Chapter 6

Self-aggregating cyclic systems

Chapter 4 and 5 discuss the utility of cyclic peptide and peptidomimetic systems as ion carriers and drug carriers. So far the usage of such compounds in monomeric form has been discussed. However some cyclic peptide systems are prone to self aggregation.

6.1 Introduction to self aggregated systems

Self aggregating systems offer a variety of potential applications: from fabricating materials, to optoelectronics, to formation of nanotubes and drug delivery systems [137-142].

Carbon nanotubes are also one such exciting example: These have been extensively studied experimentally as well as theoretically [137, 143-147]. These self aggregated forms have also been used for carriage of various types of materials [148-153].

Organic nanotubes have been synthesized by self aggregation of cyclic peptides [154-158]. Recent studies have highlighted the use of these organic nanotubes as mimics of biological channels, molecular adapters for pore forming proteins, transport vehicles in drug delivery systems and other nanostructural materials [159-170]. Fig. 22 and 23 depict some usages of such self aggregated systems. The interesting aspect is that the dimensions and properties can be toned according to needs.

In this chapter conditions for self aggregation in cyclic systems have been explored keeping in mind their prospective usage in aggregated form.
Self-assembling cyclic peptides as structure guides for nanostructured soft materials.

Peptide subunit can be assembled as transmembrane ion channels and artificial pore structures. Assemblage depends on peptide sequence and experimental conditions.

**Usage:** Solid state tubular arrays, surface supported composites, transmembrane ion channels etc.

Fig. 22 Some applications of self aggregating cyclic peptides
8, 10 and 12 cyclic peptide subunits form tubular structures with internal pore dimensions in the range of 7-13Å. They serve as conduit for water. They can function as size selective ion channel. They can be used to transport biologically relevant molecules like glucose across cell membranes.

Fig. 23 Self aggregating cyclic peptides as artificial membranes and channels
The properties of a cyclic self aggregated peptide depend upon the backbone and type of substituents used [171-175]. However, changes in stacking behaviour with slight change in backbone and number of residues have not been clearly understood. We have first studied some peptide and peptidomimetic systems in their monomeric form to understand change in properties with size and type of backbone and substituents.

6.2 Effect of substituent and backbone on monomer shape and size

Fig. 24 depicts optimized conformations for different monomers with peptide or peptidomimetic backbone. All monomers containing peptide backbone and same number of residues show same inner diameter of 12.9 Å. Outer dimensions obviously depend on substituent. (Ala)$_{12}$ monomer with CH$_2$NH backbone or CO-O- backbone shows inner diameter of 10.1 Å and 10.9 Å respectively. This is how the type of backbone and the size of backbone determines the diameter of the pore formed by aggregated form of these cyclic systems.

6.3 Dimerization tendency

Dimerization tendencies of various monomers judged by intermolecular interaction calculations are shown in figs. 25 - 27. This interaction has been calculated after complete optimization of dimer. (Ala)$_{12}$ system with peptide backbone shows good dimerization tendency when both monomers are in eclipsed orientation as well as when both the monomers are in staggered orientation (c.f. fig 25). Staggered orientation is preferred due to obvious alleviation of repulsive interactions between alanine substituents of one monomer with that of the other.
Fig. 24 Effect of backbone on size of cyclic system
Interaction energy = -75.58 kCal/mol  
System-Dimer (Ala)$_{12}$  
Back bone - Peptide linkage

Interaction energy = -84.50 kCal/mol  
System-Dimer (Ala)$_{12}$  
Back bone - Peptide linkage

Repulsive interaction  
System-Dimer (Val)$_{12}$  
Back bone (-CO-NH-) linkage  
No dimerization tendency with large hydrophobic substituent

Fig. 25 Effect of hydrophobic substituents on dimerization tendency of cyclic peptides
Fig. 26 Effect of polar substituents on dimerization tendency of cyclic peptides
Fig. 27 Effect of backbone on dimerization tendency of cyclic peptides
(Val)$_{12}$ system does not show any dimerization tendency even in staggered orientation due to large size of substituents which cause repulsion and steric hinderances. (Gln-Ala)$_6$ system also shows appreciable dimerization tendency as the glutamine residues of monomers can interact by H-bonding and electrostatic interactions (c.f. fig. 26). Steric problem is alleviated due to alternating alanine residues. A twisted arrangement of monomers is preferred in this case.

