Proton, copper(II) and nickel(II) complexes of some Amadori rearrangement products of D-glucose and amino acids

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Abstract

The formation of the copper(II) and nickel(II) complexes of six Amadori rearrangement products of D-glucose with amino acids ('fructose–amino acids') was investigated by potentiometry, CD and EPR spectroscopy. The −I effect of the fructose residue in the ligands was reflected by the protonation constants of their amino groups In the complexes ML formed with the copper(II) ion, besides the amino acid-type coordination a weak interaction with non-deprotonated alcoholic hydroxy groups was demonstrated by CD studies. Increase of the pH may lead to transformation of the species ML to give complexes ML_2 and/or MLH_{-1}. The former contains two nitrogen donor atoms in the copper(II) coordination sphere, while in MLH_{-1} the ligands are coordinated through their carboxyl, amino and deprotonated alcoholic OH groups, as revealed by equilibrium, CD and EPR studies. Above pH ~7, a redox reaction takes place between copper(II) and the ligand. The nickel(II) ion forms amino acid-type parent complexes ML and ML_2, while deprotonated species predominate in solution above pH ~9.

Introduction

Amadori [1] demonstrated that the condensation of D-glucose with an aromatic amine yields two structurally different isomers which are not α,β anomeric pairs. Kuhn and Weygand [2] proved that the stable isomer (named after Amadori) was 1-arylamino-1-deoxy-D-fructose. Amino acids readily react with D-glucose to give 'fructose–amino acids' through an analogous rearrangement.

An intensive study of the chemistry of non-enzymic browning by Anet and Reynolds [3] led to the conclusion that this type of Amadori rearrangement is the key process in the Maillard reaction. This assumption was supported by the studies by Deifel [4] on these reactions in honey.

Fructose-amino acids have also been detected in liver extracts by Abrams *et al.* [5]; they stimulate amino acid incorporation into the proteins of rabbit reticulocytes even under *in vitro* conditions.

In spite of the biological relevance of these compounds, study of their metal complexation has been rather neglected in the literature. The calcium(II) ion binding by some Maillard reaction products in food models was monitored as a function of pH by Rendleman [6]. Terasowa *et al.* [7] studied the copper(II) ion chelation by fractions of the same reaction products with glycine. Chen *et al.* [8] prepared the platinum(II) and palladium(II) complexes of fructose–glycine (FRU–GLY) and fructose–β-alanine (FRU–β-ALA) and from IR spectra concluded that the ligands are coordinated to the metal ions through the amino and carboxyl groups.

For a better insight into the complex-forming equilibria of these compounds, we have studied the coordination properties of Amadori rearrangement products of D-glucose and six amino acids (Fig. 1). Their proton, copper(II) and nickel(II) complexation was investigated in aqueous solution by potentiometry, EPR and CD spectroscopy. The results are presented below.

Experimental

Materials

All reagents used for the measurements were Reanal products, except for copper(II) perchlorate and nickel(II) perchlorate (Fluka). The concentrations of

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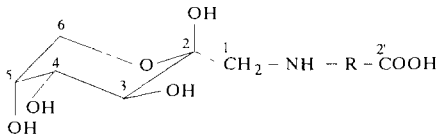

Ligand	R =
FRU - GLY	$^{1}CH_2$
FRU β - ALA	$^{1'}CH_2$ $^{3'}CH_2$ -
FRU VAL	$^{1'}CH$ $^{3'}CH(^{4'}CH_3)_2$
FRU LEU	$^{1'}CH$ $^{3'}CH_2$ $^{4'}CH(^{5'6'}CH_3)_2$
FRU ILE	$^{1'}CH$ - $^{3'}CH$ $^{4'}CH_2$ $^{5'}CH_3$ $^{6'}CH_3$
FRU - PHE	$^{1'}CH$ $^{3'}CH_2$ - $^{4'}$ $^{9'}$PHE

Fig 1. Structure of fructose–amino acids investigated, with numbering of atoms.

the standard metal-ion solutions were determined complexometrically.

Preparation of the fructose–amino acids

A large number of prescriptions [3, 5, 9-11] and two reviews [12, 13] report the modes of preparation of Amadori rearrangement products. The most frequently used and cited reference is that of Hodge and Fischer [10]. Our substances were also prepared by their method.

After the reversible reactions of amino acids with aldoses to yield N-substituted glucosylamines, irreversible isomerization to Amadori compounds takes place. The reaction is autocatalytic: no additional acid catalyst is needed. The presence of sodium pyrosulfite ($Na_2S_2O_5$) was found to increase the conversion.

The purity of the ligands was checked by IR and ^{1}H and ^{13}C NMR spectroscopy. The IR spectra (KBr pellets) displayed the characteristic bands [14] of Amadori products in pyranose-ring form, at about 3500 cm^{-1}. The signals in the ^{1}H and ^{13}C NMR spectra were assigned after Altena *et al.* [15] and Roper *et al.* [16]. They found that mutarotated aqueous solutions of fructose-amino acids contain ~64% of β-pyranose, ~14% of β-furanose, ~14% of α-furanose, ~6% of α-pyranose and only a small percentage of the open-chain form. In our spectra, recorded shortly after the dissolution of fructose-amino acids in D_2O, only the lines of the β-pyranose forms could be assigned. The recorded chemical shifts are in good agreement with data given in the above references.

