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# Comparative study of antitumor active cyclo(Gly-Leu) dipeptide: A computational and molecular modeling study

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ABSTRACT

The conformational behavior and vibrational spectra of cyclo(Gly-Leu) dipeptide, which is an important biological active compound and a therapeudic agent, have been investigated by computational methods. The theoretically possible stable conformers of free cyclo(Gly-Leu) dipeptide in electronic ground state were obtained by performing conformational analysis following DFT calculations. Further, to predict the intermolecular hydrogen bonding interactions in solid phase, various dimer structures were modeled. The optimized geometry and the wavenumbers for cyclo(Gly-Leu) and its dimers have been calculated by DFT method with B3LYP functional, 6-31++G(d,p) basis set. The complete assignment of the bands was performed based on the potential energy distributions (PED%) and experimental wavenumber shifts upon N-deuteration. General agreements between the observed and calculated frequencies are shown. Chemical interpretation of hyperconjugative interactions was carried out by natural bond orbital (NBO) analysis. Finally, HOMO and LUMO energy levels have been calculated.



## 1. Introduction

Cyclic dipeptides commonly found in nature are the simple peptide derivatives. The cyclic structure is formed by linking one end of the peptide (amino-terminus) and the other (carboxyl-terminus) with an amide bond. Generally, cyclic peptides show better biological activity compared to their linear counterparts, due to the conformational rigidity. Many cyclic dipeptides show important bioactivity, acting as anti-angiogenesis and anti-cancer agents [1–5]. Cyclo(glycine-leucine), ($C_8H_{14}N_2O_2$), belongs to the cyclic dipeptides family representing a fertile group of biologically active chemical compound. It has various biological activities such as a great activity against cervical carcinoma cells (HeLa), acting as a dopaminergic neuromodulator, inhibites the growth of colon carcinoma and breast carcinoma tumour cell lines (HT-29 and MCF-7), blocks the narcotic-induced dopamine receptor super-sensitivity, exhibits antimicrobial, antibacterial and antifungal activity, prevents the development of symptoms of neuroleptic-induced tardive dyskinesias and schizophrenia, decreases heart rate, coronary flow rate and ventricular pressure of isolated rat hearts, blocks physical dependence on morphine and facilitates memory consolidation [6–14]. Finally, it was found that cyclo(Gly-Leu) interacs with the dopamine receptors and that the central dopamine receptors may play a role in the pathophysiology of hypertension [15].

Since cyclic dipeptides have many potential biological functions, investigation of preferred conformations is very important to explore their functionary mechanism. Recently, Zhu et al. reported the stable conformations of many cyclic homo-dipeptides including cyclo(Gly-Gly) and cyclo(Leu-Leu), using quantum chemistry calculations [16]. Mendham et al. reported detail vibrational spectroscopic and DFT calculations on cyclo(Ala-Gly), cyclo(Ala-Ala), cyclo(Ser-Ser) and cyclo(Met-Met) dideptides [17–20]. Li et al. reported conformational investigations of cyclo (Arg-Tyr(OMe)) dipeptide by ab initio and chiral experimental methods [21].

Despite the biological importance of cyclo(Gly-Leu), little is known about the complete description of its molecular structure. The NMR results on cyclo(Gly-Leu) indicated that the leucine side chain is in the unfolded conformations [6]. In that study only amide group (amide-I-II and-III) experimental IR wavenumbers were given. Literature survey reveals that conformational analysis, the vibrational spectra, and the theoretical calculations of cyclo(Gly-Leu) have not been reported earlier. Although there are a number of studies on the structure of diketopiperazine crystal [22–24], up to our knowledge no crystal data on cyclo(Gly-Leu) has been reported yet. Thus the dimer is thought to be a good model for the crystal,

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since the diketopiperazine crystal contains chains of molecules connected by hydrogen bonds [22,25]. The aims of this study are to give a complete description of the molecular geometry and molecular vibrations of monomeric and dimeric units of cyclo(Gly-Leu) in the framework of conformational analysis and the density functional method, and to investigate the hydrogen bonding interactions and the coordination effects on the investigated molecule. HOMO-LUMO energy gap and NBO properties of the investigated molecule in monomer and dimer forms were also investigated. The HOMO-LUMO analysis were used to find out information regarding charge transfer within cyclo(Gly-Leu). The NBO analysis gives clear evidence of stabilization originating from the hyper conjugation of various intra-molecular interactions.

