Homochirality

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Anisotropy Spectra of Amino Acids**

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In memory of Kurt Dehnicke (1931–2011)

Biopolymers such as enzymes and nucleic acids are composed of homochiral monomers; their molecular symmetry is broken.[1] The origin of biomolecular symmetry breaking—a crucial step in the origin of life—remains unknown. Among various random[2] and deterministic[3] hypotheses that have been proposed, one well-known hypothesis is based on a photochemical model by which chiral photons, in the form of circularly polarized (CP) light, induce an enantioenrichment by interacting with racemic organic molecules, a process known as enantioselective photolysis.[4–7] According to this model, asymmetric photoreactions took place in the extreme vacuum of interstellar space, prior to the delivery of enantioenriched chiral organic molecules to the early Earth.[8] The hypothesis proposes that CP electromagnetic radiation, such as that detected in the Orion molecular cloud,[5,9] interacts asymmetrically with chiral organic molecules in interstellar ices[10] and with the early precursors of carbonaceous meteorites.[11] Both enantiomers absorb CP photons triggering photolysis, but one enantiomer has a slightly smaller absorption coefficient. This enantiomer is photo-destroyed less rapidly than its optical antipode and it will therefore become enantioenriched. The induced enantiomeric excess (*ee*) is determined by the extent of reaction ξ and is function of the anisotropy factor g, defined by $\Delta\varepsilon/\varepsilon$, the ratio between the differential extinction coefficient $\Delta\varepsilon$, and the extinction coefficient ε. However, the sign and magnitude of g depend on the wavelength of the CP light. Here we report anisotropy spectra of amino acids yielding $g(\lambda)$ values, which were recorded for solid amorphous films in a wavelength range between 130 and 350 nm. The anisotropy spectra were measured with a new experimental setup at the synchrotron radiation facility ASTRID at Aarhus University (Denmark). The anisotropy spectra obtained for amino acids in the solid phase show well-resolved zero-crossings, extrema, and g values up to 0.024. These data allow: 1) the prediction of the sign of the induced *ee*, 2) the determination of the kinetics and the *ee* values of the enantioselective photolysis, and 3) the selection of the wavelength of the CP light best suited for inducing enantioenrichment.

The enantioselective photolysis of a racemic mixture by CP light is an asymmetric transformation that can be represented by two competitive pseudo-first-order reactions with unequal rate constants, k_R and k_S, for the *R* and *S* enantiomer, respectively.[4] The rate constants are proportional to the molar absorption coefficients (ε_R and ε_S, respectively), and the efficiency of the enantioselective photolysis depends on the difference between k_R and k_S or, in this case as Kuhn already outlined,[12] on the anisotropy factor g [Eq. (1)].[4,6] More recently it has been shown by Nakamura et al. that Equation (1) is valid even for non-first-order kinetics.[13]

$$g = 2\frac{\varepsilon_R - \varepsilon_S}{\varepsilon_R + \varepsilon_S} = 2\frac{k_R - k_S}{k_R + k_S} \quad (1)$$

Buchardt described three different photochemical mechanisms for inducing an enantiomeric excess, distinguishing between asymmetric destruction, partial photoresolution, and asymmetric synthesis. In each case the optical yield is dependent on the optical anisotropy factor g, underlining its importance.[14]

Until now, however, only single anisotropy values have been reported for amino acids in aqueous solution.[15] The wavelength dependence of the anisotropy factor g has been impossible to determine. This is because in the low-wavelength region down to 130 nm a small differential absorption $\Delta\varepsilon$ of CP light by enantiomers is to be divided by large values—often three or four orders of magnitude higher—for their absorption coefficient ε, which made the precise recording of anisotropy spectra impossible up to now. Above 220 nm the absorption coefficient ε is difficult to determine because

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of its weak intensity and scattering effects, which induce large errors in the anisotropy spectrum as well. We have now succeeded in the determination of anisotropy spectra for several amino acids in the UV and vacuum-UV (VUV) spectral region. This has been achieved by using a synchrotron source for CP light which allows $\Delta\varepsilon$ to be determined with high precision over the entire spectral range, and by measuring the differential absorption of isotropic amorphous amino acid films immobilized on MgF_2 windows. Suitable films of amorphous amino acids were obtained by sublimating chiral enantiomers under controlled conditions. It is of particular relevance here that the UV and VUV anisotropy spectra of amino acids were recorded from films that were prepared by mimicking interstellar and circumstellar conditions, where organic molecules sublimate and condense by a process known as the interstellar dust cycle.