The molecular weights calculated from potentiometric titrations indicated that all compounds had a purity of more than 99.5%.

pH-metric measurements

Both the protonation and metal ion coordination equilibria were investigated by potentiometric titration in aqueous solution. The ionic strength was adjusted to 0.1 mol dm^{-3} with $NaClO_4$, and the cell was thermostated at 298±0.1 K. In order to remove carbon dioxide and molecular oxygen, high-purity nitrogen gas was bubbled through the solution during the titration. The electrode system (Radelkis OP-0718P glass electrode and Radelkis OP-0831P silver–silver chloride reference electrode) was calibrated before each measurement in the following way. A mixture of TRIS (tris(hydroxymethyl)methylamine) and strong acid ($HClO_4$) of known composition was titrated with standard sodium hydroxide solution. The electrode potential was recorded with a Radelkis OP-208/1 precision digital pH-meter in a full automatic titration set. From the e.m.f. values (E) obtained, the parameters of the modified Nernst equation (1):

$$E = E_0 + K \log[H^+] + J_H[H^+] + J_{OH}[H^+]^{-1}K_W \qquad (1)$$

were calculated, where J_H and J_{OH} are fitting parameters in acidic and alkaline media for the correction of experimental errors, mainly due to the liquid junction and to the alkaline and acidic errors of the glass electrode; K_W is the autoprotolysis constant of water: $10^{-13.75}$.

The species formed in the systems studied can be characterized by the general equilibrium process (2), while the formation constants for these generalized species are given by eqn. (3). A detailed description of the equilibria can be found in a previous paper [17].

$$pM + qL \rightleftharpoons^{\beta_{M_pL_qH_{-r}}} M_pL_qH_{-r} + rH$$

or

$$pM + qL + rH_2O \rightleftharpoons^{\beta_{M_pL_qOH_r}} M_pL_q(OH)_r + rH \qquad (2)$$

$$\beta_{M_pL_qH_{-r}} = \beta_{M_pL_q(OH)_r} = \frac{[M_pL_qH_{-r}][H]^r}{[M]^p[L]^q}$$

$$= \frac{[M_pL_q(OH)_r]K_w^r}{[M]^p[L]^q[OH]^r} \qquad (3)$$

(Charges are omitted for simplicity.)

The equilibrium constants were determined from five independent titrations in each system with the computer program PSEQUAD [18]. The metal to ligand ratios were varied from 1:1 to 1:5 for the copper(II), and from 1:2 to 1:5 for the nickel(II)-containing systems.

The metal ion concentration ranged from 2×10^{-3} to 1×10^{-2} mol dm^{-3}.

EPR measurements

The EPR spectra were recorded on a JEOL-JES-FE 3X spectrometer in the X-band at 298 K with 100 kHz field modulation. Manganese(II)-doped MgO powder served as field standard. The EPR parameters were calculated with the program package described by Szabó-Plánka *et al.* [19]. The copper(II) ion concentration was 5×10^{-3} mol dm^{-3}, while the metal to ligand ratio varied from 1:1 to 1:5.

CD measurements

CD spectra were recorded on a Jobin-Yvon Mark VI spectropolarimeter (Regional Instrumental Center, Szeged) in the wavelength interval from 200 to 800 nm. The metal ion concentration was 1×10^{-2} mol dm^{-3} in a cell with 1 cm optical pathlength in the visible region, and 5×10^{-3} mol dm^{-3} in a cell with 0.2 cm optical pathlength in the UV region. The CD data are given as the differences in molar absorptivity between left and right circularly polarized light, based on the metal ion concentration, in dm^3 mol^{-1} cm^{-1} units.

Results and discussion

pH-metric measurements

The protonation constants determined from the potentiometric titrations in the pH range 1.5–10 are listed in Table 1. In this pH range two deprotonation processes of the ligands were observed. The first is the deprotonation of the carboxyl group at about pH ~2 and the second is the deprotonation of the protonated amino group at about pH ~8, except for FRU–β-ALA, which deprotonates at slightly higher pH. Both deprotonation processes occur in a more acidic pH region than those of the appropriate amino acids. The order of the deprotonations as a function of the amino acids is the same in the two series. The protonation constants of the amino groups in fructose–amino acids were found to be about 1.5–1.8 log unit lower than those of the corresponding amino acids in spite of the higher basicity of the secondary amino groups than that of the primary ones. The negative inductive effect of the sugar moiety may cause this change in the protonation constants, similarly to that shown by Gajda *et al.* [21] for 2-(polyhydroxyalkyl)-thiazolidine-4-carboxylic acids. However, they did not observe the same effect on the deprotonation of the carboxylate group, in contrast with our present observation, because of the presence of strongly electron-attracting sulfur in the thiazolidine ring. The −I effect of the sugar moiety in fructose–amino acids directly affects the basicity of the nitrogen donor atom and only through this that of the carboxylate group.

