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A novel approach to stabilize collagen analogs through L→D configurational changes

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Abstract

Tanning plays a vital role in leather manufacture. There are several tanning agents which stabilizes the leather making protein collagen against thermal and enzymatic degradation, the classical being chromium and vegetable tannins. Collagenase is an enzyme which has great specificity to degrade collagen; the important parameter for degradation is conformation and peptide sequence. Collagen is a fibrous protein which consist of three left handed polypeptide chains oriented as right handed triple helix with the characteristic triplet sequence Gly-X-Y, where X and Y can be any L- amino acid mostly L- imino acids. Leather industry of future needs to be environmental friendly for the user. One of the ways by which this is possible is to resort to biological crosslinking without affecting the environment and depending on natural resources. In this study, an attempt is being made to stabilize the collagen like peptides against collagenase by changing its conformation through configurational changes. An approach has been proposed for such stabilization by creating an L-D, D-D and D-L configuration in polypeptide chains. It is expected that such conformational changes in polypeptide chains would reduce the accessibility of enzymes. Experimental analysis reveals the substitution of D-Leucine instead of L-Leucine induces structural changes from beta sheet to turn. Temperature interval studies show the substitution of D-Leucine leads to drastic structural changes in the overall spectrum than the host peptide.

1. Introduction

Though eukaryotic systems use almost exclusively L-amino acids in protein synthesis, D-forms as well as unnatural amino acids are known to occur in some animal peptides and microorganisms. Structurally, most of them have a unique alkyl chain bound to a short peptide chain (6-7 amino acids), a cyclic structure, and composed mainly of hydrophobic and acidic L- and D-amino acids. The origin of chiral selectivity within biological systems still remains unclear. The conversion from L- to D-form within peptides and proteins is thought to be the most subtle of all post-translational modifications, which nonetheless may invoke profound biological implications. The importance of amino acid substitution is thought to play a considerable role in the progression of many diseases like Alzheimer's and Parkinson's diseases. Most natural proteins are comprised of 19 L-amino acids and glycine, which is achiral. Although not usually found in natural proteins, D-amino acid residues have conformational attributes that are useful for the imposition of conformational stability and as structural probes. D-Proline, in particular, has been a demonstrably valuable component of artificial reverse turns. Moreover, the non-natural stereochemistry of D-amino acids endows resistance to proteolytic degradation, an attribute important in chemotherapeutic applications. Indeed, many compounds with antimicrobial and antitumor activity contain D-amino acids.

Collagen consists of three individual peptide strands folded into a right-handed triple helix. Each strand is a left-handed polyproline II helix with repeats of the sequence: X_{aa}–Y_{aa}–Gly, in which X_{aa} is often 2S-proline (Pro) and Y_{aa} is often (2S,4R)-4-hydroxyproline (Hyp). Of these residues, the glycine is perhaps the most important, as the structure of a collagen-like peptide containing a single glycine-to-alanine substitution suffers from substantial distortion and destabilization. Moreover, genetic

mutations due to the replacement of a glycine residue greatly destabilize the triple helix which leads to human diseases. Dannenberg and coworkers suggested that replacing the glycine residues in collagen strands with D-alanine or D-serine residues would stabilize the triple helix. Moreover, these workers suggested that D-serine would have a larger stabilizing effect than D-alanine because of the formation of a hydrogen bond between its side-chain hydroxyl group and a carbonyl group in another strand of the triple helix. The implications of these suggestions are enormous, as the glycines of collagen comprise; 10% of the amino acid residues in humans (that is, one-third of the protein in humans times one-third of the residues in collagen).

Leather industry of future needs to be environmental friendly for the user. One of the ways by which it is possible is to resort to biological crosslinking without affecting the environment and depending on natural resources. In this study, an attempt is being made to stabilize the collagen like peptides against collagenase by changing its conformation through configurational changes. An approach has been proposed for such stabilization by creating an L-D, D-D and D-L configuration in polypeptide chains. It is expected that such conformational changes in polypeptide chains would reduce the accessibility of enzymes.

