Amplified vibrational circular dichroism

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INTRODUCTION

1.1 THE DIFFERENCE BETWEEN LEFT AND RIGHT

In 1811 the French physicist Francois Jean Dominique Arago, observed for the first time optical rotation of light passing through a quartz crystal placed between crossed polarizers. Optical activity was then defined as the ability of certain materials to rotate the polarization plane of a plane-polarized electromagnetic field. Around the same time, another French physicist, Jean Baptiste Biot, observed the same phenomenon in liquids and gases of organic substances such as turpentine, an extract from pine trees. The English astronomer Sir J. F. W. Herschel realized in 1822 that there was a correlation between optical rotation and non-superimposable mirror-image crystals when he observed that mirror-image forms of quartz crystals rotate linearly polarized light in opposite directions.

In 1948, Louis Pasteur solved a long standing problem concerning the nature of tartaric acid. A solution containing naturally occurring tartaric acid derived from wine lees exhibited optical activity whereas a solution containing tartaric acid obtained through chemical synthesis was optically inactive. Pasteur noticed that the synthetically-made crystals came in two asymmetric forms that are mirror images of one another. He separated the two forms of the compound by selecting crystals by hand and by preparing separate solutions of the two forms he observed rotation of the polarization in opposite directions. An equimolar mix of the two, on the other hand, did not give rise to optical activity.

In 1874, Jacobus Henricus van ’t Hoff and Joseph Achille Le Bel independently proposed an explanation for optical activity based on a model in which the chemical bonds between carbon atoms and their neighbors are directed towards the corners of a regular tetrahedron. This 3D structural interpretation introduced the concept of the asymmetrical
carbon atom and could explain the occurrence of multiple isomers. At that time, however, a formal connection between the property of handedness related to mirror-image molecules and/or objects and optical activity was not made yet. The first and currently accepted designation was given by Lord Kelvin during the Baltimore Lectures on Molecular Dynamics and the Wave Theory of Light in 1904.

"I call any geometrical figure or group of points chiral, and say that it has chirality if its image in a plane mirror, ideally realized, cannot be brought into coincidence with itself." (Lord Kelvin, Baltimore Lectures, 1904).

Two non-superimposable mirror-image forms of a chiral molecule, such as our right and left hands, are referred to as enantiomers (from the Greek words for opposite and part). Because enantiomers of a chiral molecule exhibit opposite-signed optical activity of equal magnitude they are also called optical isomers (Fig. 1) and designated according to specific naming conventions. The R/S nomenclature is the preferred within the chemistry community. However, the D/L naming system remains in use in some areas of biochemistry.

The D/L nomenclature has been proposed by M. A. Rosanoff in 1906, where he arbitrarily assigned the dextrorotatory (dextro=right) (+)-glyceraldehyde to the D configuration, and the levorotatory (levo=left) (−)-glyceraldehyde to the L configuration. In the early sixties, R. S. Cahn, C. K. Ingold and V. Prelog introduced the R/S system. The R/S system is more general than the D/L system since it does not involve a reference molecule such as glyceraldehyde and can also be extended to molecules without a chiral center. The Cahn Ingold Prelog (CIP) priority rules define which label, R (for Rectus) or S (for Sinister), to give to each chiral center according to the atomic number of the atoms directly connected to the chiral center.
It is in the realm of biology that we find the most profound effects of chirality. Living organisms contain proteins whose building blocks (amino acids) are exclusively L-enantiomers. As a consequence, the secondary structural motifs of proteins are also naturally biased towards single-handed conformations. For example, L-amino acids arrange preferably to form right-handed helices (α-helices) in proteins (Fig. 2A) as a result of favorable hydrogen bonding interactions between the NH and CO groups in the protein backbone. On the other hand, a left-handed helix can only be formed with similar stability if its building blocks are non-natural D-amino acids. Another naturally occurring handedness can be found in the nucleus of every living cell. The backbone of nucleic acids (DNA and RNA) is constructed from polymer chains formed exclusively by two naturally occurring D-sugars (D-ribose in RNA and 2-deoxy-D-ribose in DNA). Moreover, in the same way that proteins have helices of a single handedness in their secondary structure, double-stranded DNA also has a natural bias for right-handed double-helical structures (Fig. 2B). There is thus a naturally occurring hand-

Figure 2: A. Crystal structure of an Enzyme (Aspartate transaminase, PDB 1AAM); B. Crystal structure of DNA type-B (PDB BDNA).
edness in the fundamental molecular structures that constitute living organisms.

Many theories have been proposed to explain this natural selectivity\(^2\). However, at present there is no general agreement among scientists regarding this matter. Our current understanding is far too limited to know if an initial enantiomeric bias has been imposed in prebiotic chemistry, or at an earlier stage, and most of all what the underlying reason for this bias would be.