TABLE 1 Protonation constants of the fructose-amino acid ligands and the corresponding amino acids [20]

Ligand	log β_1	log β_2	log K_2
FRU–β-ALA	8.74±0.03	12.06±0.03	3.32±0.03
β-ALA	10.10	13.63	3.53
FRU–GLY	8.10±0.02	10.16±0.03	2.06±0.02
GLY	9.57	11.93	2.36
FRU–VAL	7.71±0.02	9.60±0.04	1.90±0.03
VAL	9.42	11.68	2.26
FRU–LEU	7.83±0.01	9.85±0.04	2.02±0.03
LEU	9.57	11.92	2.35
FRU–ILE	7.89±0.02	9.82±0.03	1.96±0.03
ILE	9.62	11.87	2.25
FRU–PHE	7.28±0.02	9.08±0.04	1.80±0.04
PHE	9.11	11.29	2.18

The copper(II) ion forms stable complexes with fructose–amino acids, but above pH ~7 the ligand reduces the metal ion and the system becomes undefined. Therefore, our investigations were restricted to the pH region 1.5–6.5. The stability of each system under these conditions was checked by back-titration. The formation constants of the copper(II) complexes of the fructose–amino acids are given in Table 2.

In the acidic interval (pH < 3), only the deprotonated carboxylate group was shown to coordinate to the metal. The stabilities of the protonated complexes MLH were almost equal within experimental error for all systems. All $\log K_{MLH}$ values were slightly higher than those of analogous complexes involving only carboxylate coordination. This indicates that, similarly to some sugar complexes, even at low pH protonated donor grops may participate in the coordination. The $\overline{H_{-1}}(mol_{OH}/mol_{Cu})$ versus pH curves, calculated from the differences between the titration curves of the ligands in the presence and absence of copper(II), started to rise at about pH = 3 (pH ~4 for FRU–β-ALA) and reached a value of ~2 at pH ~5.5 in two slightly separated steps. The most obvious assumption was that the fructose–amino acids react with copper(II) in the same way as amino acids, chelating in the complexes ML and ML_2 through their carboxylate oxygen and amino nitrogen donor atoms, since the amino acid moiety of the ligands does not contain other donor groups. The titration curves display very similar patterns in almost all cases (with only one inflection point, when the hydroxide ion consumption is two equivalents per copper(II) ion), however, independently of the ligand to metal ratio. Since quantitative formation of the species ML_2 is impossible in systems with equimolar ligand

TABLE 2 Overall formation constants of species formed in copper(II)–fructose–amino acid systems (log K values are given in parentheses)

Species	log β			
	ML	ML_2	MLH	MLH_{-1}
FRU–β-ALA	6.31 ± 0.05		10.50 ± 0.04 (1.76)	2.06 ± 0 04 (9.50)
β-ALA	7.04	12 54		
FRU–GLY	7.38 ± 0.02	13.26 ± 0.05 (5.87)	9.89 ± 0.15 (1 79)	1.90 ± 0 05 (8 27)
GLY	8 15	15 03		
FRU–VAL	6.83 ± 0.06	11 54 ± 0.09 (4.71)	9 65 ± 0.10 (1 94)	1.76 ± 0.06 (8.67)
VAL	8.11	14.90		
FRU–LEU	7 34 ± 0 03	12.48 ± 0.06 (5.13)	10 14 ± 0.12 (2.34)	2 11 ± 0.04 (8.51)
LEU	8.2	15 0		
FRU–ILE	7 22 ± 0 06	12 35 ± 0.09 (5.12)	10.12 ± 0 11 (2.23)	2 23 ± 0 03 (8 76)
ILE	8.40	15.40		
FRU–PHE	7.01 ± 0.05	11.27 ± 0 11 (4.26)	9.46 ± 0.15 (2 18)	2.22 ± 0.05 (8 96)
PHE	7.86	14.77		

and metal ion concentrations, a further deprotonation process must be assumed in the system, which may be due either to the deprotonation of an alcoholic hydroxy group of the sugar moiety or to the formation of mixed hydroxo complexes (through deprotonation of the co-ordinated water molecule). Potentiometric equilibrium studies alone do not permit a distinction between these two possibilities. Both complexes will therefore be formulated as MLH_{-1}. The above considerations suggest that the main species in the copper(II)–fructose–amino acid systems in the pH range from 3 to 6 are ML, ML_2 and MLH_{-1}.

The stabilities of the parent complexes ML reflect well the sequence of the protonation constants of the secondary amino groups, with the exception of the FRU–β-ALA complex, which is of lower stability in spite of the higher basicity of its amino-N than those for the other ligands. This behaviour may be due to the difference between the stabilities of six- and five-membered chelate rings. Because of the difference in the protonation constants of these ligands and the corresponding amino acids, only stability constants calculated on the basis of equilibrium (4) can be compared to show the effect of the sugar moiety on the stability

$$M^{2+} + HL \rightleftharpoons ML^{+} + H^{+} \quad (4)$$

constants in the systems. The latter constants, presented in Table 3, reveal the extra stability due to the sugar residue in the complexes, as evidence of the weak coordination of their alcoholic OH groups. The smaller

TABLE 3 Basicity-adjusted stability constants for species ML in copper(II)– and nickel(II)–fructose–amino acid systems, compared with the corresponding constants for the amino acids

Ligand	$\log K_{CuL} - \log K_{HL}$	$\log K_{NiL} - \log K_{HL}$
FRU–β-ALA	−2 43	−4 61
β-ALA	−3.06	−5.52
FRU–GLY	−0 72	−3 13
GLY	−1.42	−3 79
FRU–VAL	−0.88	−3 80
VAL	−1.31	−4 00
FRU–LEU	−0 49	−3 39
LEU	−1 46	−4.12
FRU–ILE	−0 67	−3.85
ILE	−1 22	−4.22
FRU–PHE	−0 27	−3 20
PHE	−1 25	−3.96

effect for the complexes containing side-chain-carrying amino acids is probably due to the steric hindrance of the latter moiety.