In this paper, stabilization of collagen like peptide (FALGPA) against enzymatic degradation by inverting the stereochemistry of the amino acid (Leucine (L)) from L to D has been investigated. This study reports the structural changes on the collagen mimics containing D-Leu in place of L-Leu. Experimental analyses reveal the presence of D-amino acids in the peptides stabilizes the peptides against enzymatic attack.

2. Experimental

Materials
Chemical reagents were obtained from Sigma Aldrich.

Methods
Structural analysis
CD spectra were recorded on a Jasco J-815 spectrometer, using a Jasco MCB 100 Peltier thermoelectric temperature controller. Peptide solutions used were of 0.5mM concentration and prepared using tris buffer (pH 7.4), equilibrated at 4 °C for more than 48 h prior to analysis. For wavelength scans, the signal was collected from 200 to 350 nm at 1 nm intervals, for 3 s at each point at 25 °C.

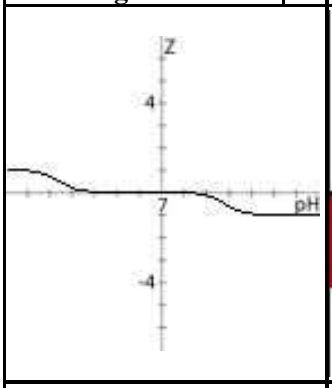
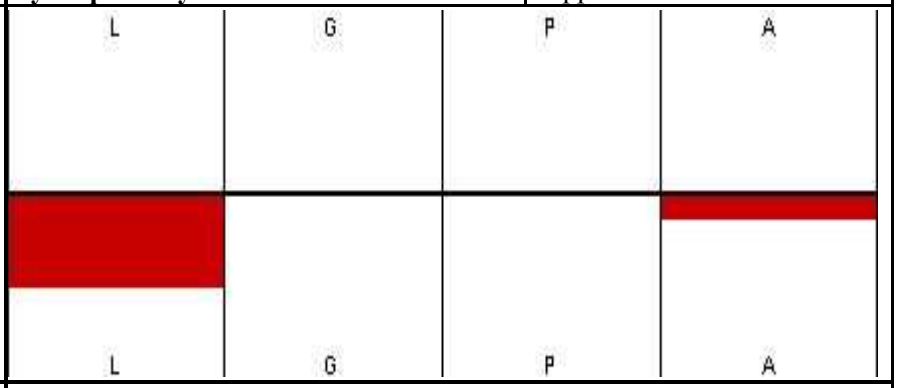
For equilibrium melting temperature (*T*m) transitions, the ellipticity at 303, 231 and 202 nm were monitored for FA(L-L)GPA peptide and 301, 234, 192 nm for FA(D-L)GPA peptide while the sample temperature was increased from 15 to 65 °C at intervals of 1°C. To know the thermal reversibility of the peptides, samples were cooled from 65 to 15 °C at intervals of 1 °C. The data was collected for 10 s at each point.

For enzymatic stability analysis, the peptides were subjected to real time analysis at 37 °C at pH 7.4 for thirty minutes. The concentration of peptide and collagenase was kept constant with increase in co-factor concentration from 2 to 10 mM.

3. Results and Discussion

To address the effect of D- Leucine in collagen like peptide the host- guest approach was selected. The selected peptides and its properties were given in table 1.

Table 1. Peptide properties

Host Peptide: N-(3-[2-FURYL]ACRYLOYL)-(L-LEU)-GLY-PRO-ALA (FA(L-L)GPA)		
Guest Peptide: N-(3-[2-FURYL]ACRYLOYL)-(D-LEU)-GLY-PRO-ALA (FA(D-L)GPA)		
1-letter code: LGPA		
3-letter code: Leu-Gly-Pro-Ala		
Number of residues:	4	
Molecular weight, MW:	356.4	
Net charge	**Hydrophilicity**	Hopp & Woods
		
