VCD spectroscopic study of flexible molecules and molecular complexes

Ph.D. thesis

Sándor Góbi

Supervisor: *Dr. György Tarczay, associate professor*

Roland Eötvös University
Institute of Chemistry
Department of Inorganic Chemistry
Laboratory of Molecular Spectroscopy

Ph.D. School of Chemistry
Theoretical and Physical Chemistry, Structural Chemistry

Head of doctoral school: *Dr. György Inzelt professor of chemistry*
Head of doctoral program: *Dr. Péter Surján professor of chemistry*

Budapest, 2015

I. Introduction

Most of the important biomolecules are chiral, i.e. they have (at least) two non-superposable structures that are mirror images to each other called enantiomers. It has long been known that the two structures show differences in their effects on living organisms (e.g. the Contergan scandal). Thus the determination of absolute configuration (AC) of chiral molecules is an important aspect of molecular stereochemistry. The first traditional method for determining the AC based on the anomalous scattering of X-rays in crystallography. This method requires a perfect single crystal, however, in case of the macromolecules this requirement is not easily met. Moreover, this method requires at least two or three oxygen or heavier atoms. In NMR spectroscopy, chiral shift reagents can be used to obtain different chemical shifts for the enantiomers and identify them. However, in order to achieve this the proper reagent has to be found and the structure of the formed complex must be known.

Compared to X-ray crystallography the vibrational circular dichroism (VCD) spectroscopy does not need the sample to be crystalline; it can be measured in solvent or even in gas phase. Furthermore, the use of chiral reagents is not necessary. One of the further advantages of VCD technique is that the investigated chiral molecules do not need to have any chromophoric groups, in contrast to related methods (e.g the electronic circular dichroism (ECD) spectroscopy). In addition, VCD spectra have more bands than ECD spectra, facilitating AC determination. Due to the availability of efficient electronic structure techniques and codes, interpretation of VCD spectra is more straightforward and more reliable than that of ECD spectra. The AC is determined by comparing the signs of experimental VCD to the calculated spectrum. If they are the same, AC is the calculated configuration; if the signs are reversed the AC is the opposite of the calculated one. VCD can also be used to study conformational behaviour of optically active molecules, because the signs and the intensity of VCD signals are sensitive to molecular geometry and the evaluation can also be made by comparing the calculated to the experimental spectra. VCD spectroscopy have advantages over classical structure determination methods (like FT-IR) as well.

The quantum chemical program packages with VCD capabilities give accurate predictions for VCD spectra of simple rigid molecules without intermolecular

interactions. However, in other cases (like the C=O vibration of the carboxyl and amide groups or flexible molecules) one should be concerned with the reliability of the calculated VCD signs and the possible sources of discrepancies between computations and experiments, such as the effect of intermolecular interactions present in the sample during measurements. This can make the proper assignment difficult, therefore, in order to estimate the reliability of sign and intensity (i.e., the rotatory strength) of computed VCD bands a quantitative measure have been developed called robustness.

The investigations have been performed on molecules with non-reliable VCD signs. Thus the developed robustness estimation method has been tested on mono- and oligomers (and their complexes, respectively) of flexible molecules. The ACs of the experimentally studied compounds were known, this also helped the verification of calculated results.

During my work I have studied the structure of various optically active biomolecules by matrix isolation VCD (MI-VCD) spectroscopy, which was supported by other spectroscopical methods (e.g. FT-IR). Moreover, the effects of perturbations (considering different solvents, formation of dimers or complexes, calculation using different basis sets) on VCD spectra have been evaluated. Finally, a new theory has been established that can indicate the VCD signs that are very sensitive to even the slightest changes in either the experimental conditions or the computational method. If these signs are excluded from the evaluation the determination of the AC and the molecular structure becomes more confident.

The following molecules have been examined:

1. mono-, di- and trimers of N-formyl-N'-methyl amino acids,
2. conformers of N-acetyl-N'-methyl-β^3-homo-proline amide (Ac-β^3-HPro-NHMe),
3. and 2-chloropropionic monomers, dimers and their $CHCl_3$ complexes

II. Experimental and Computational Details

During my work MI-IR and -VCD spectroscopy were used, supplemented by quantum chemical calculations. MI-IR spectra were recorded by a Bruker Equinox 55 and a Bruker IFS 55 FT-IR spectrometer. VCD spectra were obtained by using a Bruker Equinox 55 FT-IR spectrometer combined with a PMA37 VCD module. All spectrometers were equipped with an MCT detector. In the case of MI measurements the samples were studied in Ar and Kr matrix, the solution phase experiments were taken place in BaF_2 and CaF_2 cells with different pathlenghts and depended on the examined compound and its concentration.