As the next step in the equilibrium system, ML is transformed to ML_2 or MLH_{-1}. In a solution with 1:1 ligand to copper(II) ratio (shown in Fig. 2) the species MLH_{-1} predominates for almost all ligands. On increase of the L:Cu ratio, formation of the species ML_2 becomes more favoured. In the 5:1 systems, FRU–GLY forms mainly ML_2, whereas the other amino acid-containing ligands yield ML_2 and MLH_{-1} in nearly equimolar

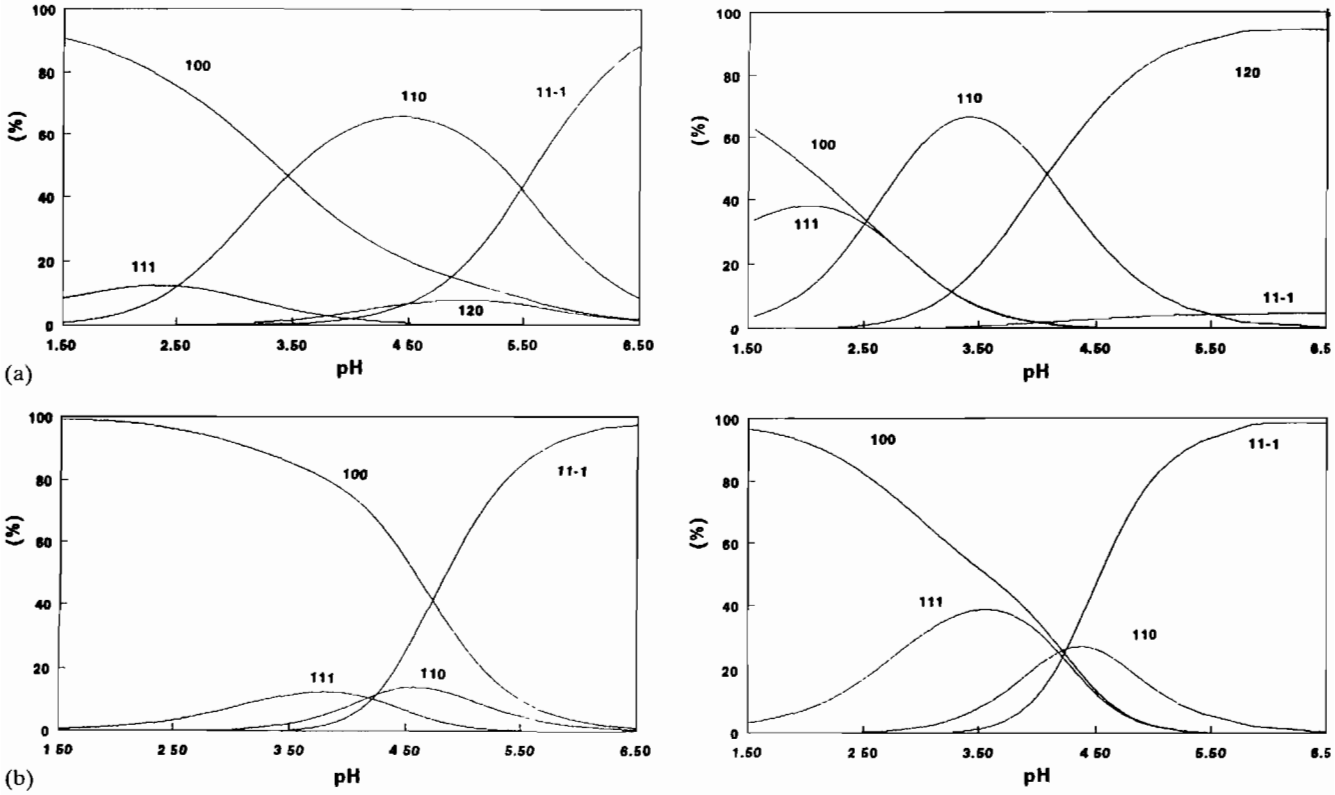

Fig. 2. Copper(II) distribution diagrams in fructose–amino acid–copper(II) systems, copper(II) concentration 0 008 mol dm^{-3}; (a) FRU–GLY and (b) FRU–β-ALA at metal to ligand ratios of 1.1 and 1·5.

amounts, while even under such conditions FRU–β-ALA prefers to form MLH_{-1}. The high stability of the complex MLH_{-1} of FRU–β-ALA is probably due to steric reasons, i.e. to the six-membered chelate ring permitting a more preferable position of the alcoholic OH groups in this compound than in the others.

Further increase of the pH led to further deprotonation processes, but in parallel with this oxidation of the ligand molecule started and prevented a correct evaluation of the titrations.

