Circular Dichroism in the Ion Yields of Amino Acids

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Introduction

A chiral molecule is one which lacks internal symmetry and so cannot be superposed on its mirror image; with each mirror image said to be enantiomers of each other. In 1848, Louis Pasteur postulated that most of the molecules in living systems are homochiral, that is, they possess only one enantiomer. In fact, in a recent study of proteome-wide data, it was shown that D-isomers of amino acids are an extremely rare occurrence with only 837 entries in a database of protein sequences containing over 188 million amino acids[1]. The origin of nature's preference for L-amino acids and D-sugars over their mirror images is still widely debated[2-4]. This enantioselectivity facilitates a stereospecific interaction between amino acids and other chiral molecules. Such findings have important pharmacokinetic implications, since approximately 50% of marketed drugs are chiral and of these 50% are sold as racemic mixtures[5]. Consequently, production and detection of pure enantiomers is an essential concern in the pharmaceutical industry.

Given that enantiomers have almost identical physical and chemical properties, identifying each molecule can be an arduous task. However, chiro-optical techniques can be employed which rely on the differential absorption of left- and right-circularly polarized light as it passes through a chiral molecule, known as circular dichroism (CD). Combining this phenomenon with mass spectrometry allows the CD of a resonant transition to be mirrored in the ion yields upon absorption of a further photon(s) required to ionize the molecule. This opens up the potential for sensitive enantio-selectivity of pure or complex mixtures of chiral molecules. If the ionization step does not give rise to a CD effect, then the asymmetry parameters measured via ion yields and conventional CD are the same[6]. This effect is termed circular dichroism in ion yields (CDIY), and has been recently observed in the chiral molecules, 3-methylcyclopentanone (3-MCP), methyloxirane, and phenylethanol[6-8]. 3-MCP is a monocyclic ketone which exhibits an unusually large CDIY of up to 20% due to a forbidden $n \rightarrow \pi^*$ transition at 324nm[9], making it the convenient workhorse to develop this technique.

However, in order to highlight the wider applicability of CDIY as a sensitive chiral analysis method, a greater range of other molecules needs to be explored. Thus, we proposed to bridge this gap by studying the aromatic amino acids phenylalanine, tryptophan and tyrosine for CDIY, by exploiting the chromophores in their side chains.

Experimental Setup

The experiment was performed using the STFC Central Laser Facility UFL2 Loan Laser which is a Coherent Libra Ti:Sapphire laser operating at 800 nm, 100 fs, 1 mJ and 1 kHz. This output was used to produce third harmonic pulses (267nm) through frequency conversion in non-linear BBO crystals. The pulses were circularly polarized (CPL) by first passing the 267nm beam through a Glan-Laser prism, then through a zero-order quarter-wave plate (266nm, Halle Optics) at 45° and 135° to the optical axis to generate left- and right-CPL, respectively.

The third harmonic output was used for ionization of the resulting plume of neutral molecules. A 100 mW CW laser was also used for desorption of the thermally labile amino acids, whilst a leak valve was employed to inject more volatile compounds into the interaction region. The experimental time of flight mass spectrometer, shown in Figure 1, was used to acquire mass spectra and has been described in detail elsewhere[10].

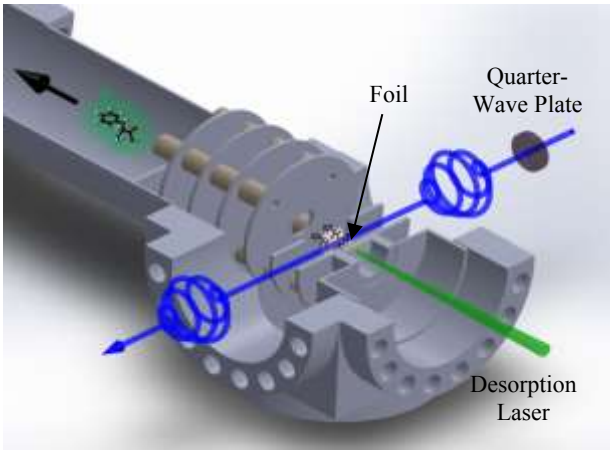

Figure 1: The experimental interaction region. The solid sample is placed on a thin foil (10 μm) which is irradiated with a desorption laser on the reverse side. The resulting plume is ionized by a circularly polarized femtosecond laser and the ions extracted into a time of flight mass spectrometer.

Reference Correction

To reduce any systematic asymmetries, an achiral reference molecule was ionised at the same time as the analyte by introducing it into the chamber via a leak valve. Since an achiral molecule has an internal plane of symmetry, its CD should be zero. Any non-zero CD values for achiral molecules are due to systematic asymmetries arising from polarization ellipticities less than 1 or differences in pulse energies for the left- and right-CPL. For an achiral reference with a similar ionisation rate, these systematic uncertainties can be corrected by taking the apparent CD value as a zero point and measuring all CD relative to this value.

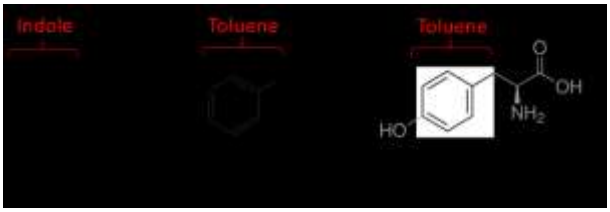

Figure 2: aromatic amino acids with their respective achiral reference molecules.

To ensure asymmetries caused by pulse energy variations were minimized, we chose reference molecules with similar chromophores to the chiral molecules under investigation; since small fluctuations in pulse energy are amplified in the ion yield due to the non-linear resonantly enhanced multiphoton ionisation (REMPI) process. Hence, we chose indole as tryptophan's reference, and toluene for phenylalanine and tyrosine, as these molecules are model chromophores for the respective amino acids (see Figure 2).

