The pH dependence of the anisotropy factors of essential amino acids †

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Received (in Cambridge, UK) 20th September 2001, Accepted 5th December 2001
First published as an Advance Article on the web 18th January 2002

In order to examine the pH dependence of the anisotropy factor (g) of all the essential amino acids, at pHs 1, 2, 7 and 11, the molar absorption coefficient (ε) and circular dichroism ($\Delta\varepsilon$) were determined in aqueous solution. The g factors were high, and except for Asn and the aromatic and sulfur-containing compounds (Tyr, His, Trp, Cys and Met) the g_{max} values are larger than 0.01 in the pH range 1–7. Large g factors of aliphatic amino acids are observed even at pH 11. On the whole the magnitudes of the g factors at pH 1 are 2–3 times those at pH 7. The n,π* transition of the carboxylic group is the major contributor to the large g factors in amino acids such as Ala and Val. It is thought that in connection with the possible origin of amino acid homochirality generated by circularly polarised light irradiation, the % ee (≡ % op) of the amino acid generated will also be pH dependent in a manner similar to that previously reported for Leu.

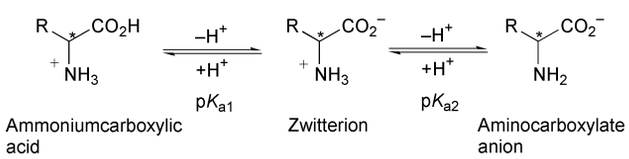
The origin of the homochirality of biomolecules in the biosphere is one of the most controversial and poorly understood issues in the chemical evolution of Earth. Since the time of Le Bel and Van't Hoff researchers have proposed various mechanisms for the occurrence of this phenomena. Some experimentally observable, such as spontaneous resolution, preferred crystallization of one enantiomer, or production of a labile chiral compound;[1,2] others are, to date, theoretical in nature, such as parity-violating neutral currents.[3,4] It is thought that one of the most feasible mechanisms for the existence of biological homochirality may be by enantiomeric enrichment *via* circularly polarized light (CPL) irradiation of amino acids. This proposition has received significant attention as, to an extent, it has been experimentally verified that the enantiomeric enrichments of many compounds, including some amino acids, can be attained by CPL irradiation.[5–10] Enantiomeric enrichment by CPL irradiation is known in photochemical terms as absolute asymmetric synthesis (AAS). The principle of AAS is that asymmetric photoreactions are brought about through the preferential excitation–decomposition of one enantiomer over the other, *via* either left- or right-handed CPL (*l*- and *r*-CPL) irradiation.[5,7,8,11] The factor that determines the degree of preferential excitation is called the anisotropy factor or g factor, and is defined as the relative difference in the molar absorption coefficients of each enantiomer towards *l*- and *r*-CPL at a given wavelength: $g = (\varepsilon_l - \varepsilon_r)\varepsilon = \Delta\varepsilon/\varepsilon$, where $\varepsilon = (\varepsilon_l + \varepsilon_r)/2$ and $0 \leqslant g < 2$.[12,13]

It has been reported that optically active amino acids have been detected in the organic mantle of the Murchison meteorite.[14] Based on these reports, Bonner proposed that the AAS of amino acids may proceed by exposure to CPL from neutron star remnants of supernovae,[1,2] whilst Bailey *et al.* proposed amino acid enantio-enrichment in the protosolar system *via* CPL irradiation generated by the scattering of light from a nearby star.[15] It was recently further reported that the Murchison meteorite contained amino acids in its organic mantle that are not found in the biosphere that also occurred in a significant enantiomeric excess (ee).[16,17] As a consequence of this work significant attention has been paid to the idea that the enantiomeric enrichment of amino acid proceeds by extra-terrestrial CPL irradiation.[1,2,15,18,19]

Recently, we reported the pH dependence of the enantio-enrichment of leucine (Leu) on irradiation with CPL; that it proceeds at pH 1 but not at pHs ⩾ 7, although the g factor of Leu is large over the pH range 1–7.[10] This was unexpected as irrespective of the solution pH, in principle, if a significant g factor exists at the irradiation wavelength and the photoreaction proceeds *via* the excited state then an ee should be observed. If it is the case that the CD is immeasurably small at a certain pH, AAS enantio-enrichment does not occur because the relationship between the g factor and the conversion determines the ee obtained.[5,6,20,21] For several essential amino acids, a few at limited pHs, the g factors have been reported.[5,9,22,23] However, the pH dependence of the g factors of all essential amino acids has not been thoroughly reported.

For essential amino acids, the UV and CD spectra have been measured under different conditions,[24] for example, in both organic and aqueous solutions,[25,26] with the solution pHs controlled by either hydrochloric acid and sodium hydroxide[25,27] or by ammonium hydroxide and phosphates.[26,28] Except for the report of less common amino acids,[29] only Legrand and Viennet in 1965 reported the $\Delta\varepsilon$'s of a series of common amino acids (albeit a limited sample).[28] As a result, to date, it has not been possible to access or rigorously compare the optical properties (including g factors) of all the essential amino acids, and there have been disagreements on the interpretation of those reported.[24]

The pH dependence of the g factors of essential amino acids directly relates to the issue of whether the origin of biological



Scheme 1 Structural change of amino acid depending on the solution pH.

† Electronic supplementary information (ESI) available: a table giving the precise pH and concentrations of amino acids, the UV spectra of Gly at various pHs, the UV, CD and g spectra of Pro at pH 13, the UV spectra of propane-1-thiol at various pHs and the CD spectra of L-Leu and L-Phe at various concentrations. See http://www.rsc.org/suppdata/p2/b1/b108575c/

 DOI: 10.1039/b108575c

homochirality may arise from CPL irradiation or not.[2,16–18] In this report, we examine the pH dependence of the chiroptical properties of all the essential amino acids over the pH range 1–11. We wish to clarify how the pH-dependent ionic composition change of amino acids affects the corresponding chiroptical properties, particularly the *g* factor. These results and considerations of the pH dependence of the *g* factors contributes to the understanding of the possible origin of homochirality *via* CPL irradiation, and will be a useful base of information for researchers to examine, rationalize and utilize the chiroptical properties of amino acids.

Results and discussion

Chiroptical properties of Ala, Leu, Val, Ile and Pro at various pHs

For this series of aliphatic amino acids the chromophores are the carboxy and amino groups; pK_{a1} expresses the equilibrium between the carboxylic acid and carboxylate anion form, with pK_{a2} expressing the equilibrium between the amino and ammonium cation form (pK_{a1} = 2.35, pK_{a2} = 9.78).[30]

Fig. 1a shows the chiroptical properties of D- and L-alanine (D- and L-Ala) at pHs 1, 2, 7 and 11. The observed UV and CD spectra showed significant deviations with the variation in solution pH. These changes were in good agreement with the reported literature,[24,25,31] and correspond to the pH dependent changes in ionic composition of D- and L-Ala chromophores. At pH 1 (in the carboxylic acid–ammonium cation form) the UV and CD bands (ε_{max} 205 nm, $\Delta\varepsilon_{max}$ 208 nm) derive from the n,π* transition of the carboxy group.[24,25] As the pH changes from 1 to 7 the form of the carboxy group changes from carboxylic acid to carboxylate anion, correspondingly it is observed that the maxima of the UV and CD bands shift to <200 nm with an isosbestic point observed in the UV spectra at 210.5 nm, Fig. 1a (UV and CD). The observed bands above 200 nm are assigned to be from the n,π* transition of carboxylate.[24,32,31] At pH 11, D- and L-Ala exist as the amino-carboxylate anion. It is reported that the absorption near 240 nm in the UV and CD spectra predominantly arises from the n,σ* transition of the amino group.[24,25] Fig. 1a (*g*) shows the *g* factors of D- and L-Ala as derived from the UV and CD spectra at various pHs, having a magnitude of g_{203} (the *g* factor at 203 nm) at pH 7 in agreement with that of the literature (g_{203} = 0.007 in water).[22] Table 1 summaries the ε_{max}, $\Delta\varepsilon_{max}$ and the g_{max} of L-Ala at pHs 1, 2, 7 and 11. Table 1 and Fig. 1a (*g*) show that the g_{max} values of D- and L-Ala at pH 1 are −0.027 and +0.027, respectively, at 222 nm. Ala possesses a high g_{max} in excess of 0.01 at 215 nm over the range of pH 1–11.