The anisotropy spectra and corresponding ee_L values of several chiral α-amino acids recorded in the UV and VUV spectral region are shown in Figure 1. Figure 1a shows the anisotropy spectra for D- and L-alanine. The anisotropy spectrum of L-alanine (blue line on red background) is characterized by a maximum at 157 nm, a second maximum at 185 nm, a minimum at 200 nm, and a third maximum at 221 nm. As expected, the anisotropy spectrum of D-alanine (red line on blue background) shows an anisotropy spectrum of nearly equal magnitude but opposite sign, a mirroring effect highlighting the consistency of the data. The pseudo-minimum at 310 nm, where the wavelength of the CP light matches the film thickness of the alanine condensate, is an artifact caused by scattering processes enhanced by interference in the film.

The anisotropy spectrum of L-valine is characterized by a maximum at 188 nm and a minimum at 206 nm (Figure 1b); L-leucine displays a minimum at 165 nm and a maximum at 226 nm including a small shoulder at 196 nm (Figure 1c). The anisotropy spectra of L-serine and L-proline show maxima at 172 and 188 nm, respectively; their minima were recorded at 226 and 203 nm (Figure 1d). The anisotropy spectrum of L-isovaline features a minimum at 173 nm and a negative plateau between 200 and 220 nm; L-methyl valine shows minima at 161 nm and at 210 nm. The sections in Figure 1a with a colored background are included in Figure 1b–e. It can be seen that the spectra for the L enantiomers of the selected proteinaceous amino acids tend to be within the red areas, whereas those of the D enantiomers lie within the blue regions. This observation favors the theory that CP light of a given wavelength and handedness can induce an *ee* of the same sign in the proteinaceous amino acids studied here.