1.2 HOMOCHIRAL BIOCHEMISTRY OF THE HUMAN BODY AND THE DRUG INDUSTRY

Single-enantiomer drugs are generally more efficient binding to biological targets than the equivalent racemic drugs. The reason is that our bodies consider enantiomers as different molecules, even though, apart from their stereochemistry, they have the same structure. The biochemistry of the human body is thus stereoselective. The cycle of a drug through the body involves amongst other processes, absorption, transport and binding to the receptors. All these events have the potential to be stereoselective, thus biasing the biological activity of enantiomers. This asymmetry has tremendous implications in the design and development of drugs by the pharmaceutical industry. For this reason, it is nowadays standard practice during and after the synthesis of new chiral drugs to determine their absolute configuration. Until a few years ago this analytical step in the production of new drugs was exclusively performed using X-ray crystallography methods. Today, a significant number of absolute structures of chiral drugs is determined using vibrational optical activity. [2]

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\(^2\) Enantiomeric preference could have been imposed on the interstellar cloud from which our solar system was formed by exposure to circularly-polarized radiation from a neutron star. As a consequence, the organic inventory of early Earth could have originated from an extraterrestrial source such as comets or asteroids. The synchrotron-radiation hypothesis would then connect terrestrial biochemistry with the extreme physics of collapsed stars.[1]
1.3 MIRROR IMAGE ASYMMETRY AND OPTICAL ACTIVITY: A CLASSICAL PICTURE OF CIRCULAR DICHROISM

In a simple classical explanation of circular dichroism[3], the chiral molecule is modeled as a conducting coil, see Fig. 3 (the light wave is propagating perpendicularly out of the paper). Both the electric field $\mathbf{E}(t)$ and the magnetic field $\mathbf{B}(t)$ exert a force on the electrons in the wire. The magnetic field $\mathbf{B}(t)$ exerts a force because the magnetic flux through the coil changes with time, leading to an electromotive force. For left-circularly polarized (LCP) light, the electromotive force works in the same direction as the electrical force, whereas for right-circularly polarized (RCP) light, the two forces have opposite directions. Hence, a larger current is generated, and more energy dissipated ("absorbed") in the coil for LCP light than for RCP light. Although this is a simplified explanation, it may be noted that a box filled with 1 cm long copper coils exhibits strong optical activity in the microwave region.[4] Circular Dichroism (CD) is one of the classical forms of chiroptical spectroscopy, which measures the differential absorption between LCP and RCP light in the ultraviolet and visible regions of the spectrum, involving electronic transitions of molecules.

1.4 VIBRATIONAL CIRCULAR DICHROISM

Vibrational circular dichroism is an extension of the classical form of CD to the infrared region of the electromagnetic spectrum. This part of the spectrum contains vibrational transitions that involve a large number of vibrational modes ($3N - 6$ for a molecule with $N$ atoms), whereas in the ultraviolet and visible part of the spectrum one generally observes a limited number of electronic transitions. It is this tremendous increase in the number of available transitions, each of which is essentially a probe of structure, that has enabled VCD to revolutionize molecular stereochemical analysis. These vibrational modes are structurally sensitive to conformation, thus providing sufficient stereochemical detail to reliably determine a single absolute configuration. At the same time,
the vibrational fingerprints in the VCD spectrum allow the retrieval of the solution-state conformation or distribution of conformations of the molecule. This sensitivity to molecular conformation has been exploited in studies involving relevant complex biological systems such as protein fibrils[5, 6, 7, 8, 9, 10], nucleic acids[11, 12] and even viruses[13]. The study of biological systems however also puts some fundamental drawbacks of VCD in the spotlight. One of the drawbacks – and perhaps the most striking and limiting one – is the intrinsic small magnitude of VCD signals. In general, the intensity of VCD bands is $10^{-4} - 10^{-5}$ smaller than the associated infrared absorption bands. These small signal intensities have significant repercussions on the actual measurement. The noise level in these measurements is often comparable with the intensity of some of the VCD features, which thus remain hidden within the noise if the measuring strategy is not changed accordingly. To some extent, the problem may be solved by averaging over longer periods of time. Other approaches that have been taken are based on the use of thin films or working with highly concentrated sample solutions, but in these cases the added value of VCD being one of the few techniques that allows stereochemical structure determination under biologically...
relevant conditions becomes severely compromised. The potential that VCD can offer for stereochemical structure analysis thus as yet has not been fully exploited. Clearly, the field would benefit tremendously from approaches that would enhance VCD signal intensities at the molecular level.

1.5 FROM THEORY TO PRACTICE

Overcoming the above-described small signal barrier in VCD spectroscopy is the main theme covered in this thesis. We identify the physical origin of the low intensity of VCD signals and devise an experimental approach to manipulate the electronic structure of the chiral molecules. As will become clear throughout this thesis, vibronic coupling is responsible for the VCD signal magnitudes. Therefore, a controlled manipulation of the energies of the electronic manifold is expected to induce an amplification of the VCD intensities, as will be shown in Chapter 2 where we introduce the physical quantities necessary to understand the origin of VCD intensities. In Chapter 3 we describe the methodologies of a Fourier-Transform VCD measurement and of the auxiliary setup we have developed to manipulate the electronic structure of molecules electrochemically. Chapter 4 reports on the application of conventional VCD to the study of synthetic foldamers, a class of artificial molecular architectures that performs conformational changes upon photo-excitation with ultraviolet light. We show how VCD can be used to probe conformational changes by modeling the VCD spectrum using a simple coupled-oscillator model. In Chapter 5 we report the first implementation of an electrochemical apparatus, which in combination with the VCD spectrometer allows us to induce an amplification of VCD intensities for a prototypical molecular system. In Chapter 6 we report the observation of enhanced VCD intensities in chiral molecular crystals. We find a common origin in the magnitude of the nonlinear response of these crystals and the amplified intensity of the VCD signals, giving new insights in the role of electronic charge-transfer states and VCD signal-magnitudes. Chapter 7 reports on VCD amplifications by several orders of magni-
tude observed for amino acids and peptides when a paramagnetic en-
vironment is used to tune the electronic manifold. We demonstrate that
accurate conformational information can be retrieved from the amplified
signal response. In Chapter 8 we propose and implement the concept of
a local VCD amplifier, a molecular entity that can be covalently coupled
to a target region within a larger molecular system. We show that incor-
poration of such an amplifying unit can be used to locally probe active
sites in biomolecules.