For leucine (Leu), valine (Val) and isoleucine (Ile) the molecular structures are very similar to that of Ala, only differing in the length of the alkyl side-chain, therefore it is reasonable that the differences in the isotropic and anisotropic transitions between Ala and these amino acids are small.[24,33] Each pK_{a1} and pK_{a2} is nearly equal to that of Ala (pK_{a1}: Leu 2.33, Val 2.29, Ile 2.32, pK_{a2}: Leu 9.74, Val 9.72, Ile 9.76),[30] showing that the ionic compositional changes of these D- and L-amino acids with pH are also similar to those of Ala. Correspondingly the spectral changes of the UV, CD and *g* factor spectra with pH occur in a similar manner to those of Ala, Figs. 1b–d. Again, the ε, $\Delta\varepsilon$ and *g* factors of these amino acids over the pH range 1 to 7 reflect the change of the carboxy group with the solution pH, and those over the pH range 7 to 11 reflect the change of the form of the amino group.

The structure of proline (Pro) is different from those of Ala, Val, Leu and Ile because the amino group exists as a secondary amine within a constrained ring structure. Fig. 2e shows the UV and CD spectra of Pro at pHs 1, 2, 7 and 11. It was reported that a negative Cotton effect of L-Pro was observed below 210 nm,[24,28] which was also the case in our experiments. The

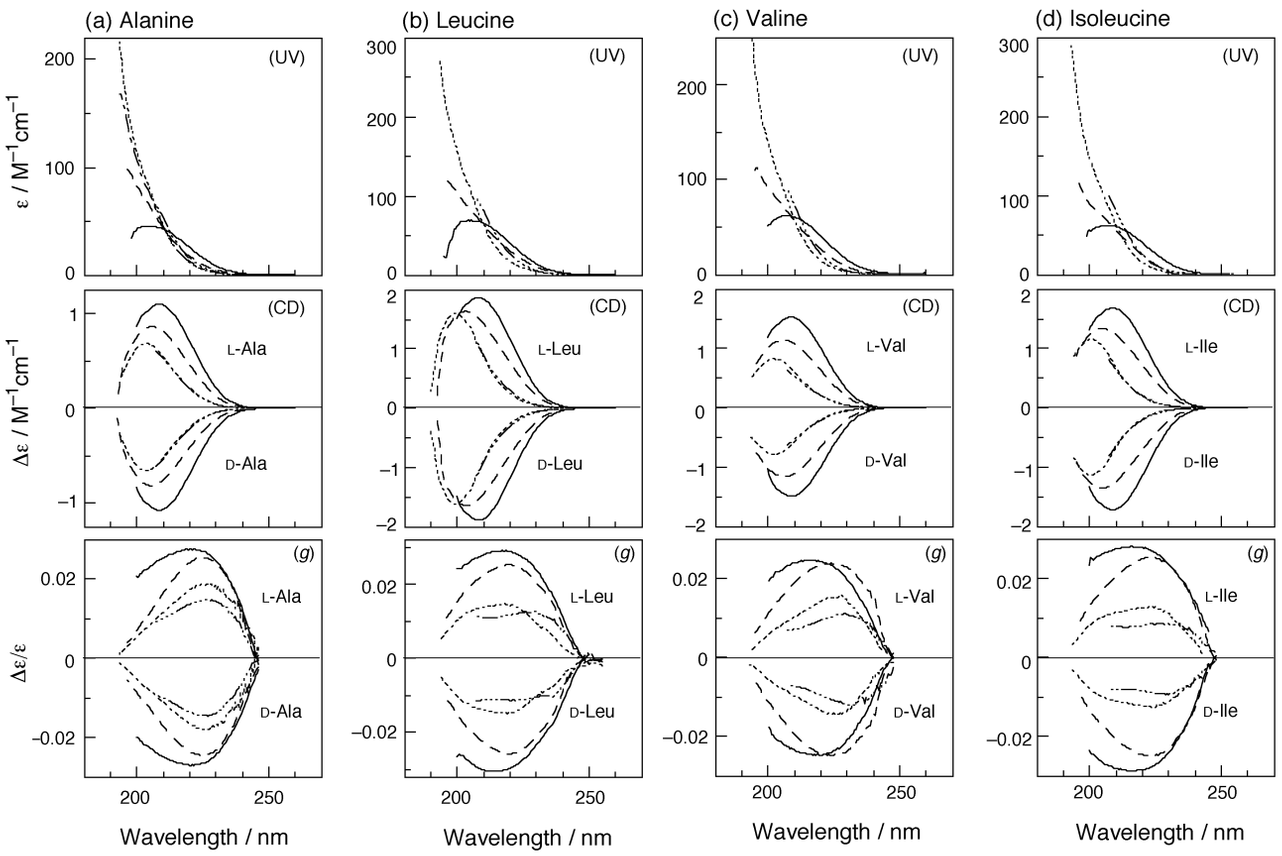


Fig. 1 Chiroptical properties of (a) D- and L-Ala, (b) D- and L-Leu, (c) D- and L-Val and (d) D- and L-Ile at pH 1 (solid), 2 (dash), 7 (dot) and 11 (chain).

Table 1 The ε_{max}'s, $\Delta\varepsilon_{max}$'s, and g_{max}'s of L-Ala, L-Leu, L-Val, L-Ile and L-Pro at various pHs

Amino acid	pH	ε_{max}/M^{-1} cm^{-1} (λ_{max}/nm)	$\Delta\varepsilon_{max}$/M^{-1} cm^{-1} (λ_{ext}/nm)	g_{max}(λ_{ext}/nm)
L-Ala	1	45.9 (205)	1.10 (208)	0.027 (220)
	2	(<200)	0.86 (205.5)	0.025 (226)
	7	(<200)	0.68 (203.5)	0.018 (226)
	11	(<207)	(<207)	0.015 (227)
L-Leu	1	69.4 (205)	1.86 (208)	0.029 (217)
	2	(<200)	1.63 (203.5)	0.025 (220)
	7	(<200)	1.60 (200)	0.015 (218)
	11	(<207)	(<207)	0.013 (230)
L-Val	1	62.9 (207.5)	1.53 (208)	0.025 (217.5)
	2	(<200)	1.14 (206)	0.024 (224)
	7	(<200)	0.82 (202.5)	0.016 (228)
	11	(<207)	(<207)	0.011 (230)
L-Ile	1	62.8 (207)	1.69 (208.5)	0.028 (215.5)
	2	(<200)	1.33 (205)	0.025 (223.5)
	7	(<200)	1.16 (200.5)	0.013 (223.5)
	11	(<207)	(<207)	0.0086 (227)
L-Pro	1	49.8 (202.5)	0.97 (209)	0.026 (222)
	2	(<200)	0.59 (211.5)	0.024 (228)
	7	(<200)	−0.74 (194)	−0.005 (197)
			0.28 (213.5)	0.027 (226)
	11	(<207)	0.29 (213)	0.013 (227)