Net charge at pH 7.0:0 Iso-electric point, pI:6	Average hydrophilicity: -0.6 Ratio hydrophilic residues / total number of residues: 0%	

Hydropathy plot predicts the free energy and hydrophobicity in the natural peptide sequence which was shown in Fig.1. The inclusion of the free energy contributions (ΔG_{Hbond}) of H-bonded peptide bonds in hydropathy plot is important because ΔG_{Hbond} determines the decision level for TM helix selection. The total side chain contribution to the free energy of transfer (−0.54 kcal/mol) was calculated using the octanol interface scale. Estimated ΔG_{Hbond} was 0.47 kcal/mol, which fall in the alpha-helix range from 0.6 to 2 kcal/mol.

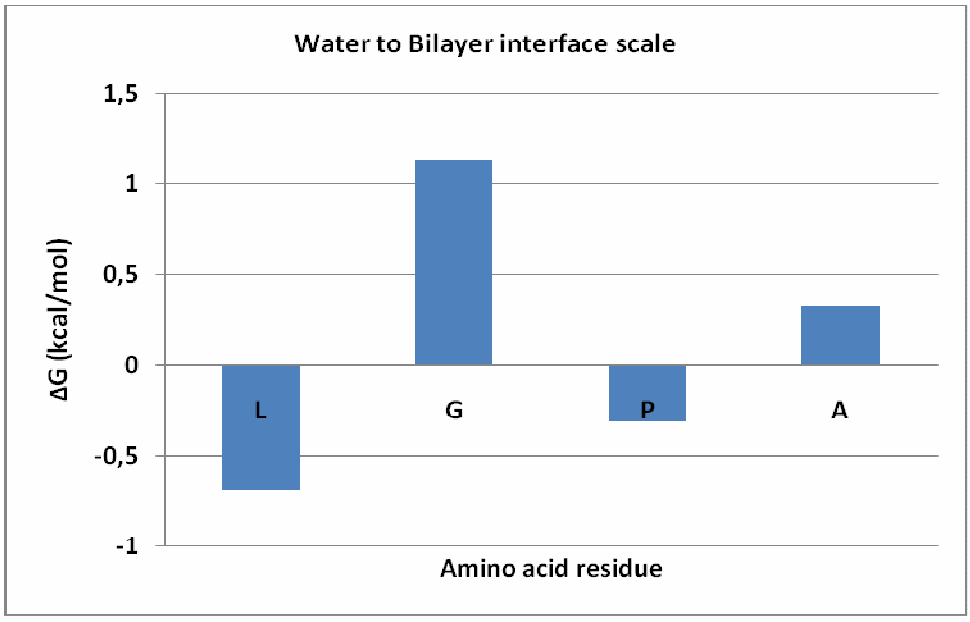


Fig. 1 Hydropathy plot at the water-octanol interface

Structural analysis

To understand the structural changes in the collagen like peptide due to the substitution of D-Leucine instead of L-Leucine in FALGPA CD experiment was carried out. The circular dichroism was measured with respect to wavelength ranging from 190 to 350 nm which was shown in Fig. 2.