The initial geometries were first optimized in the Gaussian program package at the B3LYP/6-31G* level of theory. Then the obtained structures were optimized further by PQS (Paralel Quantum Solutions) software using larger 6-31++G** or aug-cc-pVTZ basis sets. These optimizations were followed by the calculations of harmonic vibrational frequencies, IR intensities, rotatory strengths, their robustness and thermodynamical properties of the molecules. To simulate IR and VCD spectra the calculated frequencies were weighted by using the scaled quantum mechanical (SQM) force field. For spectrum simulations Lorentzian line shapes with 3 cm^{-1} half width at half maximum were applied. These were weighted by the Boltzmann ratios of conformers, which were computed from the Gibbs free energies at sample inlet temperatures. The reliability of VCD signals was estimated by using robustness method introduced by our research group.

In order to take the solvent effect into account optimizations, frequency and rotatory strength calculations were also performed combined with a polarizable continuum solvent model based on the integral equation formalism (IEF-PCM).

Transition state structures and barrier heights between conformers were obtained in Gaussian by the Synchronous Transit-Guided Quasi-Newton (STQN) method at the B3LYP/6-31++G** level of theory.

III. Results and Conclusions

1. A new, theoretically better, gauge-independent measure for the robustness was suggested and has been tested on different flexible molecules. It turned out that the computed signs of robust modes agree well with the experimental VCD signs and take only them into account results similar spectrum to the experimental one. However, signs of non-robust modes (e.g. C=O, C–Cl, C–O) may be different than those of the experimental ones.

2. Robustness greatly helps the assignment of VCD spectra by showing whether the computed signs of vibrational modes can be trusted. Projecting the magnetic transition moment vector onto the electric one of a vibrational mode results a gauge-independent quantity and carries reliable information about robustness. The robustness of a vibrational band can be dubious if its absolute value is under 10 ppm. In this case the band can show unexpected sign changes and should be disregarded during the assignment or must be taken into account with caution. The majority of bands detected in VCD spectra was found to be robust in the case of our experiments.

3. The vibrational frequencies, VCD rotatory strengths and their robustness have been calculated by quantum chemical calculations for the peptide models, denoted by ForAANHMe (where AA denotes the amino acid residues Asn, Asp, Cys and Val, respectively). Dependence of calculated rotatory strengths on the applied basis set, the backbone and side chain geometry of amino acids and the chain lenght of peptide have been examined. Special attention has been paid to the robustness of signs and their changes.

4. The rotatory strength and robustness depend on the applied basis set, but calculations at the B3LYP/6-31G*, B3LYP/6-31++G**, and B3LYP/aug-cc-pVTZ level of theories gave similar results. Furthermore, the rotatory strength and robustness of α- and β-peptides varies greatly and VCD signs of the former ones are more robust. With increasing chain length the calculated VCD spectra show increased similarity with the experimental spectra.

Only conformers with the lowest energy (the most abundant ones) should be taken into account as their signals can only be detected in the experimental spectra.

5. The conformational landscape of Ac-β^3-HPro-NHMe was investigated by using MI-IR and -VCD spectroscopy combined with quantum chemical calculations and the study of robustness. These were supplemented by measurements in solution and solvent model calculations. The results undoubtedly indicate that multiple conformers are present both in noble gas matrices and in solutions.

6. In Ar and Kr matrices three *trans* and one *cis* conformers could be confidently identified. Theoretical calculations were in agreement with experimental observations and showed that the abundance of the *cis* form is exceptionally large; it is above 10% (at 345 K) even for isolated molecules. Larger entropy factors originating from increased flexibility of the elongated peptide backbone chain can explain this increased abundance compared to those of α-proline. It is worth noting that both solvent model calculations and solution IR and VCD spectra revealed that the abundance of *trans* amino acids have been decreased by comparison to MI experiments. Even in a less polar solvent like DCM, the *cis* is the predominant peptide form.

7. On one hand, results clearly indicate that (unlike α-proline, which is an important γ-turn forming residue of natural peptides) the β^3-HPro residue is a much less reliable pseudo-γ-turn forming element. On the other hand, the β^3-HPro residue seems to be a promising *cis* peptide building block of synthetic β-peptides.

8. In addition to results related to the conformational behavior of β^3-HPro residue it must be emphasized that it is crucial to consider both the robustness of rotational strengths and the solvent effects when experimental VCD spectra of flexible molecules are interpreted based on computations.

9. The influence of formation of strongly (dimers) and weakly (complexes with $CHCl_3$) hydrogen-bonded complexes on the VCD spectrum of *S*-(–)- and *R*-(+)-2-chloropropionic acid was investigated. For both the monomers and dimers several conformers and structures were found by computations. For each of these structures IR and VCD spectra were computed, analyzed, and discussed. From the experimental side 2-chloropropionic acid monomers, 2-chloropropionic acid–$CHCl_3$ complexes and also 2-chloropropionic acid dimers were prepared in low-temperature Ar matrix and identified mainly based on the comparison to computed spectra.