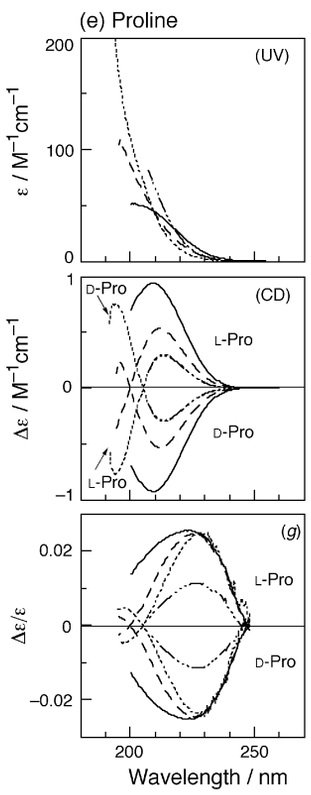


Fig. 2 Chiroptical properties of (e) D- and L-Pro at pH 1 (solid), 2 (dash), 7 (dot) and 11 (chain).

pK_{a2} of Pro is 10.60 (pK_{a1} 2.00),[30] thus, in Pro a significant degree of the composition at pH 11 is still the zwitterionic state. Because of this the CD absorption is shifted to a longer wavelength as the solution pH changes from 1 to 7, but is largely unchanged at pH 11.[25,28] Therefore, the behaviour of the UV and CD spectra (and consequently the *g* factor) over the range of pH 7–11 is different from that of Ala, Leu, Val and Ile. At pH 13, Pro exists exclusively as the aminocarboxylate anion, and as a result the UV absorption shifts to longer wavelengths than observed at pH 11.[34] The weak negative absorption maximum of the CD spectra observed at 235 nm likely arises from the n,σ^* transition of amino group.[24,25]

However in all cases the *g* factor at pH 1, which is predominately determined by the carboxylic n,π^* transition, is the largest in the pH range of 1–7 studied here. Over the range of pH 1–11 the magnitudes of the *g* factors are almost the same for this group and surprisingly large (g_{max}: L-Ala 0.027–0.018, L-Leu 0.029–0.015, L-Val 0.025–0.011, L-Ile 0.028–0.0086, L-Pro 0.027–0.013).[30] The g_{213} value (= 0.029) of L-Leu at pH 1 obtained in this study is larger than that previously reported in the literature (g_{213} = 0.0244 in 0.1 M HCl solution).[5,9]

Chiroptical properties of Ser, Thr, Cys and Met at various pHs

For serine (Ser) and threonine (Thr), the chromophores are a carboxy group, an amino group and a hydroxy group, with the pK_{a1} and pK_{a2} nearly equal to that of Ala (pK_{a1}: Ser 2.21, Thr 2.09, pK_{a2}: Ser 9.15, Thr 9.10).[30] It is thought that the influence of hydroxy groups on the CD spectra over 200 nm is small, as they are distant from the chiral centres. The UV and CD spectra, which are shown in Figs. 3f and 3g, are similar to those observed for Ala owing to the similar contributions made by the n,π* and n,σ* transitions. For Ser, the $\Delta\varepsilon_{max}$'s at pH 1 and 7 are in agreement with those in the literature.[28] The ε_{max} at pH 1 was observed at 205 nm, and an isosbestic point was observed at 210 nm (Fig. 3f), therefore, it is thought that at pH 1 the carboxylic n,π* transition produces the absorption peak at 210 nm.

For threonine (Thr), Fig. 3g shows that it was not possible to clearly observe the ε_{max} values at pH 1 over the range 200 to 270 nm, however, the CD spectral changes with pH were very similar to those of Ser. It is therefore thought that the carboxylic n,π* transition also makes a major contribution to the UV and CD spectra of Thr. The *g* factor spectra of Ser and Thr, shown in Figs. 3g and 3f, show similar characteristics to those of Ala and Leu, with g_{max} magnitudes also of the same order of magnitude as those of Ala and Leu over the pH range 1 to 11 (L-Ser 0.035–0.019, L-Thr 0.028–0.008) (Table 2).

For cysteine (Cys) and methionine (Met) the common chromophores are the carboxy and amino groups, in addition to a thiol group and a sulfide group as the third chromophore, respectively. The pK_{a1} and pK_{a2} values are pK_{a1}: Cys 1.86, Met 2.28, pK_{a2}: Cys 8.35, Met 9.21. In Cys, the pK_{a3} = 10.34 which corresponds to the equilibrium between the thiol and thiolate form also influences the chiroptical characteristics of Cys.[30] For Cys, there are frequently disagreements between the chiroptical data in the literature because the thiol group is very sensitive to oxygen,[26,27,29] therefore in this case, the UV and CD spectra of Cys were measured after nitrogen purging. Figs. 3h and 3i shows the UV and CD spectra of Cys and Met at various pHs. In the literature,[35] it has previously been reported that it is impossible for Cys to detect the presence of the weak carboxy

Table 2 The ε_{max}'s, $\Delta\varepsilon_{max}$'s, and g_{max}'s of L-Ser, L-Thr, L-Cys and L-Met at various pHs

Amino acid	pH	ε_{max}/M^{-1} cm^{-1} (λ_{max}/nm)	$\Delta\varepsilon_{max}$/M^{-1} cm^{-1} (λ_{ext}/nm)	g_{max}(λ_{ext}/nm)
L-Ser	1	45.9 (205)	1.34 (208.5)	0.035 (226)
	2	(<200)	1.09 (205.5)	0.033 (227.5)
	7	(<200)	0.88 (203)	0.023 (229)
	11	(<207)	(<207)	0.019 (230.5)
L-Thr	1	(<200)	1.41 (209)	0.028 (222)
	2	(<200)	0.83 (209)	0.024 (226.5)
	7	(<200)	0.24 (208)	0.014 (240)
	11	(<207)	0.19 (210)	0.008 (230.5)
L-Cys	1	(<200)	1.87 (208)	0.007 (226)
	2	(<200)	2.16 (202.5)	0.006 (218.5)
	7	(<200)	2.37 (202)	0.004 (212)
	11	(<207)	3.30 (216)	0.0008 (215.5)
		4870 (235.5)		
L-Met	1	(<205)	1.51 (207.5)	0.0019 (227)
	2	(<200)	1.42 (202.5)	0.0011 (225)
	7	(<200)	1.62 (199)	0.0008 (200)
				−0.0007 (235)
	11	(<207)	0.84 (208)	0.0005 (222.5)

n,π* transition due to overlapping of the thiol band. However, in this case, it is thought that the blue shift observed on changing the solution pH from 1 to 7 indicates the contribution of the carboxy n,π* transition to the CD band at 208 nm (pH 1), although this may partially overlap with the thiol band (Fig. 3h (CD)).[24,27,29] At pH 7 Cys adopts two conformations,[26,36] with a negative weak Cotton effect due to L-Cys observed at 260 nm arising from the n,σ* transition of the thiol group in one of these conformations. At pH 11 the contribution of the thiolate dominates the UV spectrum, which can be seen in the UV absorption band at 238 nm.[29,34]

Fig. 3i shows the UV and CD spectra of Met at various pHs. The CD band at 208 nm (pH 1) is thought to arise from the transition of the sulfide group.[29] The ε_{max} and $\Delta\varepsilon_{max}$ values are shifted to shorter wavelengths on changing the solution pH from 1 to 7, as a result of the conversion from the carboxylic acid to carboxylate anion form. At pH 7, a negative Cotton effect of L-Met was observed at 250 nm, which was in agreement with the reported literature.[27–29] For Cys and Met, the UV spectra have absorption bands with relatively large ε values (~10^2 M^{-1} cm^{-1}) in the range 190–250 nm; as a result the g_{max} values, which are shown in Figs. 3h and 3i and Table 2, are small (L-Cys 0.007–0.0008, L-Met 0.0019–0.0005) over the range of pH 1–11.

