Conformational Analysis of Resin Bound Peptides

Vibrational spectroscopy (IR and Raman) has a long history of application to molecular structural studies. Since the introduction of the two methods employed to investigate the vibration spectra, viz. IR absorption and light scattering (Raman spectra), the former is more powerful as it is easy to perform and can be applied to any material given in $10^{-3}$ to $10^{-7}$ g levels. But Raman spectra can give valuable information on the low frequency vibrations. The use of vibration spectra to determine the molecular structure is based on the assumption that each distinct type of molecule will have a characteristic vibration spectrum. Though it is possible to derive strict selection rules for small molecules, it is difficult to determine the structure of complex molecules. A non-linear molecule with $n$ atoms possesses $3n-6$ fundamental vibration frequencies and as $n$ increases, the complexity of the spectrum also increases. Since IR spectroscopy makes use of the vibrational bond energy, no external probes are needed to study molecular structure. However, the arrival of FTIR techniques in multidimensions and the availability of fast computers for the analysis of spectra have made it possible to apply this technique vary reliably even to complex molecules like peptides,
and proteins. Recently, Bing Yan reported the usefulness of single bead FTIR as a TLC equivalent analytical method for on resin monitoring and quantitation in solid phase organic synthesis.3

The first systematic work on the vibration spectra of amino acids in relation to their structure was done by Edsall et al.4 Later a very thorough study of the amides and of the simple -CO-NH- link (peptide bond) was carried out by Thomson et al.5 They showed that all compounds of the type R-CO-NH-R', exhibit strong absorptions near 3300 cm⁻¹ (N-H₄⁺), 1670 cm⁻¹ an 1560 cm⁻¹ (N-H₃⁻). But the systematic investigations of Miyazawa et al. on N-methylacetamide helped us to understand clearly the amide modes of vibration of a peptide.6 This knowledge can be extended to peptides and proteins also. Elliot and Ambrose correlated the IR absorption bands of an amide bond to the protein conformation.7 IR spectroscopy has been extensively used for the study of peptides and proteins in the following aspects.

- Conformation of oligopeptides and poly-amino acids,8 9
- Conformational analysis of peptides during solution-phase and solid-phase synthesis,8 10
- Effect of PEG upon the conformation of peptides11
- To monitor the kinetics of reactions on resin supports,3
- Aggregational behaviour of peptides in various solvents,12 14
- Effect of solvents upon the conformation of peptides,15 16
- Effect of non-codable amino acids and side chain protecting groups upon the conformation of peptides,17 20
- The structure of peptide hormones in lipid environments,21
- Structural stability of hydrated polypeptides,22
- Investigation of polypeptide chain folding and denaturation of proteins,23
- To recognise some amino acid incorporation into polypeptides and proteins,24
- To distinguish between DL-form and D or L form of amino acids,25
Since N-methylacetamide (NMA) is the smallest molecule containing trans amide bond (Figure 5.1), the study of this molecule can provide important insight into the nature of the amide bond of a peptide. The conformation of a polypeptide chain is essentially described by the dihedral angles $\phi$ and $\psi$ at the $C^\alpha$-atoms as depicted in Figure 5.2. The geometry and dimensions of the peptide bond have been derived by Pauling from the crystal structure of molecules containing few peptide bonds.

![Figure 5.1. Schematic diagram of NMA molecule](image)

![Figure 5.2. Perspective drawing of the L-\(\alpha\)-polypeptide chain (trans-form) illustrating the torsion angles $\phi$ and $\psi$.](image)

An isolated planar CO-NH bond can have nine bands of stretching frequencies usually called amide A, B and amide I-VII. In the case of NMA, these modes could be described in terms of displacement coordinates as shown in Figure 5.3.
A summary of the characteristic bonds associated with peptide linkages is given in Table 5.1.