Analogous investigation of the nickel(II)–fructose-amino acid complexes allowed extension of the pH region which can be monitored. The formation constants obtained from the potentiometric titrations are given in Table 4. The stability constants are about three orders of magnitude lower than those for copper(II). The $\overline{H_{-1}}$ versus pH curve (Fig. 3) shows that the first deprotonation processes start at pH > 3–4. Formation of the parent complexes ML and ML_2 occurs between pH 4 and 7. The calculated basicity-adjusted stability constants (Table 3) for these species are slightly higher than those for the amino acids. FRU–GLY and FRU–β-ALA, where no steric hindrance is caused by amino acid side-chains, form complexes that are considerably more stable than those of glycine and β-alanine, respectively. The high stability of the complexes ML_2 of FRU–β-ALA can be explained on the basis of steric effects, similarly to the stabilization of $CuLH_{-1}$. The $\overline{H_{-1}}$ versus pH curve attains a maximum between pH 7 and 8. The maximum approaches a value of 2.0 with increasing ligand to metal ratio. Since the difference between the titration curves in the presence and absence of nickel(II) ions does not disappear even above this pH, i.e. after a minimum the $\overline{H_{-1}}$ versus pH curve again increases, we may assume that at higher pH further deprotonation processes take place. These processes, however, differ strongly from those for the copper(II) complexes, since the percentage of ML at higher pH (see Fig. 3) is too low for MLH_{-1} to be formed. It is more likely that complexes ML_2H_{-1} and ML_2H_{-2} are formed, with deprotonation of the co-ordinated water molecules or alcoholic OH groups of the sugar residue in the axial position of the octahedron. On further increase of the pH, the base-consuming processes continue outside the investigated pH range.

CD spectroscopy

In general, the chirality of the octahedral transition metal complexes can be attributed to the following sources of dissymmetry: (i) chiral distortion within the metal ion–donor atom cluster – 'inherent dissymmetry'; (ii) chiral distributions of chelate rings around the metal

TABLE 4. Overall formation constants determined by potentiometric titration in nickel(II)–fructose–amino acid systems (log K values are given in parentheses)

Species	log β			
	ML	ML_2	ML_2H_{-1}	ML_2H_{-2}
FRU–β-ALA	4 13 ± 0.03	7 92 ± 0 06 (3.79)	−0.33 ± 0 10 (5 50)	−10 02 ± 0.06 (4.06)
β-ALA	4.58	7 95		
FRU–GLY	4.97 ± 0.05	8.97 ± 0.07 (4.00)	1 37 ± 0 09 (6 15)	−8 25 ± 0 12 (4 13)
GLY	5.78	10.58		
FRU–VAL	3 91 ± 0 03	6.91 ± 0 03 (3 00)	−0.58 ± 0.08 (6 26)	−10 59 ± 0.12 (3 74)
VAL	5 42	9 72		
FRU–LEU	4 44 ± 0 03	7.71 ± 0.04 (3.27)	0 44 ± 0 07 (6 48)	−9.36 ± 0.12 (3.95)
LEU	5 45	9.71		
FRU–ILE	4.04 ± 0 02	7.28 ± 0.06 (3 24)	−0 25 ± 0.11 (6.22)	−10 30 ± 0.13 (3 70)
ILE	5 4	9.7		
FRU–PHE	4.08 ± 0.02	7.38 ± 0.05 (3.30)	−0 21 ± 0.09 (6 16)	−10 41 ± 0 12 (3.55)
PHE	5 15	9 59		

ion – 'configurational dissymmetry'; (iii) 'conformational dissymmetry' due to the chiral conformations within the individual chelate rings; (iv) asymmetric centres on the coordinated ligand molecules – 'vicinal dissymmetry' [22]. The first two sources are important when kinetically inert complexes are formed, and the configurational isomers are separable. Thus, in our systems they do not contribute to the optical activity of the complexes. The chelate rings formed in the amino acid complexes are usually nearly planar and the non-coordinated side-chains of the amino acids do not induce strong conformational chirality [23]. These assumptions were supported by theoretical calculations [24]. Hence, the main contribution to the optical activity must be due to the vicinal effects of the substituents, assuming amino acid coordination of the fructose–amino acids.

The substitution of one amino group hydrogen by a fructose moiety causes only a slight change in the optical activity of the complex, as was shown for N-methylamino acid complexes [25]. Coordination of the alcoholic hydroxy groups (deprotonated or not) may lead to a contribution by conformational dissymmetry to the optical activity.

CD spectra were recorded as a function of pH in the FRU–GLY, FRU–β-ALA and FRU–VAL systems, in the presence and absence of copper(II) and nickel(II) ions.

The potentiometric measurements reveal that FRU–GLY forms a complex ML with copper(II) ion in the pH interval 2–5 in equimolar systems. In contrast with the glycine and N-methylglycine systems, the copper(II) complex of FRU–GLY has a weak positive CD maximum ($\Delta\epsilon = +0.01$) at about 680 nm, clearly indicating that a non-deprotonated alcoholic OH group from the sugar residue must be coordinated, which is also demonstrated by the basicity-adjusted stability constants. As the pH is increased to about 6, a negative CD extremum ($\Delta\epsilon = -0.14$) appears at the same wavelength. Since the species MLH_{-1} would not exhibit a CD effect if it was a mixed hydroxo complex, one of the sugar hydroxy groups must be deprotonated and coordinated to the copper(II). The sign of the CD effect relates to the absolute configuration of the carbon atom bearing this hydroxy group, the most extensive contribution to optical activity being that of vicinal dissymmetry. Thus, the configuration of this carbon atom is S. If we assume that the sugar residue exists in the β-pyranose ring form even in the complex, similarly to the nickel(II) complexes of some glycosylamine [26] derived from ketoses, where crystal structure studies support such a pattern, the only carbon atom with S configuration is C(3). One cannot exclude the possibility that due to the complexation inversion of the sugar ring may occur resulting in the S configuration of C(2)

The CD spectra recorded in the UV region display a similar behaviour. Up to pH ~ 4.5, no CD effect is observed. At higher pH values (~ 6.0), three CD extrema appear in the spectra, with $\Delta\epsilon \sim +0.30$ at <200 nm, $\Delta\epsilon = -0.29$ at 246 nm and $\Delta\epsilon = +0.49$ at 287 nm in the charge-transfer transitions.