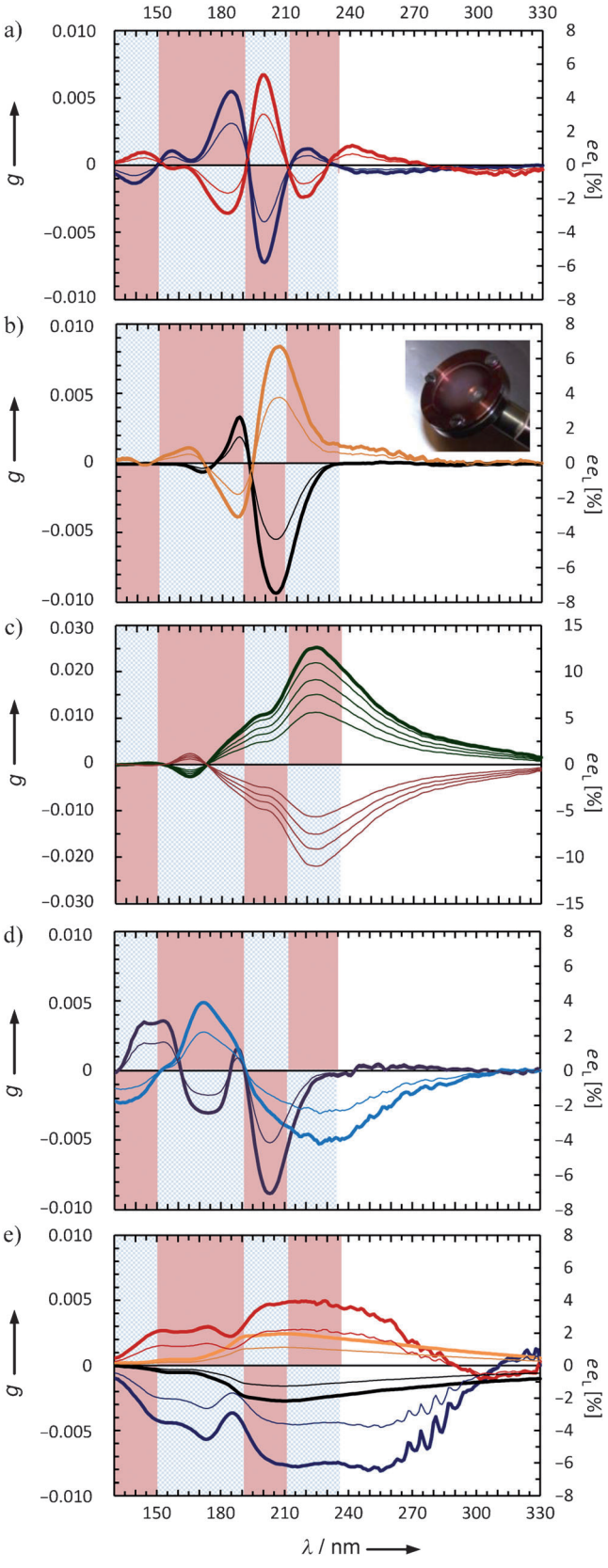

Figure 1. Anisotropy spectra (thick lines) of α-amino acids in the vacuum-UV and UV spectral region. a) Anisotropy spectra of isotropic amorphous D-Ala (red) and L-Ala (blue); b) D-Val (orange) and L-Val (black); c) L-Leu (dark green); d) L-Ser (light blue) and L-Pro (violet); e) D-Iva (red), L-Iva (blue), D-methyl Val (orange) and L-methyl Val (black). Thin lines represent the corresponding ee_L plots inducible by either left or right circularly polarized light at $\xi = 0.9999$. Additional values of ee_L are given for leucine at $\xi = 0.9995$, 0.998, and 0.99. Amino acids were sublimated using a temperature-controlled UHV chamber and condensed in the form of isotropic amorphous films on an MgF_2 window (see inset in (b); scale 1:2).

The zero-crossings of the recorded anisotropy spectra are in reasonable agreement with previous work on circular dichroic transitions of zwitterionic amino acids in the UV and vacuum-UV spectral region.[16] We note that the positions of the extrema as well as their intensity in the newly reported anisotropy spectra differ from those in previously described circular dichroism spectra.

The data in Figure 1 indicate that as the proximity of the chiral center of the α-amino acids becomes more complex, the magnitude and position of the anisotropy bands change dramatically. In the 170–190 nm region each L-enantiomer of α-H amino acids has a maximum that decreases with the size of the side chain and shifts slightly to lower energies. This maximum lies at 172 nm for L-serine, 185 nm for L-alanine, 188 nm for L-valine, and 188 nm for L-proline, but is strongly red-shifted to 196 nm for L-leucine because of the absorption band shift in this particular amino acid.[17] Detailed information on the absorption measurements is given in Figure S1 in the Supporting Information. While the problem may be more complex in the case of leucine and the α-methyl amino acids, these maxima can be assigned to the first π–π* transition of the carboxylate anion.[18] The absolute anisotropies of α-H amino acids between 190–210 nm increase with the size of the side chain based on the decreased intensities in their absorption bands (Figure S1 in the Supporting Information). These bands are acknowledged to be due to the *n*–π* transition in the carboxylate anion mixed with the *n*-(COO^-)–σ*(N-H) transition.[18] In L-leucine, however, this minimum is absent. This band is thought to overlap with the neighboring weak absorption band at lower energies corresponding to the anisotropy maximum at 225 nm.

The highest value of *g* measured for these selected amino acids is 0.024 for L-leucine, which is almost one order of magnitude greater than for all other amino acids investigated in this study. This large magnitude of *g* is due to the small absorption cross section of leucine, and according to Kuhn[19] only weak absorption bands can generate high anisotropy factors. Anisotropies of amino acids are smaller than literature values for camphor in hexane,[4] which has a maximum of $g_{310\,nm} = 0.095$. The highest known anisotropy value of an optically active compound has been reported for *trans*-hydrindan-2-one where $g_{313\,nm} = 0.24$.[20] Only strand-shaped supramolecular assemblies of conjugated polymers such as poly(aryleneethynylene) exhibit higher anisotropies up to $g_{432\,nm} = 0.38$.[21]

In order to know how the sign and intensity of the *ee* vary as a function of anisotropy *g* and extent of reaction ξ, one can calculate *ee* either numerically by iterative solution of Equation (S1) as outlined by Kagan et al.[4] or, if the anisotropies are close to zero, to a first approximation by Equation (2) (see the Supporting Information).

$$ee \geq (1-(1-\xi)^{g/2}) \times 100\,\% \qquad (2)$$

The quantitative prediction of the inducible ee_L for the tested amino acids by asymmetric photolysis at a given extent of reaction of $\xi = 0.9999$ is shown in Figure 1 and Table 1. The extent of reaction represents 99.99% photolysis of the racemic reactants. Note that at λ_{185}, right circularly polarized light induces an ee_L in all tested proteinaceous amino acids.