Table 5.1. Characteristics bands associated with amide linkage

<table>
<thead>
<tr>
<th>Symmetry</th>
<th>Designation</th>
<th>Frequency (cm(^{-1}))</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>In plane</td>
<td>Amide A</td>
<td>3200-3500</td>
<td>N-H(_{str}) (100%)</td>
</tr>
<tr>
<td></td>
<td>Amide B</td>
<td>3000-3200</td>
<td>N-H(_{str}) (100%)</td>
</tr>
<tr>
<td></td>
<td>Amide I</td>
<td>1600-1700</td>
<td>C=O(<em>{str}) (80%), C-N(</em>{str}), C-C-N(_{def})</td>
</tr>
<tr>
<td></td>
<td>Amide II</td>
<td>1500-1600</td>
<td>NH(<em>{ip}) (60%), C-N(</em>{str}) (40%), C=O(<em>{ip}), C-C(</em>{str}), N-C(_{str})</td>
</tr>
<tr>
<td></td>
<td>Amide III</td>
<td>1300</td>
<td>CN(<em>{str}) (40%), N-H(</em>{ip}) (30%), C-C(<em>{str}) (30%), C-O(</em>{op})</td>
</tr>
<tr>
<td>Out of plane</td>
<td>Amide IV</td>
<td>625</td>
<td>CO(<em>{op}) (40%), C-C(</em>{str}) (30%), C-N-C(_{def})</td>
</tr>
<tr>
<td></td>
<td>Amide V</td>
<td>725</td>
<td>NH(<em>{op}), C-N(</em>{t})</td>
</tr>
<tr>
<td></td>
<td>Amide VI</td>
<td>600</td>
<td>CO(<em>{op}), CN(</em>{t})</td>
</tr>
<tr>
<td></td>
<td>Amide VII</td>
<td>200</td>
<td>NH(<em>{op}), CN(</em>{op}), CO(_{op})</td>
</tr>
</tbody>
</table>

\(_{str}\) = stretch; \(_{def}\) = deformation; \(_{ip}\) = in plane; \(_{op}\) = out of plane; \(_{t}\) = torsion
Amide A and amide I-III are suitable for protein studies. Amide-V band in the far and near IR region is also used for the conformational investigations of peptides bound to solid supports. Since amide I band is more sensitive and clear to conformational changes, more than 90% of the work on proteins has been carried out in this region. A correlation between different absorption bands and conformation is given in Table 5.2. Larsen et al. has used near infrared fourier transform Raman Spectroscopy to study the structural implications of growing peptide chains on SPPS. Applications of Raman spectroscopy to peptide conformational analysis has also been studied.

In the amide A region, strong peak around 3280 cm\(^{-1}\) is due to β-structure and 3410 cm\(^{-1}\) is of either α-helix or unordered conformation. In the amide II region, peak at 1540 cm\(^{-1}\) is due to α-helix and that at 1555 cm\(^{-1}\) may be due to β-structure. In the amide-V region peak due to α-helix was observed around 625 cm\(^{-1}\) and which is shifted to 710 cm\(^{-1}\) on β-sheet structure (Table 5.2).

<table>
<thead>
<tr>
<th>Table 5.2. Characteristics amide I frequencies of an amide bond</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conformation</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>α-helix</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Parallel β-sheet</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Antiparallel β-sheet</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Unordered</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

\(s = \text{strong, } w = \text{weak, } m = \text{medium, } b = \text{broad}\)
Steric factors resulting from the aggregation of the pendant peptide chains by inter and intramolecular hydrogen bonding, especially the β-sheet structure formation, was recognised as the major reason for the chronic stretches of incomplete aminoacylations and deprotections occur during the solid phase synthesis of peptides. FTIR and NMR studies of the peptidyl resins carrying such sequences (difficult sequences) were performed to confirm this point. Synthetic results of a comparative synthesis of a group of β-structured peptides on newly developed BDDMA-PS and the conventional Merrifield resin, (DVB-PS), showed that the synthesis on BDDMA-PS was straightforward and ended up with high yield and excellent purity than the products from DVB-PS. To understand the mechanistic reason behind this observation, FTIR investigations on the resin bound peptides were carried out during synthesis. Many groups of peptide chemists have already used FTIR spectroscopy to monitor the conformation of peptides bound to linear and crosslinked polymers.

For the present study nine model difficult sequence peptides characterised by high \( SP_p \) values (>5), low stepwise cumulative random coil inducing parameter \( <Pc^*> \) values (<0.9), and high β-sheet propensity \( <P> \) values were selected and built on both 2 mol% BDDMA-PS and DVB-PS resins under identical conditions. High capacity resins (~2 mmol g\(^{-1}\) chlorine capacity) were used to investigate the effect of functional group capacity on the extent of aggregation and hence the coupling difficulty.