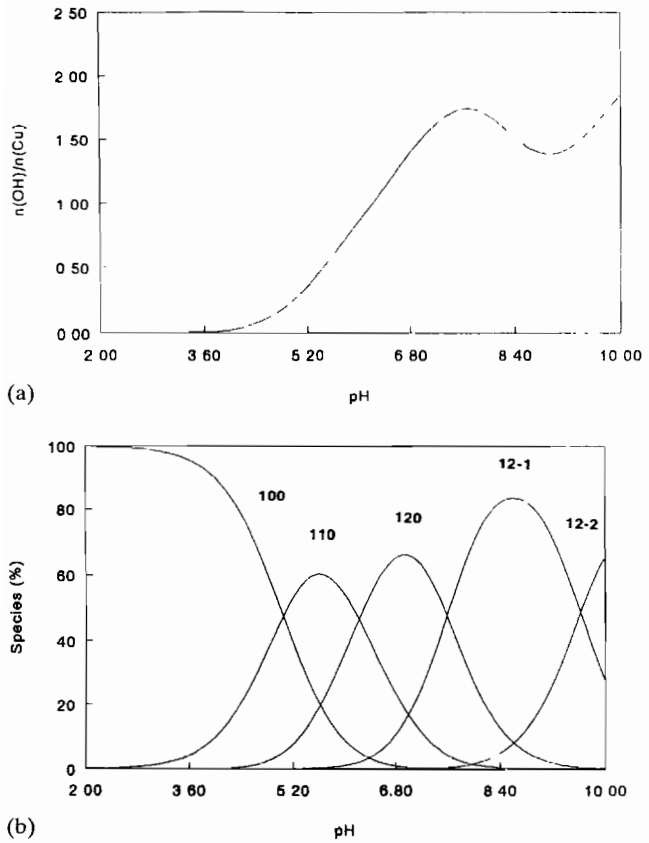

Fig 3 (a) $\overline{H_{-1}}$ vs. pH curve and (b) nickel(II) distribution diagram in nickel(II)–FRU–β-ALA 1 3 system; metal ion concentration 0.008 mol dm^{-3}

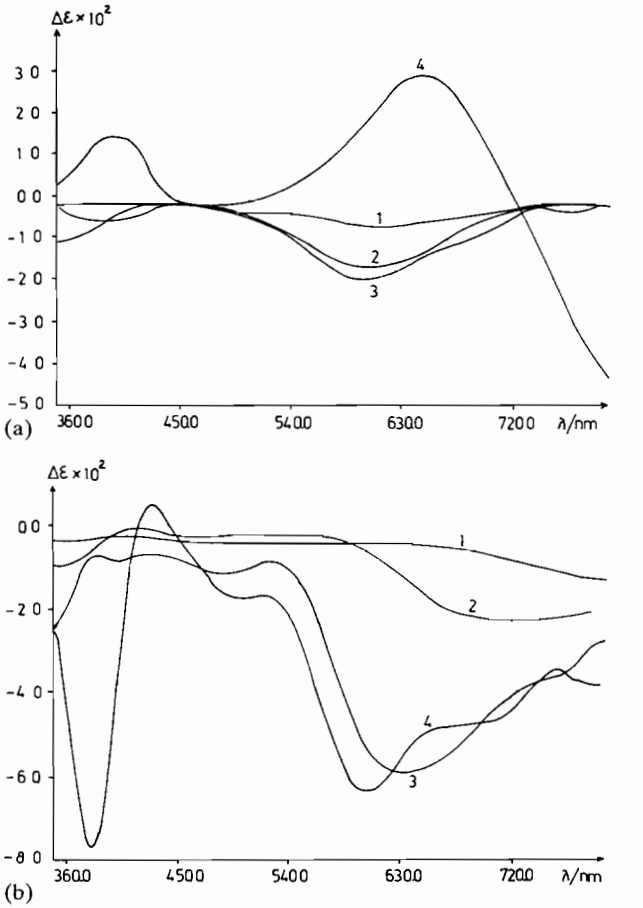

Fig. 4. CD curves for nickel(II)–fructose–amino acid 1:3 systems as a function of pH; nickel(II) concentration 0 01 mol dm^{-3}; (a) FRU–GLY at pH = 4 2 (1), 5.6 (2), 6.9 (3), 9.7 (4), (b) FRU–VAL at pH = 3 9 (1), 5 3 (2), 7.1 (3), 9.5 (4)

With increasing ligand to metal ratio, one would expect the CD effect to disappear since in ML_2 (formed under these conditions) two glycine residues coordinate to copper(II). However, this is not the case. One negative CD extremum ($\Delta\epsilon = -0.04$) remains at about 616 nm. The significantly weaker effect may suggest that in ML_2 non-deprotonated alcoholic OH groups are bound in axial positions to the metal centre.