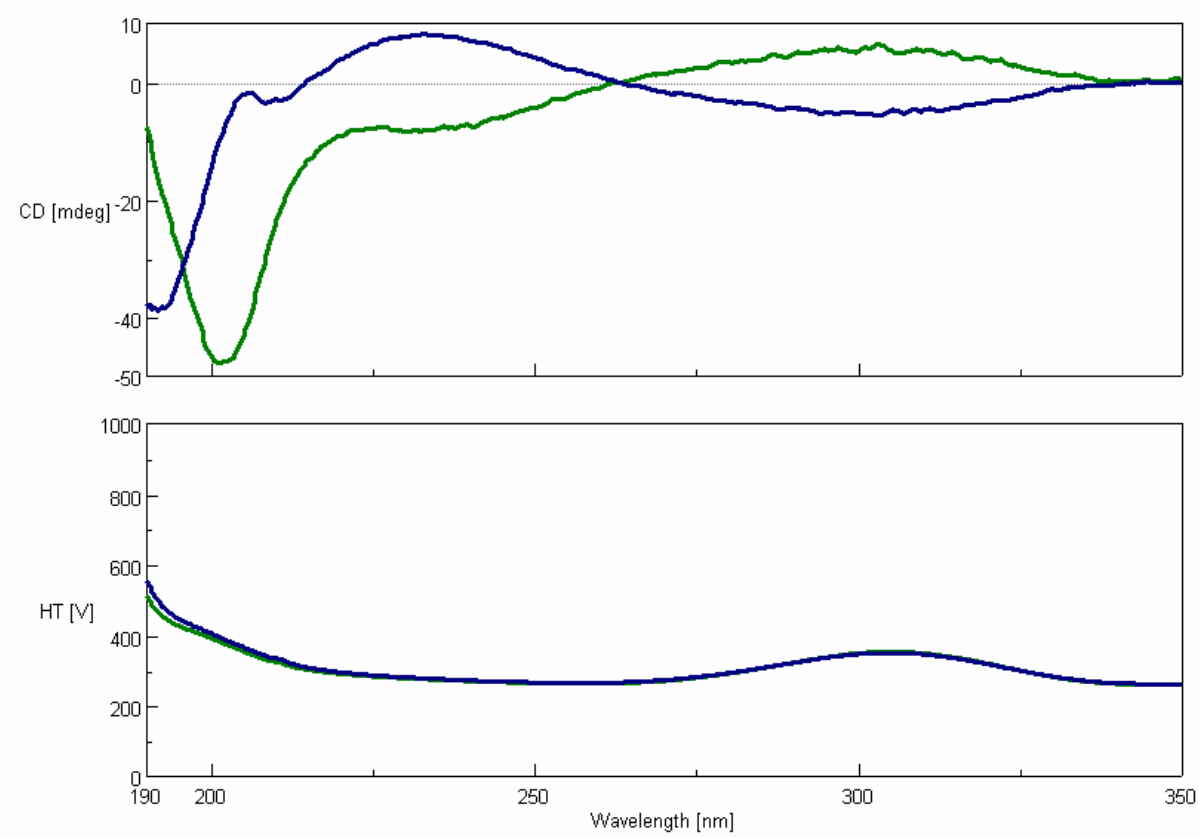


Fig.2. CD spectra of FA(L-L)GPA (green) and FA(D-L)GPA (blue) peptides

The structural analysis was carried using the Young's and Reed's reference data bank. The calculated structural values from the reference data bank was reported in table 2. According to Young's reference, D-Leucine incorporation induces structural changes from beta sheet to turn. In case of Reed's, incorporation of D-Leucine changes beta turn to random coil. These results clearly indicate, D-Leucine incorporation induces drastic structural changes in the host peptide.

To know the structural stability of host-guest peptides with respect to temperature, temperature interval scan measurement was carried out and results were shown in Fig. 3 and 4. The figure shows the structural changes induced in the host peptide by increase in the temperature from 10 to 75 °C. Prominent structural changes were observed at 202 nm.

To know the structural stability in guest peptide with respect to temperature, temperature interval scan measurement was carried out and results were shown in Fig. 5 and 6. The figure shows the structural changes induced in the guest peptide by increase in the temperature from 10 to 75 °C. Drastic structural changes were observed in the overall spectrum.

Table 2. Structural analysis for host-guest peptides

Structure	FA(L-L)GPA		FA(D-L)GPA	
	Young's Reference (%)	Reed's Reference (%)	Young's Reference (%)	Reed's Reference (%)
Helix	0	7.7	0	0
Beta	43.4	25.0	16.6	0
Turn	7.8	2.9	42.0	24.1
Random	48.8	64.4	41.5	75.9
Total	100	100	100	100
RMS Value	32.67	10.73	27.11	53.89