Many attempts have been made to identify the difficult sequences prior to their synthesis. All these attempts were based on the fact that β-sheet peptides are difficult to assemble while the peptides with disordered conformations can be synthesised easily. The first correlation between amino acid sequence and secondary structure was established by Blout et al. Later Chou and Fasman derived the helix, β-sheet and random coil conformational parameters for the 20 naturally occurring amino acids from the frequency of occurrence of each amino acid residue in the respective conformations of the proteins, whose structure had been determined by X-ray crystallography. These values can be used to predict the secondary structure of peptides from
their known primary structure. Nowick et al. studied the relation of each amino acid residues to their β-sheet propensity.35

Narita et al. classified the naturally occurring amino acids by their β-sheet structure stabilising potential (SPb).16,36 Using 77 kinds of protected tri to hepta peptide fragments of, E-Coli. ribosomal protein, he assigned each amino acid, a value SPb, known as the β-sheet structure stabilising potential. Arithmetic average β-sheet structure stabilising potential ∑SPb, of each peptide sequence can thus be calculated using the equation,

<SPb> = \frac{\sum n_i SP_{b_i}}{\sum n_i}

where n_i-number of i-th amino acid residue in the sequence.

It has been shown that peptides with <SPb> values greater than 5.0 will be highly stabilised in β-sheet structure and hence difficult to synthesise.36

The peptides used in the present work are thus typical difficult sequence peptides whose various parameters are given in Table 5.3. Peptides (P_r-P_v) are the fragments of ribosomal protein,37 P_v is from human pro-insulin fragment18 and peptides P_vii-P_vni are the fragments of acyl carrier protein.19

Table 5.3. Conformational parameters of the peptides selected in the present study

<table>
<thead>
<tr>
<th>Code</th>
<th>Peptide</th>
<th>&lt;P_a&gt;</th>
<th>&lt;P_p&gt;</th>
<th>&lt;P_b&gt;</th>
<th>&lt;SP_b&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>P_i</td>
<td>VAVI</td>
<td>1.18</td>
<td>1.46</td>
<td>0.69</td>
<td>5.50</td>
</tr>
<tr>
<td>P_n</td>
<td>KVAVI</td>
<td>1.16</td>
<td>1.32</td>
<td>0.76</td>
<td>5.00</td>
</tr>
<tr>
<td>P_m</td>
<td>NKVAVI</td>
<td>1.08</td>
<td>1.21</td>
<td>0.86</td>
<td>5.30</td>
</tr>
<tr>
<td>P_v</td>
<td>ANKVAVI</td>
<td>1.04</td>
<td>1.17</td>
<td>0.83</td>
<td>5.10</td>
</tr>
<tr>
<td>P_v</td>
<td>VAVAAG</td>
<td>1.19</td>
<td>1.17</td>
<td>0.78</td>
<td>5.30</td>
</tr>
<tr>
<td>P_v</td>
<td>QVGVQELG</td>
<td>1.06</td>
<td>1.10</td>
<td>0.91</td>
<td>4.75</td>
</tr>
<tr>
<td>P_v</td>
<td>AAIDYING</td>
<td>1.06</td>
<td>1.03</td>
<td>0.88</td>
<td>5.02</td>
</tr>
<tr>
<td>P_v</td>
<td>QAAIDYING</td>
<td>1.11</td>
<td>1.12</td>
<td>0.49</td>
<td>4.55</td>
</tr>
<tr>
<td>P_v</td>
<td>VQAAIDYING</td>
<td>1.0</td>
<td>1.15</td>
<td>0.93</td>
<td>4.70</td>
</tr>
</tbody>
</table>
All the peptides were first of all synthesised on chloromethylated BDDMA-PS and DVB-PS resins of approximately same functionalisation levels. This is very important, since the functional group capacity and functional site distribution on the polymer matrix have a pronounced effect upon the coupling difficulty. IR spectrum of each peptidyl resin was recorded in the solid state using KBr pellets. Both BDDMA-PS and DVB-PS resins have no characteristic stretching absorptions in the amide A (3500-3200), amide-I (1600-1700) region, except the peak at 1720 cm⁻¹ of the ester carbonyl group of BDDMA-PS. So these regions were selected for conformational analysis. The resin samples for the IR measurements were prepared by soaking overnight in dichloromethane, filtering and drying in vacuo. Since the changes in the conformation of the oligopeptides were noticed by the application of the shear stress both in the solid state and in the resin bound state, the same shear stress was applied to all the samples while preparing the KBr disks.

The first sequence studied (P₁) is a short peptide of only 4 residues, but it has a great β-sheet propensity as evident from the high <SPᵢ> value of 5.5 and <Pᵢ> = 1.46. Small peptide fragments can well stabilise in the β-sheet structure in the solid state. Moreover, the peptide can also be stabilised by the hydrophobic interaction of the bulky side chains of Ile and Val. The IR spectrum of the peptide bound to BDDMA-PS and DVB-PS is given in Figure 5.4.

