>Extended backbone conformation</td>
</tr>
<tr>
<td>FLSEWIG</td>
<td>The residues from F1 to E4 form a β-strand and the rest form extended backbone conformation</td>
</tr>
<tr>
<td>FSASCVPG</td>
<td>Extended backbone conformation</td>
</tr>
<tr>
<td>AVGEQELRGCNQWSGL</td>
<td>The residues from Q5 to Q12 form an α-helix and the rest form extended backbone conformation</td>
</tr>
</tbody>
</table>

Atomic coordinates of the 153-160 fragment, FSASCVPG, and the 349-364 fragment, AVGEQELRGCNQWSGL, of Hlf are available from at Protein Data Bank (http://www.rcsb.org/pdb/). The solved structure of the sequence FSASCVPG with minimum target function shows a good superposition on the reported structure of the corresponding protein fragment (Figure 4.35). The solved structure of the sequence AVGEQELRGCNQWSGL with minimum target function shows a good superposition, excepting terminal residues, on the reported structure of the corresponding protein fragment (Figure 4.43).

The predicted and experimental structures are compared as given below with the secondary structure of the sequence as part in the complete sequence.
### 5.2 Observations

a. If the peptide is small, viz. less than the critical length with 3-4 flanking residues at the terminals and sufficient middle piece to form either $\alpha$-helix or $\beta$-strand, it would deviate largely from the local structure when it forms part of the complete peptide/protein.
b. If the peptide is small, under solution conditions it would mostly form either extended backbone structure or β-strand. α-Helical structure may be possible only in high propensity sequences.

c. Prediction of secondary structure of small peptides based on protocols/algorithm for large peptides/proteins often fails and does not conform to the local structure of the peptide/protein to which the target sequence matches.

5.2.1 General (hypothetical) inference

The flanking terminal segments of well-formed α-helix dissected from complete peptide/protein shall be in irregular form suggests the following:

a. The protein folding may start from the N-terminal of the *de novo* synthesized peptide/protein, still in the phase of elongation, away from the segment, which in any environment shall form only irregular or random structure. More likely the first formed secondary structure shall be α-helical, since β-strand has to be stabilized by other β-strands which may form later only.

b. The preferred secondary structures only would guide the formation of S-S bonds, though such bonds may help the peptide/protein to regain its native secondary structure on renaturation.

c. When design of active peptides with strong secondary structural implications are considered, more importance may be given to the flanking regions of the relevant sequence and peptides without flanking segments need not be considered. Such
experimental studies may be directed to the effectiveness of the flanking residues in keeping the active segment structurally implying the predicted function.