The major goal of this thesis has been to explore the preferred conformations of the two structurally as well as functionally related immunomodulating tetrapeptides tuftsin and rigin. The occurrence of two structurally related natural peptide ligands on Ig G molecule, provides excellent biological probes for elucidating structure-function relationships of these potential pharmacophores. It may be emphasised that eversince the discovery of tuftsin, about three decades ago, by Najjar and Nishioka in 1970, its conformational investigation has been the subject of "heated scientific discussion". Our detailed comparative theoretical as well as experimental conformational analysis of tuftsin and rigin may provide some answers for their specific functions in the living systems. Our analysis described in this thesis may lead to the following conclusions:

1. One of the most interesting feature proposed is that the backbone conformation of tuftsin and rigin, as deduced from MD simulation studies in *implicit* DMSO and explicit water, is a novel type VII β-turn structure, devoid of intramolecular hydrogen bonding interaction. To the best of our knowledge, this work describes the first spectroscopic characterisation of a type VII β-turn structure in solution for a bioactive peptide.

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This β-turn structure was originally defined by Lewis *et al.* and detailed by Zimmerman and Scheraga, almost two decades ago, while analysing the chain reversals in globular proteins, whose structures have been solved by X-ray diffraction. It may be noted that the occurrence of a type VII β-turn structure is extremely rare in proteins and polypeptides. Lewis *et al.* reported the occurrence of only two examples of type VII β-turn structures from the analysis of 135 bends characterized in 8 protein crystal structures whereas, Chou and Fasman revealed the existence of eight type VII β-turn structures from the analysis of 459 β-turn structures from 29 proteins. The observed backbone torsion angles defining the type VII β-turns are summarized in Table 7.1. As evident from the Table that of the 8 turn structures, only one structure seem to be stabilized by a classical 4→1 intramolecular hydrogen bond, the N···O distance being 3.0 Å in Staphylococcal nuclease. Our conclusions about the type VII β-turn structure derived from theoretical and spectroscopic data, corroborate well with the results obtained from the analysis of protein crystal structures.

It would be of some interest to mention the findings of Prof. J.S. Richardson, who while reviewing the anatomy and the taxonomy of the protein structures although, described the occurrence of 9 examples of type VII β-turn structures, not

Table 7.1. The protein segments that are reported to adopt type VII β-turn structure. The residue numbers in the primary structure, the backbone torsion angles (°) and the relevant parameters to define the turn structures are summarized (Adopted from the review of Chou and Fasman).

Protein	Peptide Segment	$C^{\alpha}_1 \cdots C^{\alpha}_4$ (Å)	$O_1 \cdots N_4$ (Å)	ϕ_{i+1}	ψ_{i+1}	ϕ_{i+2}	ψ_{i+2}
α-Chymotrypsin (bovine)	Ser(75)-Ser-Ser-Gly(78)	5.5	5.5	-78	-25	172	127
Concanavalin A (jackbean)	Glu(166)-Gly-Ser-Ser(169)	6.9	5.3	-120	175	52	38
Hemoglobin (glycera)	Ile(118)-Gly-Gly-Lys(121)	6.5	4.2	-54	170	27	88
Lactate dehydrogenase (dogfish)	Ala(46)-Asp-Glu-Val(49)	5.9	5.7	-112	27	170	133
Papain (papaya)	Gln(114)-Pro-Tyr-Asn(117)	6.7	4.1	--	160	55	32
Ribonuclease S (bovine)	Ser(23)-Asn-Tyr-Cys(26)	7.2	5.9	-56	170	-37	-68
	Asn(34)-Leu-Thr-Lys(37)	5.1	5.0	-113	0	168	54
Staphylococcal nuclease	Pro(47)-Lys-Lys-Gly(50)	3.4	3.0	61	-2	176	-76