The copper(II)–FRU–β-ALA system exhibits CD properties similar to those of the copper(II)–FRU–GLY system at a ligand to metal ratio of 1:1; there is a negative Cotton effect with $\Delta\epsilon = -0.12$ at 680 nm in the visible, and three maxima with $\Delta\epsilon \sim +0.77$ at <200 nm, $\Delta\epsilon = -0.522$ at 245 nm and $\Delta\epsilon = +0.703$ at 282 nm in the UV region at pH = 5.6. A ligand excess does not cause significant changes in these patterns.

The copper(II)–FRU–VAL system displays fairly complicated CD spectra, because the amino acid moiety of the ligand has an optically active centre, resulting in additional vicinal and conformational dissymmetry contributions compared with FRU–GLY. The values of the Cotton effects are significantly higher than in the above systems, indicating the coordination of amino group(s) on an optically active carbon atom. In the equimolar system at about pH ~4, we observed a small positive maximum at 638 nm ($\Delta\epsilon = +0.07$) and a more intense negative maximum at a wavelength longer than 800 nm ($\Delta\epsilon \sim -0.30$), due to the species ML. The formation of MLH_{-1} yields a negative extremum at about 700 nm ($\Delta\epsilon = -0.31$). In the UV region, the ligand itself has a strong positive Cotton effect at 212 nm. Because of complex formation, this peak is covered by strong charge-transfer transitions. In the spectrum of ML, two maxima characteristic of *S*-amino acid coordination [27] were observed ($\Delta\epsilon = +1.36$ at 200 nm and $\Delta\epsilon = -0.42$ at 242 nm), while in solutions containing MLH_{-1} as predominant species three maxima appeared: $\Delta\epsilon = +1.73$ at <200 nm, $\Delta\epsilon = -2.18$ at 229 nm and $\Delta\epsilon = +1.63$ at 286 nm. With increase of the ligand excess, the spectrum in this region does not change significantly, but in the d–d transition region two lines appear with a negative maximum at 698 nm ($\Delta\epsilon = -0.37$) and a positive one at 526 nm ($\Delta\epsilon = +0.05$) probably as a consequence of the formation of a mixture of ML_2 and MLH_{-1} in the system.

TABLE 5 EPR parameters determined for copper(II)–fructose–amino acid complexes through computer simulation (the parameters are given for a mixture of Cu^{63} and Cu^{65}. g_0 values are considered accurate to ±0.002 and A values to ±0.2 G, 1 $G = 10^{-4}$ T)

Species	A_0 (G)	A_0(N) (G)	g_0	σ_M			
				−3/2	−1/2	1/2	3/2
$M(H_2O)_6$	33.0		2.206	65	65	65	65
FRU–β-ALA							
ML	60.0	10.0 (1)[a]	2.170	24	37	50	79
MLH_{-1}	72.0	10.0	2.140	16	22	35	50
ML_2		(1)					
FRU–GLY							
ML	59.7	10.5 (1)	2.162	24	37	49	79
MLH_{-1}	63.8	8.5 (1)	2.135	23	29	46	60
ML_2	68.1	10.5 (2)	2.129	16	30	55	112
FRU–ILE							
ML	60.0	9.5 (1)	2.156	22	35	63	76
MLH_{-1}	68.0	9.5 (1)	2.139	16	27	40	65
ML_2	76.0	10.5 (2)	2.126	16	30	55	112

[a]The values in parentheses are the numbers of nitrogen donor atoms in the coordination sphere in the best models for calculations.

Nickel(II) complexes display optical behaviour characteristic of octahedral complexes. In the UV region at pH = 5–7, we observed a negative Cotton effect with $\Delta\epsilon = -0.2$ at about 225 nm, similar to that for amino acids [27]. With increase of the pH, the minimum becomes more intense ($\Delta\epsilon = -0.8$) and is shifted slightly to longer wavelengths.

At pH < 9, the spectra of the fructose–amino acid complexes of this metal ion in the visible region (Fig. 4) display small negative Cotton effects at about 600 nm, suggesting a weak coordination of the non-deprotonated alcoholic hydroxy groups in the complexes ML and ML_2. At pH = 9–10, we obtained more reliable CD spectra. There were two positive extrema at about 380 and 640 nm, and a strong negative one at >800 nm for the FRU–GLY and FRU–β-ALA complexes. The spectra of the FRU–VAL complexes are more complicated, but reveal the contribution of the sugar residue to the optical activity at pH = 9–10. These results suggest that deprotonated alcoholic hydroxy groups also coordinate to nickel(II) in the complexes ML_2H_{-1} and ML_2H_{-2}. The literature on the CD spectroscopy of nickel(II) complexes of ligands with well-defined configuration [26, 28] permit the assumption that the C(3)–OH groups are probably coordinated in these systems, as was the case for the copper(II) complexes.