Chiroptical properties of Asp and Glu at various pHs

For asparatic acid (Asp) and glutamic acid (Glu) the chromophores are the amino and carboxy groups (carboxy-1) at the chiral centre, with another carboxy group (carboxy-2) distant

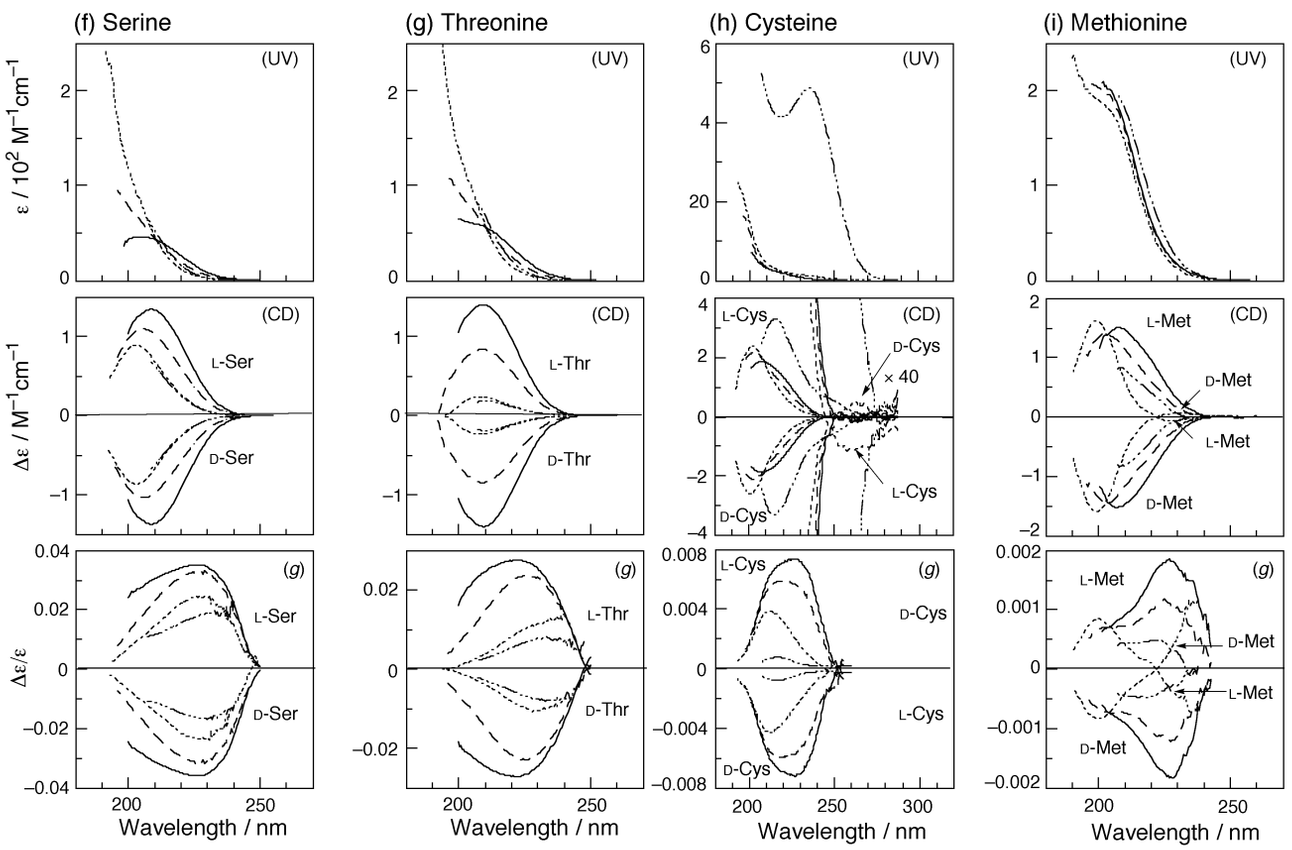


Fig. 3 Chiroptical properties of (f) D- and L-Ser, (g) D- and L-Thr, (h) D- and L-Cys and (i) D- and L-Met at pH 1 (solid), 2 (dash), 7 (dot) and 11 (chain).

Table 3 The ε_{max}'s, $\Delta\varepsilon_{max}$'s, and g_{max}'s of L-Asp and L-Glu at various pHs

Amino acid	pH	ε_{max}/M^{-1} cm^{-1} (λ_{max}/nm)	$\Delta\varepsilon_{max}$/M^{-1} cm^{-1} (λ_{ext}/nm)	g_{max} (λ_{ext}/nm)
L-Asp	1	74.7 (205.5)	0.83 (207)	0.013 (223)
	2	(<200)	0.99 (201)	0.0096 (222)
	7	(<200)	1.17 (204)	0.011 (225)
	11	(<207)	1.38 (209.5)	0.007 (232.5)
L-Glu	1	92.2 (205)	1.38 (208)	0.017 (221)
	2	(<200)	1.20 (204)	0.013 (220)
	7	(<200)	1.04 (201)	0.008 (225)
	11	(<207)	0.74 (208.5)	0.0049 (231)

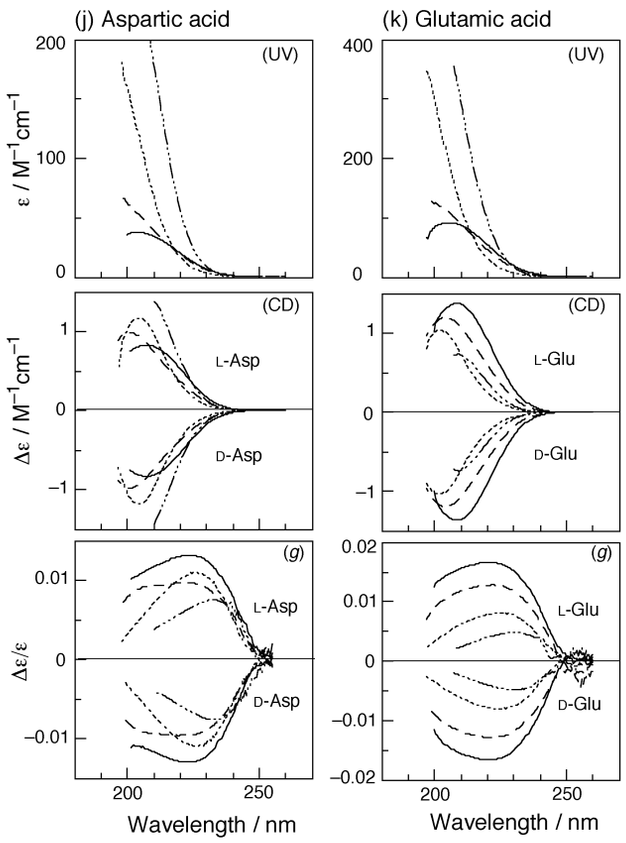


Fig. 4 Chiroptical properties of (j) D- and L-Asp and (k) D- and L-Glu at pH 1 (solid), 2 (dash), 7 (dot) and 11 (chain).

from the chiral centre. The ionic composition determining pK_{a1}, pK_{a2} and pK_{a3} values are pK_{a1}: Asp 2.10, Glu 2.10, pK_{a2}: Asp 3.86, Glu 4.07, pK_{a3}: Asp 9.82, Glu 9.47,[30] with both of the carboxylic n,π* transitions determining the UV and CD spectra over the pH range 1–7.[24] Considering these pH dependencies of the ionic composition, the UV and CD spectral changes shown in Figs. 4j and 4k in the pH range 1 to 2 correspond to composition changes of carboxy-1, which changes from the carboxylic acid to carboxylate form, with the spectral changes in the pH range 2 to 7 corresponding to the composition changes associated with carboxy-2. For both Asp and Glu the red shifts observed in the UV and CD spectra in the pH range 7 to 11 arise from the change of the amino group from the ammonium to amine form.

