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Homochirality

Circular Dichroism of Amino Acids in the Vacuum-Ultraviolet Region**

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Dedicated to Professor Henri B. Kagan on the occasion of his 80th birthday

Biopolymers such as nucleic acids and proteins are composed of chiral monomers that show identical stereochemical configuration. Naturally occurring proteins are made up of L-amino acids.[1] Hypotheses for the origin of symmetry breaking in biomolecules include the absolute asymmetric photochemistry model by which circularly polarized (CP) light induces an enantiomeric excess (*ee*) in chiral organic molecules.[2–4] This model is supported by both the observation of CP light in the star-forming region of Orion[3,5] and the occurrence of L-enantiomer-enriched amino acids in carbonaceous meteorites.[6–8] However, the differential absorption of CP light by amino acid enantiomers, which determines the speed and intensity of enantioselective photolysis, is unknown over a large spectral range. Here we show that significant circular dichroic transitions in amino acids can be observed by extending circular dichroism (CD) spectroscopy to the vacuum-ultraviolet (UV) spectral range. α-H amino acids show the same CD magnitude and sign over a large wavelength range. In a given spectral window[9] CP light is therefore capable of inducing enantiomeric excesses of the same handedness into the proteinogenic amino acids we have studied. Absolute asymmetric photochemistry might thus well have triggered the appearance of L-amino acid based life on Earth. Our results demonstrate that enantiomers of "meteoritic" α-methyl amino acids show dichroic absorption with equal magnitude, yet opposite sign to α-H amino acids. Therefore CP light cannot induce L enantiomeric excesses into α-methyl and α-H amino acids as found in meteorites.

To explain the cause of symmetry breaking in biomolecules a well-known theory[2–4,10,11] proposes that CP interstellar UV radiation—similar to that identified in the star-forming region of Orion in the infrared[3,5]—induced enantiomeric excesses into interstellar and circumstellar organic compounds by asymmetric photochemical reactions prior to their deposition on the early Earth.[12] In support of this theory chiral amino acid structures were identified in interstellar ice analogues[13] and a large number of L-enantiomer-enriched amino acids have been identified in the interior of the Murchison[6] and Murray[7] carbonaceous meteorites.[8] To verify the absolute asymmetric photochemistry model the differential CP-light absorption of proteinogenic and meteoritic amino acid enantiomers requires systematic examination.

Until now, the popular and extensively used technique of CD spectroscopy has been used to record electronic CD for chiral molecules in aqueous solution above 190 nm.[14] Water absorbs photons of $\lambda < 190$ nm, making the vacuum-UV region inaccessible for CD spectroscopy in aqueous solution. By using a synchrotron radiation source for CP light and preparing isotropic amorphous solid-state samples immobilized on MgF_2 windows, we have extended electronic CD measurements to the vacuum-UV spectral range.

We observed intense CD-active transitions of amino acids between 140 and 190 nm (Figure 1), which are much more intense than the previously known CD bands between 190 and 330 nm. Figure 1a shows the CD spectra for D- and L-alanine. As expected, the enantiomers of alanine show dichroic absorption of equal magnitude but opposite sign; the nice mirroring effect shows the high quality of the data. The CD spectra of L-alanine, L-valine, and L-leucine are characterized by maxima between 180 and 190 nm (Figure 1b), L-valine and L-leucine show minima between 160 and 170 nm, and L-serine and L-2-aminobutyric acid show maxima at 165–