Next we have considered dimerization tendency of monomers containing peptidomimetic backbone. In this case (Ala)$_{12}$ system has been studied to avoid any steric problems. This will enable us understand most suitable backbone for self aggregation of such systems. (Ala)$_{12}$ with CH$_2$NH backbone (c.f. fig. 27) does not show any possibility of self aggregation due to highly hydrophobic backbone. This system is ideal for enhancing levodopa bioavailability in monomeric form as suggested in previous chapter. (Ala)$_{12}$ system with (CO-O-) backbone shows only little tendency to self aggregate as the backbone contains enhanced flexibility and tends to pucker in a way not suitable for efficient self aggregation. These results are also depicted in fig. 27.

Self aggregation tendency of such systems is thus a close interplay of substituent type and backbone type. It is also dependent on overall size of system (i.e. inner diameter of cyclic system). However, in this study size of system studied is suitable for application under consideration.

### 6.4 Nanotube formation by self aggregation of cyclic peptide

(Ala)$_{12}$ system was chosen to study tubular formation by self aggregation. (Ala)$_{12}$ system should give rise to tubular structure that is largely hydrophobic
and inert from inside [176] as well as outside. Such an aggregated system may be used as drug delivery system to deliver contents inside cell at target.

Self aggregation in (ala)_{12} system was studied by increasing number of monomers by one each time and evaluating interaction energy until enhanced tendency for aggregation was being observed (i.e. until interaction energy was increasingly negative). As the aggregated system becomes larger, artifacts due to methodology tend to creep in. Therefore, stacking efficiency has been studied at larger basis set as well evaluating basis set superimposition error (BSSE) simultaneously each time until computational facilities at hand could support ab initio HF calculations. Results for the same are collected in table 3. Some of the results are depicted in fig. 28. An efficient aggregation of 14 monomer units was observed after which interaction starts to decrease. Therefore, a small hydrophobic substituent in a cyclic peptide allows nanotube formation of diameter ~ 14Å and length 71-75Å. This length is sufficient enough to allow usage of these systems as drug delivery systems. The optimum distance between two stacked rings is between 2.6 – 3.1Å. This is the nearest distance between carbonyl of one backbone and –NH- of the adjoining monomer backbone.

The length to width ratio of tubular structure formed could be an important parameter in judging the strength and stability of nanotube formed similar to aspect ratio for fibres. To investigate this aspect results for (ala)_{10} are shown in fig. 29. For (ala)_{12} length to diameter ratio predicted in 4 as compared to a value of 3 for (ala)_{10} system. Extrapolation of these results predicts a value of 1 for (ala)_{6}. (Glu-ala-gln-ala)_n system has been extensively prepared and studied by Ghadiri et al. They have also observed self aggregation only when n= 2 – 4[143].
<table>
<thead>
<tr>
<th>Number of Monomer units stacked</th>
<th>(Ala)₁₂ SYSTEM</th>
<th>(Ala)₁₀ SYSTEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>INTERACTION ENERGY (in kCal/mol)</td>
<td>Int. Energy (Ala)₁₀ (kCal/mol)</td>
</tr>
<tr>
<td></td>
<td>STO-3G</td>
<td>6-31G</td>
</tr>
<tr>
<td></td>
<td>Without BSSE</td>
<td>with BSSE</td>
</tr>
<tr>
<td>2</td>
<td>-14.24</td>
<td>-8.46</td>
</tr>
<tr>
<td>3</td>
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<td>-236.00</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>-183.10</td>
<td>-</td>
</tr>
</tbody>
</table>
3 monomer units stacked  
Int. energy = -28.81 kCal/mol

5 monomer units stacked  
Int. energy = -57.19 kCal/mol

7 monomer units stacked  
Interaction energy = -88.36 kCal/mol

9 monomer units stacked  
Interaction energy = -114.48 kCal/mol

11 monomer units stacked  
Interaction energy = -158.23 kCal/mol

13 monomer units stacked  
Interaction energy = -176.40 kCal/mol

14 monomer units stacked  
Interaction energy = -236.00 kCal/mol

Fig. 28 Self aggregation in (Ala)$_{12}$ system
3 monomer units stacked
Interaction energy = -27.28 kCal/mol