10. The complete analysis of MI-IR spectrum of the monomer (showing good agreement with computations) pointed out that along with the two predominant

trans conformers the *cis* form is present in a small amount in the matrix. Relatively larger abundance of the *cis* conformer compared to other carboxylic acids ($\sim$ 6%) may be explained by a weak interaction between chlorine and hydrogen atom of the O–H group. This assignment have been confirmed by a later study that investigated the selective excitation of monomers in Ar matrix and have been measured in our recently established laser laboratory.

11. In the case of 2-chloropropionic acid–$CHCl_3$ complex (in Ar matrix containing $CHCl_3$) species bonded by two weak hydrogen bonds were identified. Considering 2-chloropropionic acid dimers (in pure Ar matrix) beside the dimers bonded by two strong hydrogen bond, higher-energy ones were also identified in the annealed matrix, stabilized by a strong (C=O...H–O) and a weak (C=O...H–C) hydrogen bond.

12. VCD spectra were obtained for the monomers in Ar matrix and for the dimers in solutions. VCD spectra were also taken for 2-chloropropionic acid in Ar matrix containing $CHCl_3$ molecules. The most important conclusions on VCD computations and measurements that can be generalized (and may be important) for VCD studies of other carboxylic acids or (flexible) molecular systems capable of formation of either strong or weak hydrogen bonds can be summarized as follows: in carboxylic acids with an asymmetric α-carbon (since the C=O stretching mode does not couple with vibrational modes in which the chirality center has a large coefficient in the normal mode) the moderately intense VCD signal of the C=O stretching mode is mainly due to the large electric transition dipole of this vibration.

13. The VCD sign of C=O stretchings and all other modes in the 1900–1200 cm^{-1} region are sensitive to the X–C–C=O dihedral angle (X = Cl for 2-chloropropionic acid). Therefore, the VCD spectrum strongly depends on the conformation. The AC determination should carefully include all possible conformers. If careful analysis, e.g. a scan along the X–C–C=O dihedral angle is carried out (and external perturbations are completely excluded), then a cautious configuration analysis based on robust modes should be possible. However, for this particular acid there was not any reliable non-robust band in the spectrum of monomers.

14. Solvation model computations and measurements in un-annealed matrices containing $CHCl_3$ have both showed that averaged, non-directional external electric fields of the solvent or the $CHCl_3$ molecules trapped in the matrix can seriously affect the rotatory strengths of particular modes, mainly the signs of non-robust ones (like that of the C=O stretching and the C–O–H bending mode of

carboxylic acids). This electric effects can cause these changes directly through the altered electronic structure, or indirectly through a change in geometry.

15. The analysis of solution phase IR spectra also proved that in CCl_4 solution the dimers are predominant at 0.02 as well as at 0.1 mol dm^{-3} concentrations. In contrast to these, in $CHCl_3$ solution the monomers or (in a dynamic process) 2-chloropropionic acid–$CHCl_3$ complexes are present in considerable amount.

16. Computations show that weakly bound complexes, stabilized by non-classical hydrogen bonds can substantially change the overall shape of VCD spectrum. Regardless of the robustness of a vibrational mode the average change in VCD intensities is about 20–40%. For some of the low-intensity modes even bigger changes in intensity can be found. For measurements in $CHCl_3$ solution the use of solvation models was found to provide results less accurate than computations performed for molecular complexes. That demonstrates the presence of complexes in solution phase.

17. Calculations show that most of vibrational modes in the formerly achiral part (the $CHCl_3$ molecule) of a chiral-achiral complex are non-robust and have small rotatory strengths. There might be vibrational modes (the H–C–Cl bending of $CHCl_3$ in our case), however, that have medium rotatory strength and are relatively robust modes.

18. Dimer formation drastically changes the VCD spectrum. The spectra measured in relatively concentrated solutions are consistent with the computed spectra of dimers. For dimers larger and more robust rotatory strengths can be obtained. Comparison of the computed spectra of dimers with the experimental ones recorded in relatively concentrated ($\sim$ 0.1 mol dm^{-1}) solution are highly recommended.

19. The VCD spectrum of a carboxylic acid recorded in $CHCl_3$ is more complex and (like our case) can have lower intensities than those of the spectrum recorded in CCl_4 due to the stabilization of monomers and/or formation of weak complexes between the carboxylic acid and $CHCl_3$. Therefore, (even if it is computationally more demanding) if solubility allows, CCl_4 is a much preferred solvent over $CHCl_3$.