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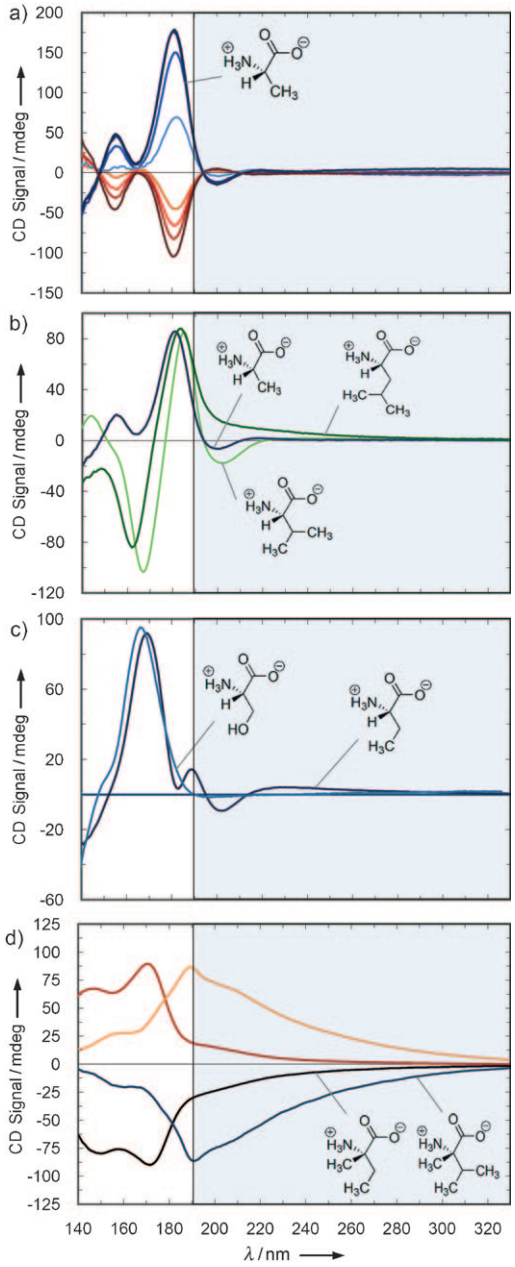

Figure 1. Circular dichroism spectra of amino acids in the vacuum-UV range. a) CD spectra between 140 and 330 nm of isotropic amorphous D-Ala (red) and L-Ala (blue) of different film thicknesses; b) L-Ala (blue), L-Val (light green), and L-Leu (dark green); c) L-Aba (dark blue) and L-Ser (light blue); d) D-Iva (red), L-Iva (black), D-methyl Val (orange), and L-methyl-Val (blue) immobilized on MgF_2. Intense CD-active transitions of amino acids occur between 140 and 190 nm, much higher than the previously known CD bands between 190 and 330 nm located in the gray-shaded spectral region. At 180 nm enantiomers of α-methylated amino acids Iva and methyl-Val show CD signals opposite to those of enantiomers of α-H amino acids. The *y* axis was normalized by mass per area.

170 nm (Figure 1c). The recorded CD spectra are in fair agreement with previous work on alanine and valine enantiomers,[15] where CD spectra were distorted by both linear dichroism attributed to the anisotropic microcrystalline samples and the CD contribution of the enantiomorphic amino acid crystals. We note that the CD spectra of the analyzed α-H L-amino acids are characterized by a maximum between 165 and 185 nm. Such strong bands might be assigned to (π*,π) transitions in the carboxyl group, while (π*,n) transitions were tentatively described to occur above 200 nm.[15]

Alternatively, and somewhat unexpectedly, we observed that the electronic CD spectra of the α-methylated amino acids L-isovaline and L-α-methyl valine show negative CD signals over the vacuum-UV spectral range (Figure 1d). The opposite enantiomers D-isovaline and D-α-methyl valine give positive CD bands of equal magnitude. The higher steric hindrance of α-methylated amino acids is assumed to provide dramatic changes in molecular orbitals, probably leading to the observed inversion of CD. According to ab initio time-dependent density functional theory calculations the CD responses of the amino acids L-valine and L-isovaline depend on the relative population of each conformer. Boltzmann population weighted theoretical CD curves recently confirmed opposite signs for non-ionic L-valine and L-isovaline in the gas phase between 130 and 180 nm.[16] Previous experimental work using a commercial CD photospectrometer indicated the potentially opposite circular dichroic sign of isovaline with valid data down to 180 nm where the absorption became saturated.[17]

The aforementioned theoretical spectra of neutral molecules in the gas phase are not directly transferable to interstellar chemistry or our experiments. The vibrational spectrum of condensed L-alanine shows strong bands associated with NH_3^+ fundamental modes (stretching at 3072 and 2978 cm^{-1} and antisymmetric deformation at 1586 cm^{-1}) and $\nu_{as}OCO^-$ and ν_sOCO^- modes, at 1621 and 1361 cm^{-1}, respectively.[18] The medium-intensity modes of NH_3^+— δ_s, ρ, and τ at 1520/1505, 1237, and 486 cm^{-1}, respectively—are additional markers for the characteristic vibrations of the zwitterionic form. The infrared spectrum of L-alanine shows no bands at 3322 and 1723 cm^{-1}, which are assigned to the νNH_2 mode as a marker for the non-protonated amine group and to the νC=O mode as marker for the protonated carboxyl group, respectively.[18] Similarly, the vibrational spectra of L-isovaline and L-methyl valine show strong bands at 1614 and 1366 cm^{-1}, and 1618 and 1366 cm^{-1}, respectively. No bands are present at 3322 and 1723 cm^{-1}, confirming that L-isovaline and L-methyl valine occur in zwitterionic form in the amorphous solid film. We conclude that both α-H and α-methyl amino acids occur in the zwitterionic state after sublimation and condensation.