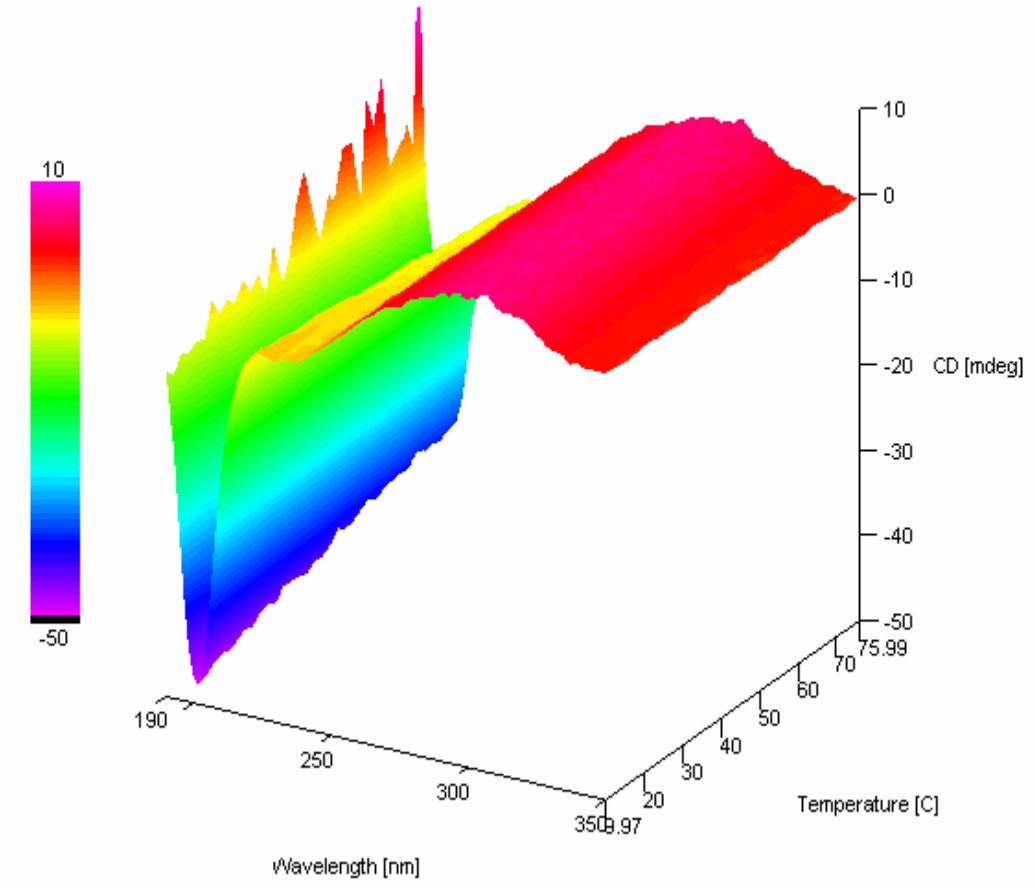


Fig. 3. CD spectra of FA(L-L)GPA (host) peptide with respect to temperature