List of Publications

Publications related to the PhD thesis

1. S. Góbi, K. Knapp, E. Vass, Z. Majer, G. Magyarfalvi, M. Hollósi, G. Tarczay *Is beta-homo-proline a pseudo-gamma-turn forming element of beta-peptides? An IR and VCD spectroscopic study on Ac-beta-HPro-NHMe in cryogenic matrices and solutions*, Physical Chemistry Chemical Physics, **12**(41), 13603–13615 (2010)
2. S. Góbi, E. Vass, G. Magyarfalvi, G. Tarczay *Effects of strong and weak hydrogen bond formation on VCD spectra: a case study of 2-chloropropionic acid*, Physical Chemistry Chemical Physics, **13**(31), 13972–13984 (2011)
3. S. Góbi, G. Magyarfalvi *Reliability of computed signs and intensities for vibrational circular dichroism spectra*, Physical Chemistry Chemical Physics, **13**(36), 16130–16133 (2011)
4. S. Góbi, G. Magyarfalvi, G. Tarczay VCD *Robustness of the Amide-I and Amide-II Vibrational Modes of Small Peptide Models*, Chirality, **27**, 625–634 (2015)

Conference posters

1. S. Góbi, G. Tarczay *Modellvegyületek vizsgálata VCD spektroszkópiával* (in Hungarian)
   Universitaes Nostrae Scientia Nostra, Budapest, Hungary 2010.
2. S. Góbi, E. Vass, G. Magyarfalvi, G. Tarczay *Effects of classical and non-classical hydrogen bond formation on VCD spectra: a case study of 2-chloropropionic acid*
   XIX. International Conference on Horizons in Hydrogen Bond Research, Göttingen, Germany 2011.

Presentation related to the thesis

1. S. Góbi, E. Vass, G. Magyarfalvi, G. Tarczay
   Klasszikus és nemklasszikus hidrogénkötésű komplexek kialakulásának hatása a 2-klór-propionsav VCD spektrumára (in Hungarian)
   Inorganic and Metallo-organic Chemistry Working Committee Meeting, Hungarian Academy of Sciences, Demjén, Hungary 2011.

Communications not related to the dissertation

Publications

1. G. Tarczay, S. Góbi, E. Vass G. Magyarfalvi *Model peptide–water complexes in Ar matrix: Complexation induced conformation change and chirality transfer*, Vibrational Spectroscopy, **50,** 21–28 (2009)

2. G. Bazsó, S. Góbi, G. Tarczay *Near-Infrared Radiation Induced Conformational Change and Hydrogen Atom Tunneling of 2-Chloropropionic Acid in Low-Temperature Ar Matrix*, J. Phys. Chem. A, **116**, 4823–4832 (2012)

3. G. Bazsó, S. Góbi, G. Magyarfalvi, G. Zügner, A. Demeter, T. Turányi, S. Dóbé, G. Tarczay *Az ELTE-TTK Lézerlaboratóriuma: Első eredmények és kutatási perspektívák* (in Hungarian), Magyar Kémiai Folyóirat, **118**(2-4), 65–71 (2012)

4. G. Tarczay, E. Vass, S. Góbi, G. Magyarfalvi *Rezgési optikai aktivitás – Abszolút konfiguráció és konformáció meghatározása* (in Hungarian), Magyar Kémiai Folyóirat, **119**(1), 53–66 (2013)

5. Cs. Kiss, Gy. Szabó, J. Horner, B. C. Conn, T. G. Müller, E. Vilenius, K. Sárneczky, L. L. Kiss, M. Bannister, D. Bayliss, A. Pál, S. Góbi, E. Verebélyi, E. Lellouch, P. Santos-Sanz, J. L. Ortiz, R. Duffard, N. Morales *A portrait of the extreme Solar System object 2012 DR30*, Astronomy and Astrophysics, **555,** A3 (2013)

6. E. Najbauer, G. Bazsó, S. Góbi, G. Magyarfalvi, G. Tarczay *Exploring the Conformational Space of Cysteine by Matrix Isolation Spectroscopy Combined with Near-Infrared Laser Induced Conformational Change*, J. Phys. Chem. B, **118**, 2093–2103 (2014)

7. Á. Kereszturi, S. Góbi *Possibility of H_2O_2 decomposition in thin liquid films on Mars*, Planetary and Space Science, **103**, 153–166 (2014)

Conference poster

1. G. Tarczay, S. Góbi, E. Vass, G. Magyarfalvi
   Model Peptide - Water Complexes in Ar Matrix: Complexation Induced Conformation Change and Chirality Transfer
   Students for Students, V. International Conference, Cluj-Napoca, Romania 2008.