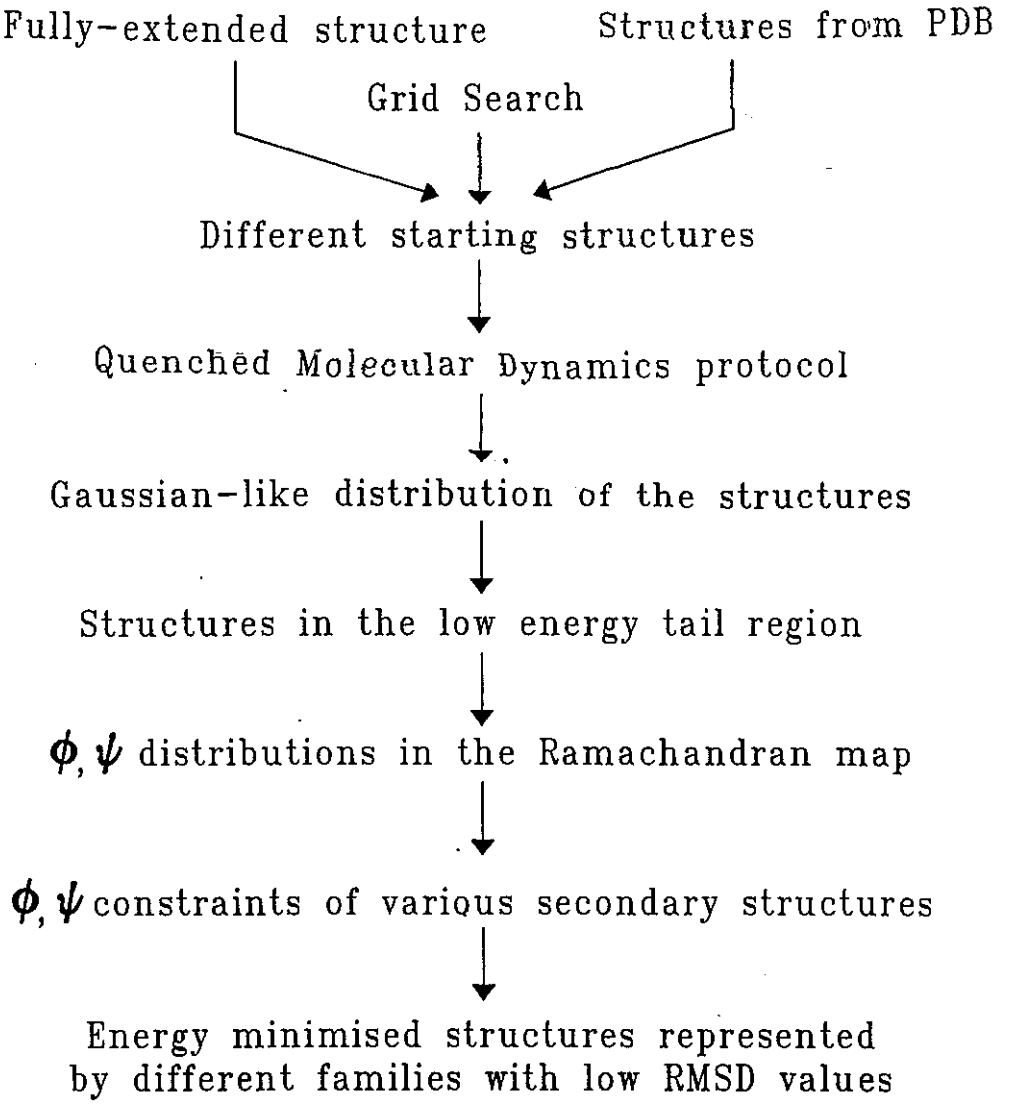
only questioned their existence but also their consideration as a distinct category of reverse turn structures.

Our extensive theoretical conformational analysis of these peptides indeed characterized the existence of type VII β-turn structure, which received strong support from the experimental data. Therefore, we advocate that the type VII β-turn should be considered as a separate class of secondary structure since, it is fully characterizable both theoretically and experimentally.