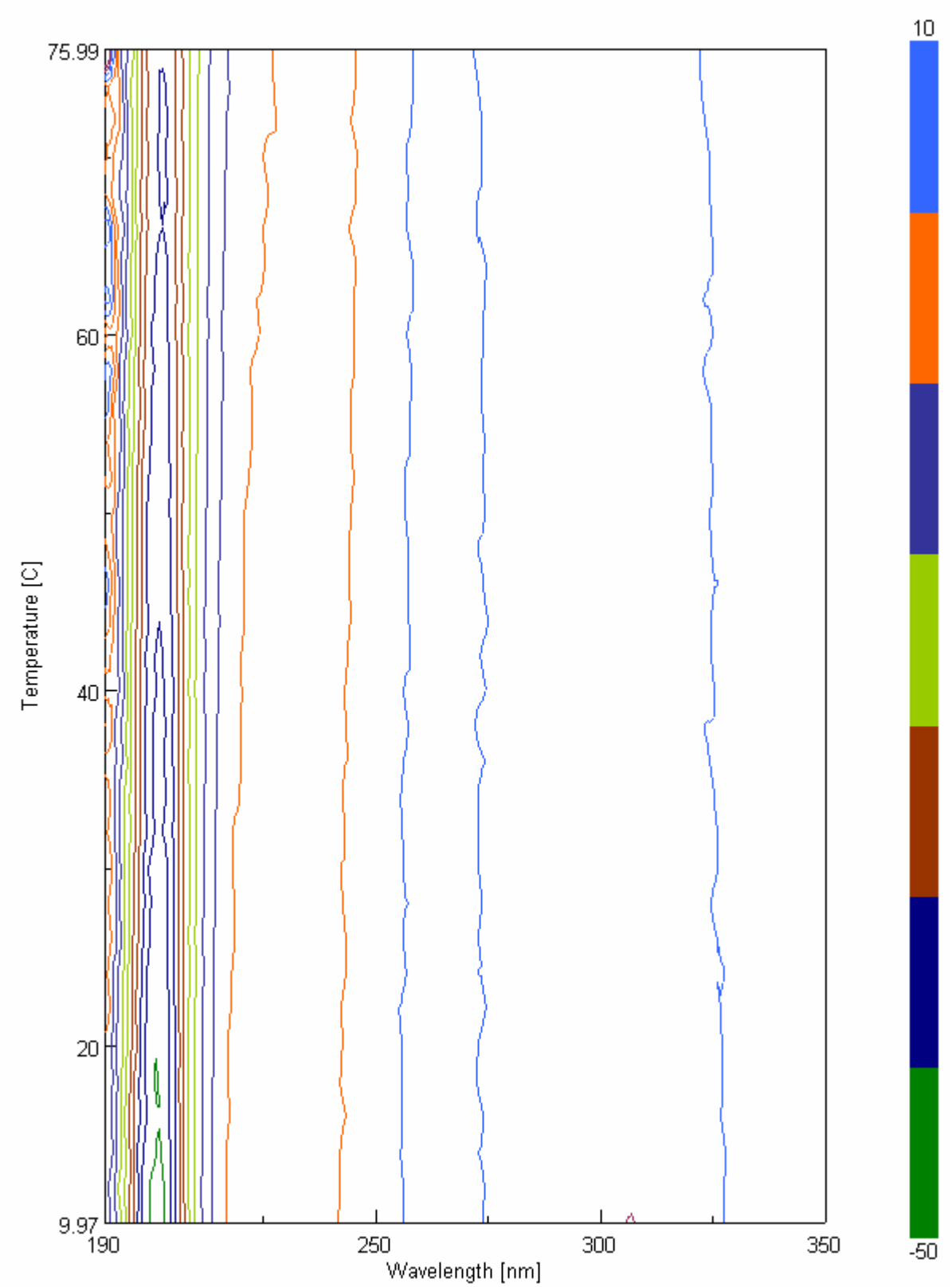


Fig. 4. CD spectra of FA(L-L)GPA (host) peptide with respect to temperature in 2D view