Figs. 4 (*g*–j and *g*–k) shows the *g* factor spectra, with the g_{225} of Glu at pH 7 in good agreement with that reported by Norden (g_{225} = 0.008 in water).[22] The *g* factors of Asp and Glu at pHs 1–3, in which the carboxylic n,π* transitions are dominant, are relatively large due to the small ε values in the UV spectra (g_{max}: L-Asp 0.013–0.0096, L-Glu 0.017–0.008) (Table 3).

Chiroptical properties of Asn, Gln, Lys and Arg at various pHs

For asparagine (Asn) and glutamine (Gln) the chromophores are the amino and carboxy group, with an additional amide group not directly bonded to the chiral centre. Each pK_{a1} and pK_{a2} is roughly the same as that of Ala (pK_{a1}: Asn 2.02, Gln 2.17, pK_{a2}: Asn 8.80, Gln 9.13).[30] Figs. 5l and 5m shows the UV and CD spectra of Asn and Gln at pHs 1, 2, 7 and 11. The UV spectral changes over the pH range 1 to 7 were very small, however significant changes were observed on changing the pH from 7 to 11. For both Asn and Gln the CD spectra underwent blue shifts on changing the pH from 1 to 7. For Asn the CD absorption became more intense on changing the pH from 1 to 7,[29] with the tendency in Gln opposite to that of Asn. For Asn at pH 11, a weak CD band was observed at 223 nm,[28] which is thought to originate from the n,π* transition of the amide group. The large differences in the behaviour of the Asn and Gln CD spectra is as a result of the structural differences between Asn and Gln, which is quite significant considering that there is only one methylene group in the chain between the chiral centre and the amide group, although, it is worth considering that at pH 11 there may be a significant interaction between the carboxy and amide groups in Asn. The ε's of Asn and Gln are large, especially for Asn, and consequently the *g* factors are relatively small even at pH 1 (L-Asn 0.005 at 226 nm, L-Gln 0.010 at 226 nm) as shown in Figs. 5l and 5m and in Table 4.

For lysine (Lys) and arginine (Arg) the chromophores are the amino and carboxy group with another amino group distant from the chiral centre. The pK_{a1}, pK_{a2} and pK_{a3} values are pK_{a1}: Lys 2.18, Arg 2.01, pK_{a2}: Lys 8.95, Arg 9.04, pK_{a3}: Lys 10.53, Arg 12.48.[30] In both cases, the pK_{a1}, pK_{a2}, and pK_{a3} values determine the equilibrium between the carboxylic acid and carboxylate anion, and between the two ammonium cation and the amine forms. The pK_{a3} values indicate that the amino group that is far from the chiral centre is, to a degree, in the form of an ammonium cation over the entire pH range used in this study. Figs. 5n and 5o show the UV and CD spectra of Lys and Arg at pHs 1, 2, 7 and 11. For Lys an isosbestic point is observed in the UV spectra at 210 nm as the solution pH is changed from 1 to 7, with the change from 7 to 11 shifting the CD to longer wavelengths. For Arg, the behaviour of the UV spectra over the pH range 1 to 7 was not clear because these absorption maxima were below 210 nm and only the tails of the absorption bands were observable. However, the CD maxima were shifted to shorter wavelengths on changing the solution pH from 1 to 7,[29] then shifted to longer wavelengths on changing the solution pH from 7 to 11.[28] These pK_{a3} values are high (especially in Arg), therefore, even at pH 11 the form of the amino groups far from the chiral centres is still predominantly ammonium. This shows that the carboxylic n,π* transition contributes considerably to the CD spectra over the pH range 1 to 7, and that the n,σ* transition of the amino group bonded to the chiral centre dominates the CD spectra over the pH range 7 to 11. Although the values of the ε's are high (*ca.* 10^3 M^{-1} cm^{-1}), the g_{max}'s of L-Lys and L-Arg at pH 1 are still relatively large (L-Lys 0.026 at 220 nm, L-Arg 0.017 at 221 nm) (Figs. 5n and 5o and Table 4).

Chiroptical properties of Phe, Tyr, His and Trp at various pHs

Phenylalanine (Phe) and tyrosine (Tyr), in addition to the amine and carboxy chromophores, have a phenyl and phenol

Table 4 The ε_{max}'s, $\Delta\varepsilon_{max}$'s and g_{max}'s of L-Asn, L-Gln, L-Lys and L-Arg at various pHs

Amino acid	pH	ε_{max}/M^{-1} cm^{-1} (λ_{max}/nm)	$\Delta\varepsilon_{max}$/M^{-1} cm^{-1} (λ_{ext}/nm)	g_{max}(λ_{ext}/nm)
L-Asn	1	(<200)	0.69 (206)	0.005 (226)
	2	(<200)	0.79 (203)	0.003 (225)
	7	(<200)	1.02 (201)	0.002 (208)
	11	(<207)	−0.104 (223)	−0.0018 (233.5)
L-Gln	1	(<200)	1.55 (208.5)	0.010 (226)
	2	(<200)	1.51 (205.5)	0.007 (224.5)
	7	(<200)	1.25 (202)	0.004 (210)
	11	(<207)	0.808 (211.5)	0.0035 (225)
L-Lys	1	(<200)	1.46 (208)	0.026 (220)
	2	(<200)	1.22(204)	0.023 (224)
	7	(<200)	1.08 (201)	0.013 (221.5)
	11	(<207)	(<207)	0.011 (226.5)
L-Arg	1	(<200)	1.77 (204)	0.017 (221)
	2	(<200)	1.79 (202)	0.013 (220)
	7	(<200)	1.91 (201)	0.006 (220)
	11	(<207)	0.82 (208.5)	0.0056 (224.5)

group for the third chromophores, respectively. The pK_{a1} and pK_{a2} values are pK_{a1}: Phe 2.58, Tyr 2.20 and pK_{a2}: Phe 9.24, Tyr 9.11,[30] being nearly equal to those of Ala. Figs. 6p and 6q show the UV and CD spectra of Phe and Tyr at various pHs. For Phe the intense π,π* band (^1L_a band) has its ε_{max} at 208 nm, with the weak aromatic forbidden π,π* band (^1L_b band) having an ε_{max} at around 250 nm.[24,37,38] The UV and CD isosbestic points were observed at 208 and 212 nm, respectively, with the $\Delta\varepsilon_{max}$ shifted from 218 nm to 216 nm and the intensities decreased on changing the solution pH from 1 to 7. Further, the g_{max} peak shifted from 229 nm to 227 nm, and its magnitude decreased. It was reported that the allowed aromatic π,π* band (^1L_a band) at 218 nm (pH 1) overlapped with the carboxylic n,π* band, with the pH dependence allowing facile interpretation of the results obtained in this study.[24] In the present case, Fig. 6p (CD) shows that the $\Delta\varepsilon_{max}$ only undergoes a 2 nm blue shift on changing the solution pH from 1 to 7, with the forbidden band (^1L_b band) at 278 nm shifting to a longer wavelength with decreased intensity. The characteristic fine structure of the band at 278 nm reveals it as a transition arising from an aromatic chromophore. These observations agree with reports that explain that this shift in the CD spectra is due to some interaction between the phenyl and carboxy groups, and the interaction is stronger than that between the phenyl and carboxylate anion.[24,37] These results indicate that the carboxylic n,π* transition contributes little to the CD signal at 278 nm.