Measurements of Circular Dichroism in Ion Yields

We measured the CDIY of the $S_0 - S_1$ ($^1\pi\pi^*$) transition located on the benzene ring of phenylalanine and tyrosine, and the $S_0 - S_2$ ($^1\pi\pi^*$) transition located on the indole group for tryptophan. The S_1 band energy for phenylalanine has been previously reported by Martinez *et al.*[11] to be 4.65eV, and theoretically calculated for tryptophan in the range 4.54 - 4.87eV[12]. Since the IE of phenylalanine has been calculated as 8.4eV[13] and experimentally determined for tryptophan as 7.5eV[14] and tyrosine as 8.4eV, this gives a 1 + 1 REMPI scheme at 267nm (4.65eV).

Measurement of the CD was achieved by firstly acquiring ion yields using left-CPL, then after 5000 laser pulses switching to right-CPL. This cycle was repeated 50 times to give a total of 250,000 laser shots for each handedness of polarization. To quantify the dichroism in the ion yields, an asymmetry factor analogous to the Kuhn g-factor[15] was used:

$$g\,(\%) = 200 \times \frac{I_{LCP} - I_{RCP}}{I_{LCP} + I_{RCP}}$$

where I_{LCP} and I_{RCP} are the integrated ion yields of one selected mass due to left- and right-circularly polarized light, respectively. An example measurement is presented in Figure 3, where the ion yield for phenylalanine, together with the corresponding reference corrected *g-factor* for each prominent peak in the mass spectra are shown. All *g*-values presented originate from peaks which have no mass interferences with the simultaneously ionized reference molecule. The measurements were taken for each enantiomer, L-Phe in red and D-Phe in blue.

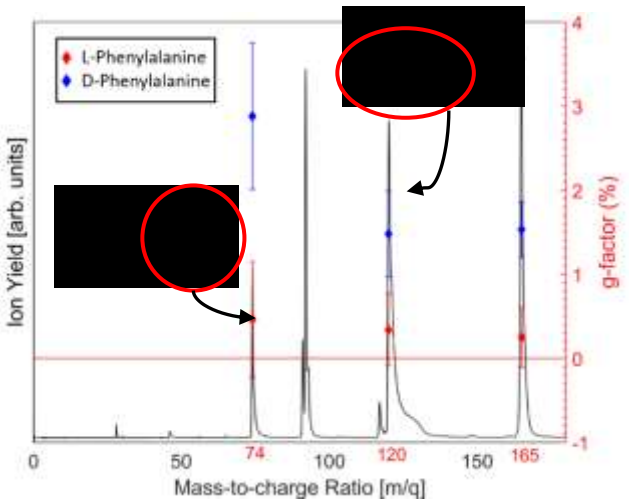

Figure 3: Mass spectrum and *g*-values for the parent (165amu) ion and 2 main fragments (120, 74amu) of L- and D-phenylalanine

In an ideal measurement, *g*-values would be equal in magnitude and opposite in sign for each enantiomer, however, as shown in Figure 3, there is still an instrumental bias. This is due to systematic uncertainties which arise from small changes in the transmission of the wave plate and the ellipticity they induce which are not completely eliminated by the use of a reference molecule. The statistical uncertainties are also high due to the large fluctuations in the pulse-to-pulse energies (≈10%). Nevertheless, there is a clear difference in the two enantiomers with the main fragments giving $g = 0.40 \pm 0.25\%$.

Table 1 shows our best g-factor results for each amino acid, along with the average for each enantiomer. Theoretical calculations for CD of gas phase amino acids are scarce since they need to take into account for the large number of possible conformers. However, calculations of L-Tryptophan by Guillaume *et al.*[16] estimate $g \approx 0.1\%$ at 267nm, which is consistent with the limited statistics of our results.

Amino Acid	Mass	g-factor (%)	Average g-factor (%)
L-Phe	165	0.75 ± 0.72	0.84 ± 0.46
	120	0.85 ± 0.75	
	74	0.97 ± 0.96	
D-Phe	165	1.53 ± 0.33	1.64 ± 0.26
	120	1.48 ± 0.51	
	74	2.88 ± 0.87	
L-Trp	204	0.20 ± 0.27	0.15 ± 0.19
	130	0.09 ± 0.27	
L-Tyr	181	0.88 ± 0.46	-0.44 ± 0.15
	107	-0.60 ± 0.16	

Table 1: g-factors for prominent peaks in the mass spectra of the amino acids enantiomers: phenylalanine, tryptophan and tyrosine.

Conclusions

We have reported, to the best of our knowledge, the first CDIY measurements on the amino acids phenylalanine, tryptophan and tyrosine. Despite the significant pulse energy fluctuations of the laser system, we managed to reduce systematic and statistical uncertainties down to the $g = 0.2\%$ level. This exceeded the amount of circular dichroism in ion yields from tryptophan and tyrosine but was sufficient to observe a value of $g = 0.4\%$ for phenylalanine. In order to reduce the uncertainties further, a laser system with a much more stable output would be required. With the advent of compact, highly stable, fs fiber lasers in the marketplace, development of an ionization based chiro-optical technique should be possible in the next few years. This is particularly true if the recent discovery of photoelectron circular dichroism (PECD), which has a large asymmetry parameter[17-21] (typically 4-10%), can be exploited as a more sensitive chiral analysis technique.

Acknowledgements

This work was supported by the CLF Laser Loan Scheme, EPSRC (EP/L005646/1), Leverhulme Trust (RPG-2012-735) and Northern Ireland Department of Employment and Learning.

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