In our vacuum-UV measurements the contribution of the crystal lattice structure to the optical activity below 190 nm is eliminated since isotropic amorphous amino acids are sublimed and condensed to form a film of defined thickness on the MgF_2 window. The amorphous form of the condensed amino acids and the isotropic orientation in the solid state were verified by electron diffraction. An epon resin was applied to the condensed amino acid film, and after polymerization 70 nm transversal sections (epon cuts) were subjected to electron diffraction spectroscopy. The recorded electron diffraction spectra were diffuse, verifying the isotropic

amorphous solid state of the sublimed L-amino acid films without long-range order.

Scanning electron microscope (SEM) images of condensed L-valine and D-α-methyl valine were also recorded. A noncrystalline, isotropic amorphous structure is visible showing sheetlike aggregates for L-valine and networks composed of fibers for D-α-methyl valine (Figure 2). For comparison microcrystalline L-valine was prepared by concentrating an aqueous L-valine solution to dryness. In this case microcrystals evolve and are visible by SEM imaging (Figure 2 a,b). Particularly relevant here is that we recorded UV and vacuum-UV CD spectra of amorphous α-H and α-methyl amino acids that were prepared under simulated interstellar and circumstellar conditions, where organic molecules sublime and condense by a process known as the interstellar dust cycle.

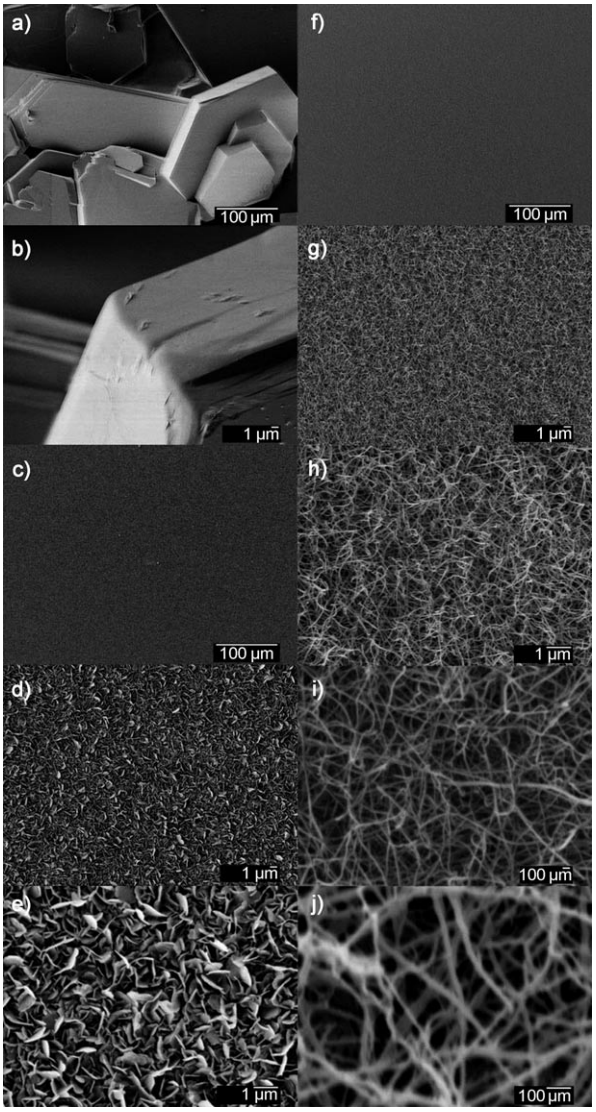

Figure 2. Scanning electron microscope (SEM) images of L-valine and D-α-methylvaline. a,b) Microcrystalline L-valine obtained by evaporation of a supersaturated aqueous solution at 1 atm; c–e) amorphous L-valine; and f–j) amorphous D-α-methylvaline. The noncrystalline isotropic amorphous films show no long-range order and were used for CD measurements.