Figure 5.4. FTIR spectrum of (P₁) bound to (a) DVB-PS and (b) BDDMA-PS.
It can be seen that the peptide assumed a typical β-sheet structure on DVB-PS as judged from their peaks at 3284 cm\(^{-1}\) (amide A) and 1642 cm\(^{-1}\) (amide I) region.\(^5\)\(^6\)\(^7\)\(^8\)\(^9\)\(^10\)\(^11\)\(^12\) But these peaks were shifted to 3425 cm\(^{-1}\) and 1658 cm\(^{-1}\) when bound to BDDMA-PS. These are the characteristic absorptions of a disordered conformation.\(^8\)\(^12\)

Similarly the next three peptides (P\(_6\)), (P\(_m\)) and (P\(_6\)) also have great tendency towards the β-sheet formation. All of them have \(<\text{SP}_p>\) values greater than 5.0 and their \(<\text{P}_p>\) values are also relatively high. Narita has shown that those peptides with \(<\text{P}_c>\) values less than 0.85 and \(<\text{P}_p>\) greater than \(<\text{P}_a>\) prefers β-sheet in the solid-state and it is very difficult to disrupt these structure even with electron-donating and electron-accepting solvents.\(^15\) But, when these peptides are assembled on BDDMA-PS, their β-sheet structure is disrupted as seen from the shift of the amide A band from 3280 to 3420 cm\(^{-1}\) and in the amide-I band from 1640 to 1665 cm\(^{-1}\). The IR spectrum, obtained for these sequences when bound to BDDMA-PS and DVB-PS are given in Figures 4.5-4.7.
The sequence (P₅), is also well stabilised in β-sheet structure. It contains only the hydrophobic amino acids and contains a AVA moiety which is a strong β-sheet inducer. The peptide dissolves only in DMSO/DCM mixture and it is again a strong indication of its β-sheet propensity. But when the support was changed from DVB-PS to BDDMA-PS, amide A band was shifted from 3285 to 3415 cm⁻¹ and amide I band was shifted from 1639 to 1666 cm⁻¹. This corresponds to a transition from β-sheet to random coil conformation (Figure 5.8).

The sequence (P₅) also has a β-sheet propensity, although its <SP> value is 4.75. But Narita has shown that for penta and hepta peptides with SP values in the range 3 to 5 will be sufficient to stabilise in β-structure. The IR spectrum of this peptide, bound to both DVB-PS and BDDMA-PS, shows a transition from β-sheet to random coil conformation (Figure 5.9).

All the peptides investigated so far (P₁ to P₅) adopt a β-sheet structure when bound to DVB-PS and assumes unordered conformation on BDDMA-PS. Solubility studies on a wide range of solvents were conducted to substantiate the β-sheet structure propensity of these protected peptide
fragments. Insolubility generally originates from their aggregation through two types of intermolecular interactions, viz. hydrogen bonding and van der Waals interaction. Narita et al. showed that solvents of high electron accepting and electron donating numbers (AN and DN) were useful for β-sheet disruption and hence dissolution.\textsuperscript{15,43,44} The presence of β-branched amino acids like Ile and Val increases the tendency for association by van der Waals forces. β-sheet forming tendency of these amino acids were well reported.\textsuperscript{13,15} Narita et al. also studied the conformation of these peptides in various solvents (Table 5.4).\textsuperscript{16}

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|c|c|}
\hline
Peptides & DCM & DCM: DMSO (9:1) & DCM: DMSO (4:1) & DMSO \\
\hline
VAVI & β (A) & γ (A) & -(A) & -(A) \\
KVAVI & β/γ (B) & β/γ (B) & β/γ (A) & γ (A) \\
NKVAVI & β (C) & β (B) & β (B) & γ (A) \\
ANKVAVI & β (C) & β/γ (C) & β/γ (C) & γ (A) \\
VAVAAG & β (C) & β (C) & β/γ (C) & γ (A) \\
QVGQELG & β (C) & β (C) & -(A) & γ (A) \\
\hline
\end{tabular}
\caption{Effect of solvents upon the conformation of the peptides}
\end{table}

\textsuperscript{A} - completely soluble, \textsuperscript{B} - soluble on heating, \textsuperscript{C} - insoluble, \textsuperscript{β} - β-sheet structure, \textsuperscript{γ} - random coil conformation.
Conformational Analysis of Resin Bound Peptides

Goodman et al. reported that the formation of helix and β-sheet structure begins at hepta or penta peptide levels in solution. But in solid state, no critical chain length has been found, though some reports have appeared.