For Tyr isosbestic points were observed in the UV spectra at 228 nm, with the spectra shifted to longer wavelengths on raising the pH from 7 to 11. The absorptions at 243 nm and 287 nm are thought to be from the phenolate.[37] It was observed that there are three bands in the CD spectra in the region 200 nm to 290 nm, which is in agreement with the literature.[39] For Tyr the CD band at 227 nm displays a clear blue shift in contrast to that of Phe on changing the pH from 1 to 7, suggesting that it is the carboxylic n,π* transition that is responsible for the CD band at 227 nm, although it was

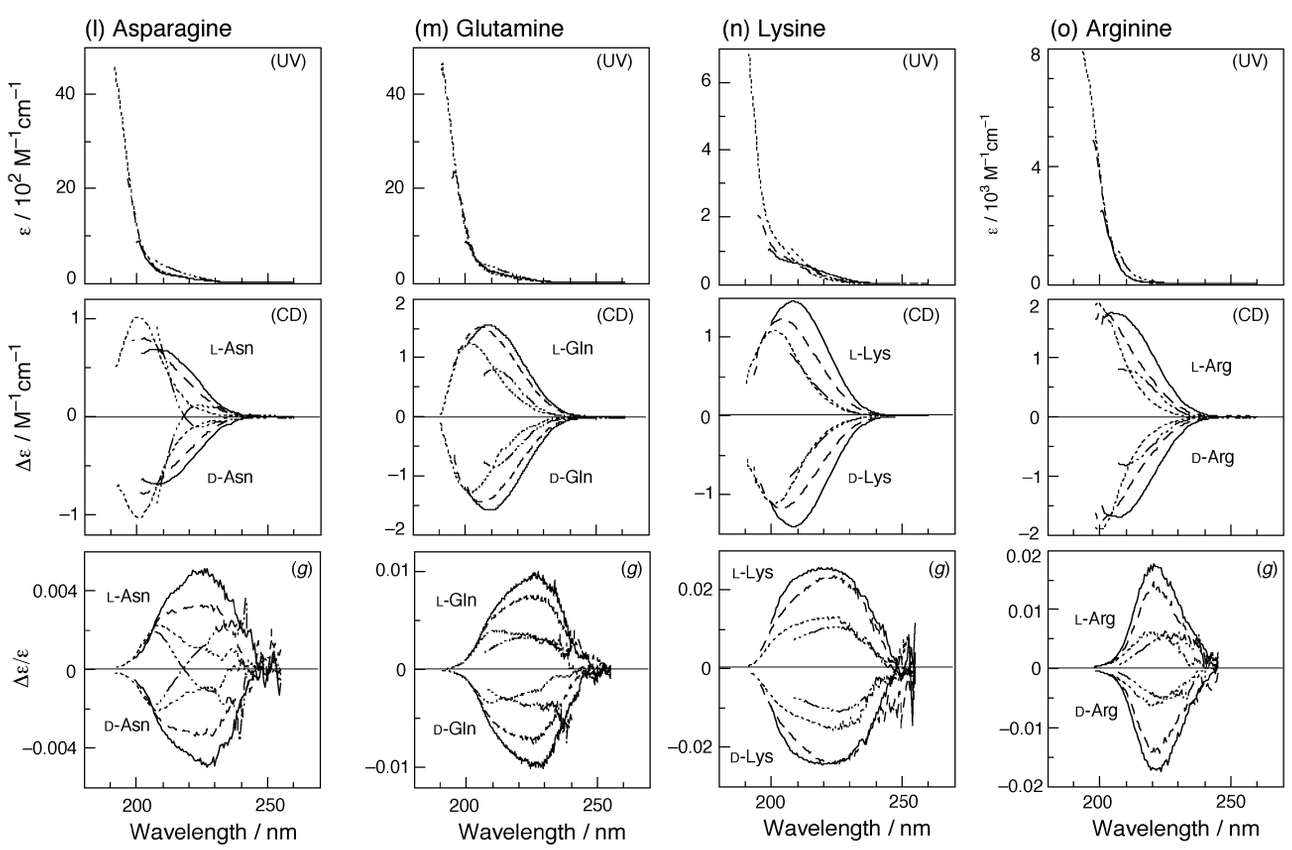


Fig. 5 Chiroptical properties of (l) D- and L-Asn, (m) D- and L-Gln, (n) D- and L-Lys and (o) D- and L-Arg at pH 1 (solid), 2 (dash), 7 (dot) and 11 (chain).

Table 5 The ε_{max}'s, $\Delta\varepsilon_{max}$'s and g_{max}'s of L-Phe and L-Tyr at various pHs

Amino acid	pH	ε_{max}/M^{-1} cm^{-1} (λ_{max}/nm)	$\Delta\varepsilon_{max}$/M^{-1} cm^{-1} (λ_{ext}/nm)	g_{max} (λ_{ext}/nm)
L-Phe	1	8580 (206)	4.43 (218.5)	0.014 (228)
	2	8650 (206)	3.90 (218)	0.011 (228)
	7	8650 (206)	2.80 (217)	0.0045 (226)
	11	8110 (208)	2.86 (216.5)	0.0021 (232)
L-Try	1	13800 (200)	2.22 (203)	0.0003 (204.5)
		8730 (223)	2.7 (227)	0.0007 (235.5)
		1530 (274)	0.3 (276)	0.0007 (240.5)
	2	29500 (196)	1.9 (203)	0.0003 (205.5)
		7640 (223)	1.8 (226)	0.0005 (240.5)
		1310 (274)	0.3 (276)	0.0002 (~270)
	7	49600 (193)	2.5 (201)	0.0003 (205.5)
		9170 (223)	0.7 (221.5)	0.0002 (~270)
		1530 (274)	0.3 (276)	
	11	7050 (225.5)	−0.382 (243.0)	0.0002 (211.5)
		6040 (240)	0.219 (287)	0.0001 (251.5)
		1500 (282.5)		0.0002 (~270)

reported to be from the ^1L_a band of Tyr for ribonuclease S.[40] For the other two bands in the CD spectra, the band at the longer wavelength undergoes a dramatic red shift on changing the pH from 7 to 11, indicating that the aromatic π,π* transition contributes to the CD band (^1L_b band). Three *g* factors were also observed corresponding to these three bands, suggesting that the aromatic π,π* transition of Tyr determines the *g* factor at 203 nm and 276 nm. Comparing the g_{max} of Phe with that of Tyr, the g_{max} of Phe is considerably larger than that of Tyr (Table 5). Considering the effect of solution pH on the CD and UV spectra, the contribution of the n,π* transition of Phe at 220 nm is thought to give rise to the large *g* factors at pH 1 and 2. For Tyr, our data ($g_{max} = g_{270} = 0.0002$) is in agreement with the *g* factor ($g_{266} = 0.000176$) estimated by Nikogosyan *et al.* using the % ee of Tyr generated by CPL irradiation using laser irradiation.[41]

For histidine (His) and tryptophan (Trp) the chromophores are the common amine and carboxy groups, with a further imidazolyl and indolyl group, respectively, as the third chromophores. The pK_{a1}, pK_{a2} and pK_{a3} values are pK_{a1}: His 1.77, Trp 2.38, pK_{a2}: His 6.10, Trp 9.39, pK_{a3}: His 9.18.[30] The pK_{a3} value embodies the equilibrium between the imidazole and imidazolium cation in His.