4 monomer units stacked
Interaction energy = -42.46 kCal/mol

5 monomer units stacked
Interaction energy = -56.06 kCal/mol

6 monomer units stacked
Interaction energy = -69.34 kCal/mol

8 monomer units stacked
Interaction energy = -97.14 kCal/mol

9 monomer units stacked
Interaction energy = -134.37 kCal/mol

Fig. 29 Self aggregation in (Ala)₁₀ system
If cyclic system is too small efficient aggregation cannot be observed due to strains in the ring and if it is too large flexibility introduced in backbone does not allow efficient self aggregation. These results are in agreement with our theoretical predictions.

6.5 **Self aggregated cyclic peptides as drug delivery Systems**

Some natural peptides of diverse microbial origin possess the ability to self aggregate at lipid phase and form trans membrane channels across lipid bilayers. Such peptides have interesting potential to be used as artificial membranes. However, the design and synthesis of artificial system is not an easy task. Considering work in progress by several groups along these lines, we have explored the usage of self aggregated cyclic peptides as drug delivery systems. Many drugs have their targets intracellular resulting in poor bioavailability. Some may lead to toxic effects when taken orally. In such cases a drug delivery system may be used to reduce toxicity and enhance therapeutic index. Carriage of antibiotic gentamicin encapsulated in peptide tubular structure has been considered in this study. Gentamicin is an aminoglycosidic antibiotic used to protect us against a wide spectrum of bacteria gram positive as well as gram negative [177]. Its use is restricted to low doses due to accumulation of its residues in kidney [178]. Delivery of gentamicin at target may reduce its harmful effects.

6.5.1 **Design of drug delivery system for gentamicin**

Chemical structure for gentamicin and its completely optimized conformation are shown in fig. 30. A minimum length drug delivery system formed by (ala)$_{12}$ cyclic peptide was taken to encapsulate gentamicin.
Fig. 30 (a) Chemical structure of gentamicin (b) Optimized gentamicin (c) Empty carrier (d) Complex and (e) Reorganized carrier
Desired amount of self aggregation may be achieved by pH control [179-180]. Complex was then subjected to complete minimization to understand reorganization in delivery system required to efficiently carry gentamicin and to understand whether interaction is strong enough to hold gentamicin until delivery at target. These results are also shown in fig. 30. Reorganizations required in carrier as well as drug are both small (carrier reorganization =1.07 kCal/mol, drug reorganization = 0.46 kCal/mol ) facilitating unhindered carriage of drug. Overall interaction energy is also reasonable to hold it until delivery at target such that it will not slip away before delivery. Release of drug at target may be natural by passive diffusion or by enzymatic disintegration of peptide carrier.

Further, to simulate more realistically we have taken drug in solvated form. Again carrier and solvated drug complex have been completely optimized. Water molecules also orient to give maximum stabilization to complex. Results for the same are depicted in fig. 31. For solvated drug carriage also results indicate low reorganization and appropriate carriage efficiency.

Overall results indicate that self assembled cyclic peptides can be used in drug delivery systems to enhance bioavailability of certain drugs acting intracellularly.
Interaction energy of solvated gentamicin and carrier = -102.97 kCal/mol

Solvation energy of gentamicin = -97.89 kCal/mol

Fig. 31 Solvation of gentamicin and carriage of solvated gentamicin in a peptide delivery system
6.6 Summary

Following inferences are drawn from this study:

1. In self aggregating cyclic systems the type of backbone and size of backbone determines the diameter of pore formed.

2. Flat cyclic systems with small substituents favoring intermolecular H-bonding show appreciable self aggregation tendency.

3. Highly hydrophobic backbone does not allow self aggregation.

4. Small hydrophobic substituent in a cyclic peptide allows nanotube formation of diameter ~14 Å and length 71-75 Å. The optimum distance between two stacked rings is between 2.6 - 3.1 Å.

5. Trimer of cyclic peptide (ala)₃ has been suggested as a carrier for antibiotic gentamicin to avoid its side effects and enhance bioavailability and thus its therapeutic value.