## 2. Experimental and computational details

### 2.1. Experimental

The polycrystalline cyclo(Gly-Leu) was reagent grade (Sigma) and used without any further purification. FT-IR spectrum of the title dipeptide was recorded on a Bruker Tensor FTIR spectrometer in the wavenumber range 4000–400 $cm^{-1}$ by KBr pellet technique with a resolution 0.5 $cm^{-1}$ based on averaging 200 sample and 30 backround scans. Micro Raman spectra were recorded by a Jasco NRS 3100 micro Raman spectrometer (1800 lines/mm grating and high sensitivity cooled CCD) equipped with a 532 nm diode laser. Rayleigh scattering was rejected by a notch filter. The spectrometer was calibrated with the silicon phonon mode at 520 $cm^{-1}$. The exposure time, the accumulation and spectral resolution were taken 2 s, 100 spectra and 3.9 $cm^{-1}$ respectively. Spectral manipulations, such as baseline corrections and band fitting procedures, were performed using GRAMS/AI 7.02 (Thermo Electron Corporation) software package. Band fitting was done using Voigt function (a convolution of Lorentzian and Gaussian functions; for Raman), and fitting was undertaken until reproducible and converged results were obtained with squared correlations better than $r^2$ ~0.99995. The second derivative profile gives information about the position of the bands and band widths. Thus for the band fitting procedure (to locate the position of the peaks), the second derivative of the IR (absorption) or Raman spectrum was used as a guide. The second derivatives of the spectra were obtained by using Savitzky–Golay function (two polynomial degrees, 9 points).

### 2.2. Computational studies

Conformational analysis of the cyclo(Gly-Leu) was carried out by searching for the low energy conformations of constitutive residues, using the program described in [26], and Ramachandran maps [27,28]. Then the energies of the preferred conformations were optimized by the Gaussian03 program [29], using DFT method, B3LYP functional and the 6-31 + + G(d,p) and cc-pvqz basis sets. Afterwards, the dimeric forms of cyclo(Gly-Leu) dipeptide were constructed by bringing together two identical monomers in possible configurations. Then we chose the lowest 4 dimeric structures to minimize using the same method and basis sets. Finally we calculated the frequencies of all the optimized structures using DFT/B3LYP/6-31 + + G(d,p) level of theory [29,30]. The harmonic force field for cyclo (Gly-Leu) dipeptide was evaluated with the scaled quantum mechanical force field procedure of Pulay et al. [31]. The definition of internal coordinates for the cyclo(Gly-Leu) is given in Table S1.

The IR intensities, Raman activities and the potential energy distributions (PED) of vibrational modes were computed and the force fields in Cartesian coordinates were converted to natural internal coordinates using by MOLVIB program [32,33]. The Raman activities of cyclo(Gly-Leu) were converted to relative Raman intensity by using the Simirra simulation program [34]. Pure Lorentzian band shapes with 10 $cm^{-1}$ band widths (FWHM) were used in theoretical calculation [35,36].

Optimised scaling factors for monomeric and dimeric forms of cyclo(Gly-Leu) are given in Table 1.

The scaling factors were transferred from the previous studies on other cyclic dipeptides [18,19] and then optimized by fitting the observed frequencies to the calculated ones.

## 3. Results and discussion

### 3.1. Structure

The crystal structure of structure of diketopiperazine (DKP) was found by Corey already in 1938 [22], and was later refined by Degeilh and Marsh [23]. Quite recently the structure was redetermined by Dorset [24]. All earlier investigators found the DKP ring in crystalline structure to be planar (except for the hydrogen atoms of the methylene group). Thus in our study initially the DKP ring is supposed to be planar. For determining the stable conformers of free cyclo(Gly-Leu) dipeptide, firstly, theoretical conformational analysis was performed, by searching for the low energy conformations of constitutive residues. Consequently the preferred conformations of cyclo(Gly-Leu) were computed as functions of side chain dihedral angles, $\chi 1$, $\chi 2$, $\chi 3$, $\chi 4$ [26,37]. The orientation of the side chains playes a key role in the stability of the conformations [16]. The theoretical conformational analysis method permit us to determine the relative positions of the side chain residues of the stable conformations of dipeptide and whole sets of energetically preferred conformers. Afterwards, the most stable conformation of the cyclo(Gly-Leu) was optimized by using DFT/B3LYP level of theory by using 6-31 + + G(d,p) and cc-pvqz basis sets. The calculated structure of the lowest energy for the isolated gas-phase molecule has a boat conformation. The calculation has also been repeated with the DKP ring constrained to be planar since the X-ray structure studies on DKP crystal indicate that the DKP ring is known to be almost planar in solid state [22–24]. But the microwave spectroscopic study on DKP molecule in gas phase reveals that DKP ring is in boat conformation [38]. The most stable form of the free DKP molecule is probably a boat conformation as we have found during the study of the title compound, consistent with previous quantum chemical

**Table 1**
Optimised scaling factors for monomeric and dimeric forms of cyclo(Gly-Leu).

| Monomeric form: | | Dimeric form: | |
|---|---|---|---|
| N—H stretch | 0.89 | N—H stretch (not involving H-bonds) | 0.89 |
| C—H stretch | 0.91 | N—H stretch (involving H-bonds) | 0.98 |
| N—H and C—H deformation | 0.92 | C—H stretch | 0.91 |
| C=O stretch | 0.90 | N—H and C—H deformation | 0.92 |
| All others | 0.98 | C=O stretch (not involving H-bonds) | 0.90 |
| | | C=O stretch (involving H-bonds) | 0.90 |
| | | All others | 0.98 |