Figs. 6r and 6s show the UV and CD spectra of His and Trp. For His the spectral changes in the UV spectra were small in the pH range 1 to 11, with a small red shift observed for the pH change 7 to 1.[24] For Trp, isosbestic points in the UV spectra were observed at 240 nm for the pH change between 1 to 7, and at 228 nm and 238 nm for the pH change between 7 to 11;[24] this change corresponds to the pH dependent ionic composition changes. From pH 1 to 7, the ammonium carboxylic acid changes to the ammonium carboxylate anion, and from pH 7 to 11, the ammonium and indolyl cations change to the amine and indole, respectively. The CD spectra in Figs. 6r and 6s show that

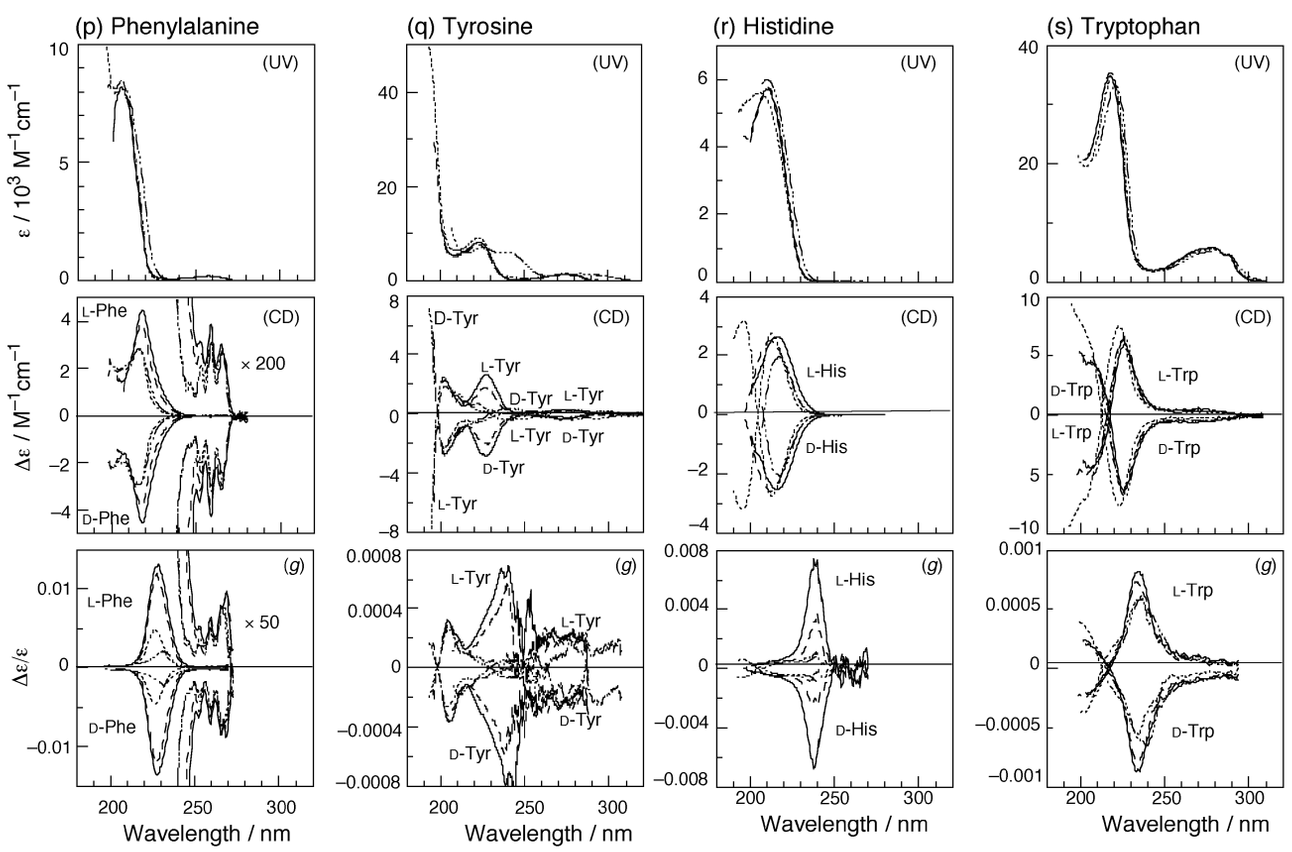


Fig. 6 Chiroptical properties of (p) D- and L-Phe, (q) D- and L-Tyr, (r) D- and L-His and (s) D- and L-Trp at pH 1 (solid), 2 (dash), 7 (dot) and 11 (chain).

Table 6 The ε_{max}'s, $\Delta\varepsilon_{max}$'s and g_{max}'s of L-Trp and L-His at various pHs

Amino acid	pH	ε_{max}/M^{-1} cm^{-1} (λ_{max}/nm)	$\Delta\varepsilon_{max}$/M^{-1} cm^{-1} (λ_{ext}/nm)	g_{max}(λ_{ext}/nm)
L-His	1	5840 (213)	2.6 (217)	0.007 (238)
	2	5930 (211)	2.5 (212)	0.0006 (215)
				0.004 (240)
	7	5800 (204.5)	−3.2 (196)	−0.0006 (195)
			2.8 (213)	0.001 (239)
	11	6010 (210.5)	1.97 (217.0)	0.0008 (~239)
L-Trp	1	35400 (218)	−4.4 (206)	0.0008 (234)
		5940 (279)	6.1 (226.5)	
		4890 (287.5)	0.6 (269)	
	2	35400 (218.5)	−5.0 (202.5)	0.0007 (233)
			6.3 (224.5)	
		5940 (279)	0.6 (269)	
		4890 (287.5)		
	7	34800 (219)	−7.1 (202.5)	0.0006 (236)
		5590 (279)	7.6 (223)	
		4720 (287.5)	0.4 (267)	
	11	33300 (221.0)	−(<207)	0.0006 (236)
		5400 (280.0)	6.73 (226.0)	
		4610 (288.0)	0.4 (269.5)	

blue shifts of the CD bands (pH 1: His 217 nm, Trp 226.5 nm) were observed on changing the solution pH from 1 to 7, whilst red shifts of the CD bands (pH 7: His 213 nm, Trp 223 nm, pH 11: His 217 nm, Trp 226 nm) were observed on changing the pH from 7 to 11. This suggests that the n,π* transition of the carboxy group contributes to the bands over the pH range 1 to 7. In the case of Trp, it is uncertain whether the n,σ* transition of the amino group mainly contributes to the bands or not, because the ^1B_b band of the indole chromophore of various substituted indoles also appears at 224 nm.[42,43] For Trp, fine structure was observed at *ca.* 270 nm in the UV spectra, which suggests that the band arises from the ^1L_b band of the indole group. In both cases, the *g* factors are relatively small because the ε's from the imidazolyl and indolyl groups are large relative to the corresponding $\Delta\varepsilon$'s.