Interstellar radiation shows asymmetric components in the infrared and is partially circularly polarized.[3,5] This circular polarization was calculated to be valid for the vacuum-UV spectral region, where it has, however, never been observed experimentally. "Chiral photons" are known to be capable of transferring their inherent asymmetry to organic molecules by means of enantioselective photolysis under specific laboratory conditions.[2–4,9,10,19] This light–matter chirality transfer depends on the differential absorption of CP light by two enantiomers. The knowledge of chiroptical properties of amino acids over a large spectral range is vital to the question, whether all amino acids are asymmetrically photolyzed at the same energy/wavelength and whether the sign of the *ee* induced is the same for all amino acids of interest.[20] We conclude from our results that CP light of a given wavelength (170–200 nm) and of a given helicity is capable of inducing enantiomeric excesses of the same handedness into the studied proteinogenic amino acids. These amino acids were selected by their assumed early recruitment into proteins.[21] Absolute asymmetric interstellar photochemistry might thus well have triggered the appearance of L-amino-acid-based life on Earth.

L-Enantiomer-enriched amino acids have been identified in the interior of the Murchison[6] and Murray[7] carbonaceous meteorites.[8] Meteoritic α-methyl amino acids such as isovaline show significantly higher L enantiomeric excesses (up to 18.5%) than α-H amino acids (up to a few percent). It is assumed that an initial *ee* of α-methyl amino acids was better conserved on account of slower racemization rates. Our results demonstrate that enantiomers of the α-methyl amino acids show CD signals of opposite sign to those of α-H amino acids. Thus CP light of a given handedness cannot simultaneously induce L enantiomeric excesses into α-methyl and α-H amino acids as they were found in meteorites. Complementary models for the understanding of the collective enrichment of the L enantiomers in meteoritic amino acids such as enantioselective adsorptions,[22] crystallization effects,[23] magnetochiral influences,[24] β-radiation products,[25] and influences of the weak nuclear interaction[26] will have to be taken into account.

We have so far investigated only amino acids, but it would be straightforward to extend our CD studies to other families of organic molecules that may show intense CD-active transitions in the vacuum-UV range.

Experimental Section

The CD spectra of the amino acids were recorded at the UV1 and the CD1 beamlines on the synchrotron radiation facility ASTRID at the Institute for Storage Ring Facilities (ISA), Aarhus University in Denmark. Linearly polarized radiation from the beamlines was converted into alternating left- and right-handed CP light by a CaF_2 photoelastic modulator, passed through the sample, and detected by a vacuum-UV-enhanced photomultiplier. Wavelength and rotational strength magnitude was calibrated with (+)-camphor sulfonic acid (CSA). MgF_2 windows were cut perpendicular to the *c* axis of a single MgF_2 crystal to minimize birefringence. Blank CD spectra were measured for the MgF_2 window and subtracted from the sample CD spectra. Each CD spectrum was measured twice. The amino acid film on the MgF_2 support was turned around the axis of the synchrotron

radiation and spectra measured at 0°, 90°, 180°, and 270°. The CD spectra recorded at these four positions were identical, allowing us to exclude disturbing effects arising from linear dichroism and linear birefringence.[27]

Amino acids were purchased from Fluka in >99.5% purity. Amino acid films were prepared by sublimation and condensation under temperature- and pressure-controlled conditions in an ultra-high-vacuum (UHV) chamber. Amino acids were placed in the sublimation source, a cylindrical 19 mm^3 quartz reservoir, which fits inside a heated metal cylinder. Its temperature was measured with a K-type thermocouple. A shutter allowed for opening and interrupting the amino acid flow. The rate of amino acid condensation (typical 10–20 $nm\,min^{-1}$) was monitored by a 5 MHz quartz-crystal microbalance (QCM) that could be inserted between the sublimation source and the MgF_2 support. The UHV chamber was evacuated by a turbo pump, backed by a scroll pump. The pressure was monitored by a full-range gauge. Amino acid sublimation–condensation datasets are shown in Table 1.

Table 1: Datasets for amino acid sublimation–condensation.

Amino acid	T_{sub} [°C][a]	t_{sub} [min]	Film thickness [nm][b]
D-alanine	149.0	30	500
L-alanine	149.0	30	500
L-valine	140.0	30	500
L-leucine	131.0	20	150
L-serine	153.0	40	250
L-2-aminobutyric acid	138.0	30	500
L-isovaline	130.0	30	400
D-isovaline	130.0	30	400
L-α-methylvaline	145.0	40	300
D-α-methylvaline	145.0	40	300

[a] Sublimation temperature recorded at $P = 10^{-7}$ mbar, optimized to obtain amino acid condensation rates of 10–20 $nm\,min^{-1}$, which were monitored with a quartz crystal microbalance (QCM). [b] Film thicknesses determined by QCM have an error of ±10 nm.

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