Table 1: Inducible enantiomeric excess ee_L [%] through asymmetric photolysis of racemic α-amino acids with right circularly-polarized light and extent of reaction $\xi = 0.9999$, as function of the irradiation wavelength λ.

	λ [nm]						
Amino acid	140	155	170	185	200	220	180–190
L-alanine	−0.62	0.45	0.33	2.52	−3.33	0.55	2.23
L-valine	−0.03	−0.03	−0.30	1.13	−3.69	−1.15	1.00
L-leucine	0.06	−0.16	−0.64	2.70	4.98	11.31	2.68
L-serine	−0.92	0.16	2.19	1.15	−1.14	−2.13	1.09
L-proline	1.26	1.57	−1.34	0.19	−3.81	−0.81	−0.05
2-methyl-L-valine	−0.06	−0.24	−0.29	−0.76	−1.20	−1.21	−0.78
L-isovaline	−1.12	−2.03	−2.49	−1.68	−3.22	−3.54	−1.84

It has been shown for the Orion molecular cloud that interstellar radiation is partly circularly polarized in the infrared wavelength region.[5,9] "Chiral photons" are known to be capable of transferring their asymmetry to organic molecules by enantioselective photolysis.[4,5,7,12,22] The induced optical yield of the chirality transfer from light to matter depends on the anisotropy spectrum of the organic molecules. We conclude from our results that CP light of a given energy and helicity may well induce an *ee* of the same sign into the tested proteinaceous amino acids, probably under interstellar conditions. The anisotropy spectra presented here show that irradiation in the UV (around 200 or 220 nm) rather than in the vacuum UV (around 160 nm), as suggested by CD spectra alone, is most likely to yield the highest *ee* values. In molecular clouds the estimated flux of UV CP light is much higher than for VUV CP light.[5] A declining distribution of the emitted CP photon flux at lower wavelengths would give a non-zero integral of the anisotropy spectrum over all wavelengths, yielding—in coherence with the Kuhn–Condon zero-sum rule—$ee \neq 0$ upon irradiation. After enantioselective photolysis in interstellar space and delivery to the early Earth, enantioenriched amino acids might have provided a nonracemic environment out of which the molecular evolution of primitive life[23] arose.[24]

Experimental Section

Anisotropy spectra of solid-state amino acids were recorded at the synchrotron radiation facility ASTRID, Aarhus University, Denmark using the UV1[25] and the CD1[26] beamlines. To record the differential absorption of CP light by amino acid enantiomers, a CaF_2 photoelastic modulator was applied to convert linearly polarized synchrotron radiation into 50 kHz alternating left and right CP light. After passing through the amino acid sample, the transmitted light was recorded using a vacuum UV enhanced photomultiplier. Prior to recording the differential absorption of the amino acid enantiomers, wavelength and rotational strength magnitude were calibrated with camphorsulfonic acid (CSA). Careful calibration ensures differential absorptions to be known to better than ±1%. In this study we recorded both the extinction ε and its corresponding Δε simultaneously on the same spot on the sample; the sample thickness and its optical density thus need not be known precisely. Conversion of the photodetector signal and its gain voltage to give absorption measurements were achieved by using the method described in Ref. [27],

comparing samples between the beam line and a calibrated photospectrometer (Evolution 300, Thermo). Supplementary measurements of anisotropy spectra were performed at synchrotron SOLEIL, Gif-sur-Yvette, France, using the DISCO beamline.

The sublimation chamber was an ultrahigh-vacuum system equipped with an amino acid sublimation source, a quartz microbalance, and a holder for the MgF_2 substrates onto which the amino acids were condensed.[16,28] SEM images of the condensed amino acids show no long-range order, the amino acids were thus considered amorphous.[16] A more detailed description of the experimental section is given in the Supporting Information.

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