2. Further, the approach of conformational analysis in the present investigation particularly, employing distance dependent dielectric, is likely to provide critical information regarding their conformation, presumably at the receptor site, bearing in mind the fact that the biologically active peptide usually function via their own specific receptors. The results of conformational analysis of tuftsin are in excellent agreement with those reported by Nikiforovich. In our in *vacuo* MD simulations, the observed molecular conformation of tuftsin is primarily stablized by an electrostatic interaction between oppositely charged Lys ε-amino and the Arg carboxylate groups, which was considered previously the basis for the rigidification to design and develop the equipotent *quasi*-cyclic analog. On the other hand, the predicted three-dimensional structure of rigin in *vacuo*, was stablized by end to end "salt-bridge" interaction. The two distinctly different backbone conformation of tuftsin and rigin however, may disposition their functional side-chains differently which can interact with their own specific receptors. This conclusion finds some experimental support from the fact that though rigin is capable of efficiently displacing [^{3}H]-tuftsin from macrophages however; it does only at high concentration (~ 10^{-4} M).

3. The protocol employed in the present investigation primarily involves the hypothesis by O'Connors *et al.* in 1992. The results of MD simulations of tuftsin and rigin indeed substantiate the hypothesis. Further to analyse the low energy structures, we employed the Ramachandran criteria which generated the peptide backbone conformations that were restricted to sterically allowed regions of the ϕ, ψ map. Subsequently, we considered the possibility of various secondary structural features (C_5-structure, γ-turns, β-turns and extended structures) that can be accessible to such short linear peptides.

The approach depicted in Scheme 7.1 yeilded statistically significant number of structures presumably representing the solution conformations. Conformationally distinct family(ies), with low energy structures, resulted from the analysis expected to exhibit low RMSD values (≤ 0.7 Å) for the backbone non-hydrogen atoms. We


Fully-extended structure
Structures from PDB
Grid Search
Different starting structures
Quenched Molecular Dynamics protocol
Gaussian-like distribution of the structures
Structures in the low energy tail region
ϕ, ψ distributions in the Ramachandran map
ϕ, ψ constraints of various secondary structures
Energy minimised structures represented by different families with low RMSD values

propose that the conformation of at least one of the families can be complemented by experimentally derived ^{1}H NMR parameters. To substantiate this, our investigation on tuftsin and rigin indicates that the conformational characteristics presented by at least one family could be complemented by most of the ^{1}H NMR parameters. It is also reasonable to conclude that the approach is likely to provide a fair amount of knowledge about the distribution of conformational heterogeneity in solution. It may be worthwhile to establish the validity of the protocol for other medium-size peptides (about 5-10 residues).

It may be stated that the precise conformational features of short linear peptides may not be entirely addressed by either MD simulations or the NMR data alone nevertheless, their combinations may permit a fair estimate of the extent of an ensemble of the probable peptide conformations.

4. The conformational analysis of tuftsin has revealed that the sequence largely favours a type VII β-turn structure centered around Lys-Pro residue, under three different environmental conditions. While, rigin tends to adopt type VII β-turn structure in DMSO and water and a distorted type III β-turn structure is proposed in *vacuo* conditions. It was our contention to analyze the CD spectra of proposed type VII β-turn structure. In aqueous media, the CD spectra of tuftsin and rigin tends to exhibit a strong negative band ≤ 200 nm and a very weak negative shoulder at ~ 230 nm, however, the spectral pattern seems to be pH sensitive. It may be noted that both the immunomodulators preferred an energetically favourable type VII β-turn structure in aqueous and non-aqueous environments, interestingly exhibited different CD spectral pattern in aqueous and non-aqueous, organic solvents. The analysis of present CD studies reported for short linear bioactive tetrapeptides clearly substantiate the conclusion that the CD spectral properties of short linear model peptides (whose structures have been established from X-ray crystallography and/or NMR spectroscopy), that are shown to adopt well-defined secondary structural features, point towards the condition that the conformational features solely based on CD spectral pattern may not be straightforward. From the investigation it is also apparent that the type VII β-turn structures are devoid of intramolecular hydrogen bonding interaction consequently, a significant amount of conformational flexibility may be associated with such structures that may get reflected in their CD spectral behaviour.

Finally, it may also be concluded from this thesis that the approach described here may signify the utility of the proposed protocol for rational designing of the peptide drugs by optimal use of computer modelling and then complementing the results by various spectroscopic data for proposing the novel conformational models as well as for the refinement of the proposed models, may be a valuable protocol in absence of crystal structure.