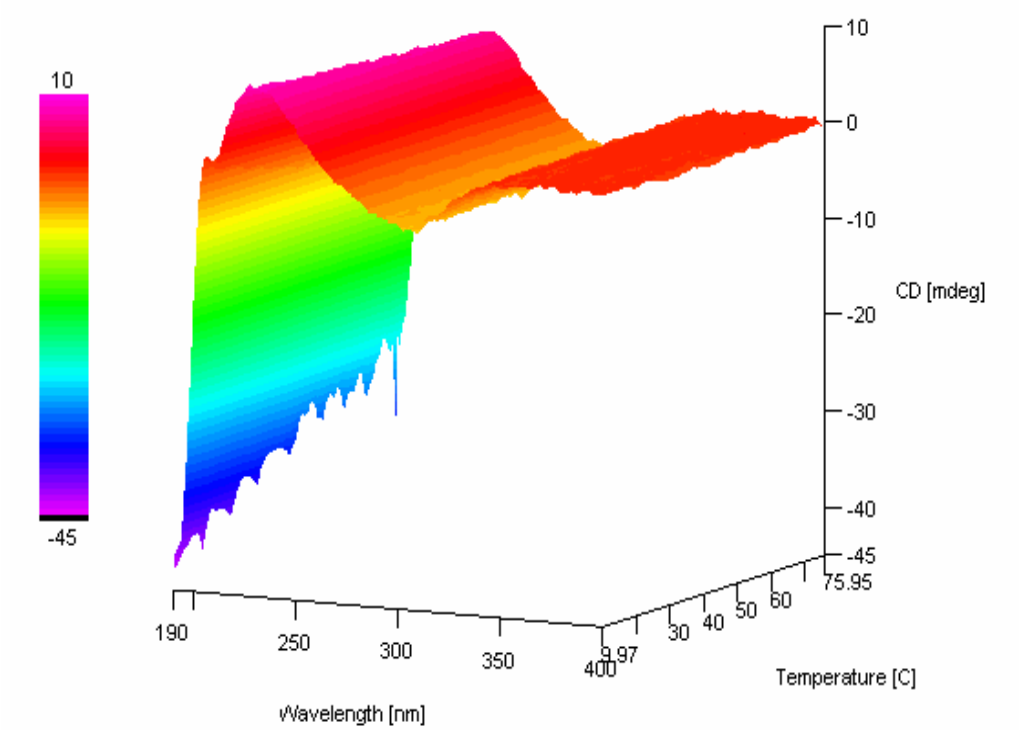


Fig.5. CD spectra of FA(D-L)GPA (host) peptide with respect to temperature

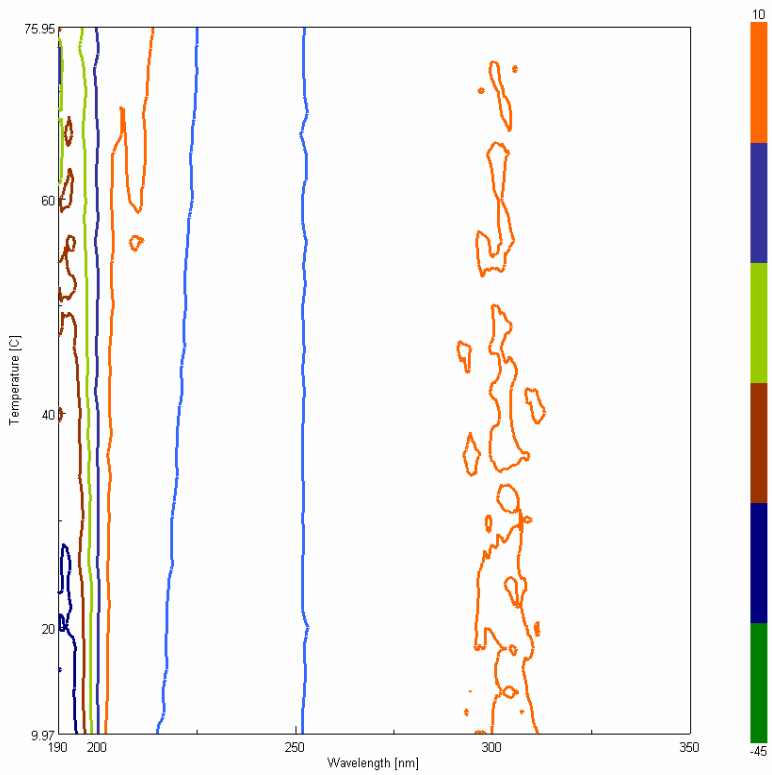


Fig. 6. CD spectra of FA(D-L)GPA (host) peptide with respect to temperature in 2D view

4. Conclusions

Host-guest peptide analysis reveals the substitution of D-Leucine instead of L-Leucine induces structural changes from beta sheet to turn. Temperature interval studies show the substitution of D-Leucine leads to drastic structural changes in the overall spectrum than the host peptide.

5. References

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