The remaining three peptides (P_{II}), (P_{II'}) and (P_{I}) used in the present study are the fragments of ACP. Solid-phase synthesis of ACP (65-74) fragment was reported to be a challenging task due to the internal aggregation of the peptide by β-sheet formation especially after the deprotection of 9th residue. So, samples were collected after the attachment of 8th, 9th and 10th residues from both DVB-PS and BDDMA-PS resin and spectrum was recorded (Figures 4.10-4.12). DVB-PS samples collected after the attachment of 8th residue (P_{II}) gave a sharp peak at 1639 cm\(^{-1}\) and a smaller peak at 1658 cm\(^{-1}\). Similarly peaks were obtained at 1646 and 1638 cm\(^{-1}\) in the amide I region for samples collected after 9th and 10th residue, (P_{II'}) and (P_{I}) respectively. The amide A region showed peaks at 3298, 3310 and 3286 cm\(^{-1}\) respectively. These peaks can be assigned to β-sheet structure. When the support was changed to BDDMA-PS, the above peaks were shifted to 1660, 1662 and 1665 cm\(^{-1}\) respectively in the amide I and
3408, 3415 and 3412 cm\(^{-1}\) in the amide A region for samples (P\(_{ii}\)), (P\(_{iii}\)) and (P\(_{iv}\)). So it was clear that a clear transition from \(\beta\)-sheet to unordered conformation does occur as the support was changed from DVB-PS to BDDMA-PS. But a shoulder was observed for (P\(_{iii}\)) bound to BDDMA-PS at 1648 cm\(^{-1}\). This shows that a fraction of the peptide chain still assumes \(\beta\)-sheet in BDDMA-PS also. Thus, it can be seen that the aggregation of the pendant peptide chains by \(\beta\)-sheet formation during SPPS can be largely suppressed on BDDMA-PS support.

![Figure 5.12. FTIR spectrum of (P\(_{iv}\)) bound to (a) DVB-PS and (b) BDDMA-PS](image)

| Table 5.4. Amide A and I absorptions of DVB-PS and BDDMA-PS bound peptides |
|-----------------------------|---------------------|---------------------|---------------------|---------------------|
| Sample Code | Sequences | Amide A (3200-3500 cm\(^{-1}\)) | Amide I (1600-1700 cm\(^{-1}\)) |
| | | DVB-PS | BDDMA-PS | DVB-PS | BDDMA-PS |
| P\(_i\) | VAVI | 3284 | 3425 | 1642 | 1658 |
| P\(_{ii}\) | KVAVI | 3282 | 3428 | 1646 | 1666 |
| P\(_{iii}\) | NKVAVI | 3281 | 3420 | 1646 | 1666 |
| P\(_{iv}\) | ANKVAVI | 3278 | 3418 | 1640 | 1662 |
| P\(_v\) | VAVVVG | 3285 | 3415 | 1639 | 1666 |
| P\(_{vi}\) | VQGVQVELG | 3282 | 3420 | 1643 | 1658 |
| P\(_{vii}\) | AAIIDYING | 3288 | 3396 | 1639 | 1658 |
| P\(_{viii}\) | QAIIIDYING | 3310 | 3412 | 1646 | 1662 |
| P\(_ix\) | VQAIIIDYING | 3286 | 3415 | 1638 | 1665 |
Though various evidences are there to believe that desegregation of resin bound β-sheets are taking place on the new support BDDMA-PS, no clear cut picture is available on the mechanism of disruption of β-sheet structure. However, in peptidyl resins, the sharp peak observed at 1720 cm⁻¹ due to the ester carbonyl group of the crosslinker BDDMA-PS was broadened and shifted to about 1705 cm⁻¹ (Figure 5.13). This may be probably due to the interaction of the growing peptide chain with this carbonyl group via NH...O=C< hydrogen bond. In presence of polar aprotic solvents, this kind of hydrogen bonding is possible due to the flexibility and low crosslink density of the matrix. Once such a bond has established, the peptides cannot acquire regular structural patterns due to the intervention of carbonyl group. β-sheet disrupting property of solvents like NMP and DMSO has been well established. Such solvents can well solvate the peptide chains by more strong interaction with the amide bonds than the intermolecular hydrogen bonding among the peptide chains. So all terminal amino groups can be well available for reaction. So, the couplings when performed in NMP-DMSO mixture (10 to 20% v/v), even the otherwise difficult couplings can be easily conducted on BDDMA-PS support. The higher homologue of BDDMA, namely hexanediol diacrylate (HDODA)-crosslinked polystyrene has also shown this property of β-sheet disruption during SPPS.\textsuperscript{50}
References


