VCD spectroscopic study of flexible molecules and molecular complexes

Ph.D. thesis

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I. Introduction

Most of the important biomolecules are chiral, i.e. they have two non-superposable structures that are mirror images to each other called enantiomers. It has been long known that the two structures show differences in their effects on living organisms (e.g., the Contergan scandal). Thus the determination of the 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 have to be found and the structure of the formed complex must be known.

As compared to X-ray crystallography, in the case of vibrational circular dichroism (VCD) spectroscopy the sample need not 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 the VCD technique is that the investigated chiral molecules do not need to have any chromophoric groups, unlike in the related methods (e.g the electronic circular dichroism (ECD) spectroscopy). Furthermore, VCD spectra have more bands than ECD spectra, facilitating absolute configuration 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 the 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 the VCD bands are altered by the molecular geometry and the evaluation also made by comparing the calculated and the experimental spectra. A 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 calculation and with 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 the sign and the intensity (i.e., the rotatory strengths) of the computed VCD bands quantitative measure was 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 of flexible molecules. The ACs of the experimentally studied compounds were known, this also helped to verify the calculated results.

During my work I have studied the structure of various optically active biomolecules by VCD spectroscopy which was supported by other spectroscopical methods (e.g., FT-IR). Moreover, the effects of perturbations (using 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. conformers, di- and trimers of N-formyl-N’-methyl derivatives of amino acids
2. conformers of Ac-β3-homo-proline,
3. 2-chloropropionic monomers, dimers and their CHCl₃ complexes
II. Experimental and Computational Details

During my work matrix isolation 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 using a Bruker Equinox 55 FT-IR spectrometer combined with a PMA37 VCD module. All the spectrometers were equipped with an MCT detector. In the matrix isolation measurements the samples were studied in Ar and Kr matrix, the solution phase experiments were running in BaF$_2$ and CaF$_2$ cells with different pathlengths depending on the examined compound and his concentration.

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

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, which has been tested on different flexible molecules. It turned out that the signs of the robust modes agree well with the experimental VCD signs and regarding only them gives a similar spectra to the experimental VCD spectra. However, the signs of the non-robust modes (e.g., C=O, C–Cl, C–O) can be different than that of the experimental ones.

2. Robustness greatly helps the assignment of the VCD spectra by showing which signs of the calculated vibrational modes can be trusted. The projection of the magnetic transition moment onto the electric one of a vibrational mode is a gauge-independent quantity and carries information about robustness reliably. The robustness of a vibrational band can be dubious if this parameter is under 10 ppm. In that case the band can show unexpected sign changes and should be disregarded during the assignment or must be take them into account with caution. The majority of bands detected in VCD spectra is found to be robust in our experience.

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

4. A rotatory strength and the robustness depends on the basis set used, but calculations at the B3LYP/6-31G* and the B3LYP/aug-cc-pVTZ level of theories gave similar results. Furthermore, VCD signs are very sensitive to the molecular geometry hence to the molecular conformation as well. The rotatory strengths and robustness of α- and β-peptides varies greatly and the VCD signs of the former one are more robust. With increasing chain length the calculated VCD spectra show increased similarity with the experimental spectra. Only the conformers with the
lowest energy (the most abundant ones) should be taken into account because their signals can only be detected in the experimental spectra.

5. The conformational landscape of Ac-$\beta^3$-HPro-NHMe was investigated using matrix isolation IR and VCD spectroscopy combined with quantum chemical calculations and the study of the robustness of VCD signals. 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 conformers and one cis conformer could be confidently identified. Theoretical calculations in agreement with experimental observations 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 the increased flexibility of the elongated backbone explain this increased abundance compared to the case of $\alpha$-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 compared to the matrix isolation experiments. Even in a less polar solvent like DCM, the cis peptide form is dominating.

7. On one hand, results clearly indicate that unlike $\alpha$-proline, which is an important $\gamma$-turn forming residue of natural peptides, the $\beta^3$-HPro residue is a much less reliable pseudo-$\gamma$-turn forming element. On the other hand, the $\beta^3$-HPro residue seems to be a promising cis peptide building block of synthetic $\beta$-peptides.

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

9. The influence of formation of strongly (dimers) and weakly (2-chloropropionic acid – CHCl$_3$ complexes) hydrogen-bonded complexes on the VCD spectrum of $S$-($-$) and $R$-($+$)-2-chloropropionic acid is investigated. For both the monomers and the 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, revealed that besides the two predominant trans conformers the
cis form is present in a small amount in the matrix. The relatively larger abundance of the cis conformer compared to other carboxylic acids (\(~6\%) could be explained by the weak interaction between chlorine atom and the hydrogen atom on the \(\alpha\)-carbon. This assignment have been confirmed by a later study that investigated the selective excitation of the 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), besides the usual strong bonded dimers a higher-energy form, stabilized by a strong (C=O…H−O) and a weak (C=O…H−C) hydrogen bond was also identified in the annealed matrix.

12. VCD spectra were obtained for the monomers in Ar matrix and for the dimers in solutions. A VCD spectrum was also measured for 2-chloropropionic acid in Ar matrix containing CHCl\(_3\) molecules. The most important conclusions on the VCD computations and measurements that can be generalized and can 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 \(\alpha\)-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 the C=O stretchings and all other modes in the 1900–1200 cm\(^{-1}\) region is sensitive to the X−C−C=O dihedral angle (X = Cl for 2-chloropropionic acid). Therefore, the VCD spectrum strongly depends on the conformation. An absolute configuration determination should carefully include all possible conformers. If careful analysis, e.g., a scan along the X−C−C=O dihedral angle in the present case is undertaken, and external perturbations are excluded, then a cautious configuration analysis based on robust modes should be possible. However, for this particular acid there was no reliable non-robust band in the spectrum of the monomers.

14. Solvation model computations and measurements in un-annealed matrices containing CHCl\(_3\) have both revealed that the averaged, non-directional external electric fields coming from the solvent or the CHCl\(_3\) molecules trapped in the matrix can affect the rotatory strengths of particular modes and even 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 alone, or indirectly through a geometry change.
15. The analysis of solution IR spectra also proved that in CCl₄ solution the dimers are predominant either at 0.02 or 0.1 mol·dm⁻³ concentration. In contrast to these, in CHCl₃ solution the monomers or (in a dynamic process) 2-chloropropionic acid – CHCl₃ 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 the VCD spectrum. Regardless of the robustness of the vibrational mode the average change in VCD intensities is about 20–40%. For some of the low-intensity modes even larger intensity changes can be found. For measurements in a CHCl₃ solution the use of solvation models was found to yield less accurate results than the calculations performed for molecular complexes, which reveal the presence of the complexes in solutions.

17. Calculations show that most of the vibrational modes in the formerly achiral part (the CHCl₃ 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 CHCl₃ in our case), however, that have almost medium rotatory strength and which 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 are obtained. The comparison of the computed spectrum of the dimers with the experimental spectrum recorded in relatively concentrated (∼ 0.1 mol dm⁻¹) solutions is highly recommended.

19. The VCD spectrum of a carboxylic acid recorded in CHCl₃ is more complex and, like in the present case, can have a lower intensity than that of the spectrum recorded in CCl₄ due to the stabilization of monomers and/or formation of weak complexes between the carboxylic acid and CHCl₃. Therefore, (even if it is computationally more expensive) if solubility allows, CCl₄ is a much preferred solvent over CHCl₃.
List of Publications

Publications related to the PhD thesis


Conference posters


Presentation related to the thesis

Communications not related to the dissertation

Publications


Conference poster

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.