The origin of homochirality on amino acid by CPL irradiation

In relation to the origin of homochirality in amino acids *via* CPL irradiation process(es), the importance of the consideration of the pH on the chiroptical properties can be clearly observed. From the results detailed above the *g* factor shows a clear dependency on the ionic state, and thus pH, of the amino acid; with the *g* factors directly affecting the magnitude of the generated ee. Applying Kagan's equation (using the pH dependent *g* factors) suggests that the % ee of amino acids at pH 7 should be non-zero. However, in our recent paper it was found that at pH 7 and above the detection of ee (≡ op) of Leu generated by CPL irradiation at 215 nm was beyond the limit of instrumentation (JASCO J-725) and therefore smaller than 0.05%, even though the *g* factor was over 0.01.[10] For the aliphatic essential amino acids, the *g* factors' pH dependence and dominant electronic transition(s) are essentially identical to those of Leu. In the case of most amino acids irradiated with CPL at 215 nm, the % op generated on CPL irradiation may also come close to zero at pH 7.

This has important consequences for the mechanism of the origin of homochilarity in amino acids. In the Murchison meteorite Val and Ala were discovered in significant ee's.[14–18] Therefore the ionic state of the amino acids found on the Murchison and other meteorites is a key issue, however, currently the ionic state of these amino acids when contained within the meteorite body is not known. Indeed, the implications from this and previous work is that the pH will play a decisive role in the generation of an ee,[1,2,10,44–46] showing the need to unambiguously determine this issue for amino acids of extraterrestrial origins.

In order to answer these questions on the origin of homochirality of amino acids more rigorous investigation of the AAS of amino acids by CPL irradiation is required, and it is hoped that the chiroptical data and interpretations reported here will greatly contribute.

Conclusions

The ε's and $\Delta\varepsilon$'s of all the essential amino acids were measured in aqueous solution at a series of pHs, and the *g* factors subsequently determined, constituting the first comprehensive report of the chiroptical properties of all the essential amino acids under identical conditions. It was revealed that the *g* factors were high, and except for Asn and the aromatic and sulfur containing compounds, *i.e.* Cys, Met, Tyr, His and Trp, the g_{max} values of these amino acids are relatively large, over 0.01 for the pH range 1–7. The rationale of this is that for the aliphatic amino acids the spectral characteristics are dominated by the n,π* transition (possessing low ε values of *ca.* 10–100), which means that the combined molar absorption coefficient ($\varepsilon_{carboxy} + \varepsilon_{amino} + \varepsilon_{aromatic}$) term in the equation $\Delta\varepsilon/\varepsilon = \Delta\varepsilon/(\varepsilon_{carboxy} + \varepsilon_{amino} + \varepsilon_{aromatic})$ is small, as there are little or no contributions from the ε_{amino} and $\varepsilon_{aromatic}$ terms, thus giving rise to larger *g* factors. For aromatic amino acids the dominant transitions are the π,π* transition (for example, ^1L_a band of Phe, possessing high ε values of *ca.* 10^3–10^4 M^{-1} cm^{-1}) resulting in a larger ($\varepsilon_{carboxy} + \varepsilon_{amino} + \varepsilon_{aromatic}$) term and subsequently lower *g* factors. Indeed for aliphatic amino acids large *g* factors are observed even at pH 11, as the n,σ* transition does not contribute to ε to the same degree as the π,π* transition, with the magnitudes of the *g* factors at pH 1, on the whole, 2–3 times that at pH 7. For L-Phe the g_{max} was 0.014 at 228 nm at pH 1, differing greatly from the other aromatic amino acids. This is due to the n,π* transition appearing in the UV absorption spectrum in a region where the intense π,π* transition is weak; thus the ($\varepsilon_{carboxy} + \varepsilon_{amino} + \varepsilon_{aromatic}$) term is smaller in this n,π* region resulting in the relatively large *g* factor of Phe.

As detailed earlier the optical characteristics of these amino acids show a significant pH dependence, with the general trend being that on increasing pH the $\Delta\varepsilon_{max}$ values become increasingly blue-shifted and less intense, and the g_{max} values are reduced, though less so for aromatic amino acids. This is as a direct result of modifications to the ionic state of the active chromophores arising from the changes in pH, which changes their optical characteristics. As discussed previously the n,π* transition of the carboxy group dominates the CD spectra (with transitions from groups such as thiol, sulfide, aromatic and alcohol making more minor contributions); as the pH is increased this contribution shifts to shorter wavelengths. This has the two-fold effect of blue shifting the $\Delta\varepsilon_{max}$ (from 200–250

nm to <200 nm) and reducing its intensity due to a greater separation with the minor n,σ* transition, which is conversely red-shifted on increasing pH.

For the aromatic containing amino acids the changes in the CD spectra are complicated by the presence of the π,π* transitions, and thus the *g* factors do not so closely follow the same pH dependent changes seen for the aliphatic amino acids. This is due to the contributions made by the π,π transitions of the aromatic moieties in the UV which have different pH dependencies.

Additionally, as it is the same pH dependent n,π transition of the carboxylic group that determines the *g* factor, UV and CD spectra for all the aliphatic amino acids, it is thought that the % ee (≡ % op) for these irradiated with CPL at 215 nm will also be zero at pH 7, as observed previously for Leu. This is an important point to solve in relation to the origin of homochirality of amino acids.

Experimental

General procedure

The UV spectra were measured using a JASCO V-560 spectrometer. The circular dichroism (CD) spectra were measured using a JASCO 720 WI instrument with a rectangular quartz cell (light-path length = 10 mm): room temperature 23 ± 1 °C, band width 1 nm, resolution 0.5 nm, response 4 sec, scan speed 50 mm/sec, number of scans 4–8 times. Each *g* factor is calculated from the correspondin ε and $\Delta\varepsilon$, both of which were independent of the amino acid concentration, ranging from 2 to 20 mmol dm^{-3} for aliphatic amino acids and from 0.03 to 3 mmol dm^{-3} for aromatic amino acids.[34]

Materials

All of the amino acids were commercially available from Aldrich (A) or Wako (W) and used without further purification; D-alanine (D-Ala, W), L-alanine (L-Ala, W), D-leucine (D-Leu, W) and L-leucine (L-Leu, W), D-valine (D-Val, W), L-valine (L-Val, W), D-isoleucine (D-Ile, W), L-isoleucine (L-Ile, W), D-proline (D-Pro, W), L-proline (L-Pro, W), D-serine (D-Ser, W), L-serine (L-Ser, W), L-threonine (L-Thr, W), D-threonine (D-Thr, W), D-cysteine monohydrochloride monohydrate (D-Cys, W), L-cysteine monohydrochloride monohydrate (L-Cys, W), L-methionine (L-Met, W), L-methionine (D-Met, W), D-aspartic acid (D-Asp, W), L-aspartic acid (L-Asp, W), D-glutamic acid (D-Glu, W), L-glutamic acid (L-Glu, W), D-asparagine (D-Asn, A), L-asparagine (L-Asn, A), D-glutamine (D-Gln, W), L-glutamine (L-Gln, W), D-lysine monohydrochloride (D-Lys, A), L-lysine monohydrochloride (L-Lys, A), D-arginine (D-Arg, W), L-arginine (L-Arg, W), D-phenylalanine (D-Phe, W), L-phenylalanine (L-Phe, W), D-tyrosine (D-Tyr), L-tyrosine (D-Tyr, W) D-histidine (D-His, W), L-histidine (L-His, W), D-tryptophan (D-Trp, W), L-tryptophan (L-Trp, W), Glycine (Gly, W) and propane-1-thiol (W).

pH Adjustment

Solutions of D- and L-amino acids and Gly were prepared with 0.1 M or 0.01 M standard HCl solution, 0.1 M or 1 M standard NaOH solution and distilled water. The precise pHs were checked using a Horiba F-12 pH meter. The actual pHs as indicated by pH 7 were 6.23–7.49 at 22.6–24.8 °C.[34] The differences between the actual pHs and pHs indicated by pH 1, 2 and 11 were within ±0.2.

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