EPR measurements

The EPR spectra of the copper(II)–FRU–GLY, FRU–β-ALA and FRU–ILE (as characteristic representative of the fructose–amino acids containing an amino acid side-chain) systems were recorded as a function of pH. The room-temperature spectra proved to be in good agreement with the potentiometric equilibrium results. No resolved nitrogen superhyperfine coupling was observed but for the computer simulation of the spectra the presence of nitrogen in the coordination sphere of the copper(II) had to be taken into consideration. The simulated g values, coupling constants and linewidths are presented in Table 5. The EPR lines of the hexaaqua copper(II) ion and the species MLH_{-1} were readily observed in the systems. The parameters for ML and ML_2 were obtained through decomposition of the overlapping spectra. The spectrum of MLH seemed very similar to that of the hexaaqua species, and could not be separated

The equimolar copper(II)–FRU–GLY system at pH < 2.5 gives a broad singlet signal due to the hexaaqua ion (see Fig. 5). On increase of the pH, the formation of new species starts, yielding an additional line shifted to higher magnetic fields. At pH ~ 4.0, this species predominates, but the hexaaqua ion still exists in solution. The difference spectrum at this pH can be characterized with $g_0 = 2.162$, $A_0 = 59.7$ G and $A_0(N) = 10.5$ G, assuming one nitrogen donor atom in

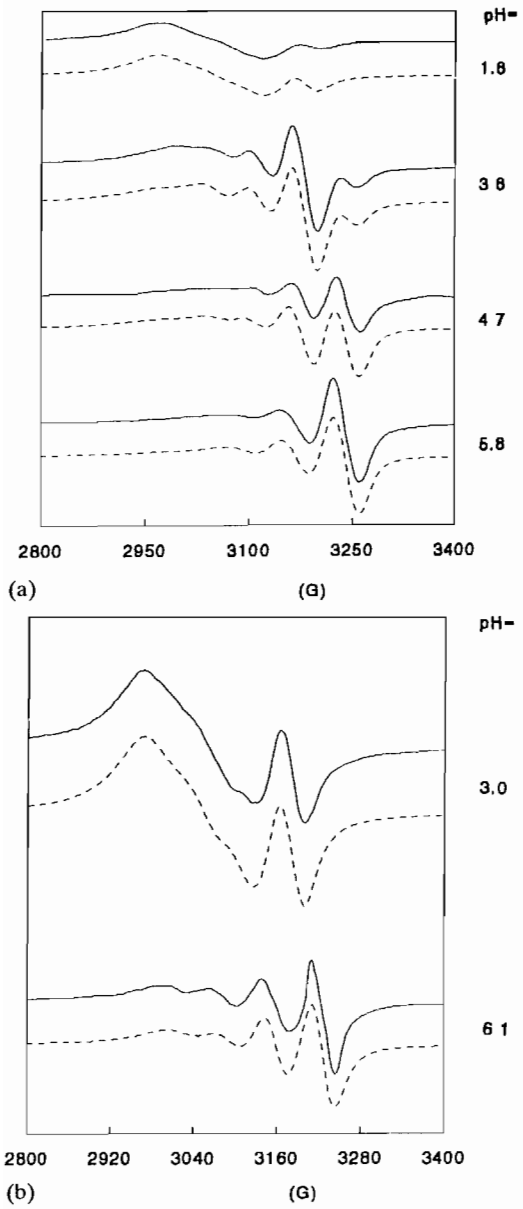

Fig. 5. Measured and simulated (dotted line) room-temperature EPR spectra for copper(II)–FRU–GLY 1·1 and 1:5 systems as a function of pH. Copper(II) concentration 0.008 mol dm^{-3}. The 1 1 simulated spectrum at pH = 3 0 was obtained through superposition of the spectra of $Cu(H_2O)_6$ (~50%) and CuL (~50%); at pH 6.1, only the species $CuLH_{-1}$ exists in solution. The 1·5 spectrum at pH = 1 8 consists of the spectra of $Cu(H_2O)_6$ (~80%) and CuL (~20%); at pH = 3.6 of the spectra of CuL (~80%) and CuL_2 (~20%); at pH = 4.7 of the 50–50% mixture of the spectra of CuL and CuL_2, and at pH = 5.8 of the spectrum of the species CuL_2 alone

the equatorial plane of the copper(II) coordination sphere as best model. These parameters are in good agreement with those of systems containing one nitrogen donor atom, such as complexes ML of amino acids. The distribution diagram (Fig. 2) indicates that at this pH the predominant species is ML.

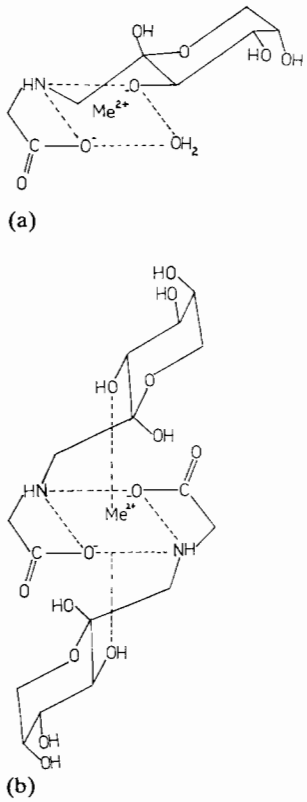

Fig. 6. Structures proposed for species MLH_{-1} (a) and ML_2 (b) formed in copper(II)–fructose–amino acid systems

On increase of the pH to ~5.0, a new species appears, which is the only copper(II) complex at pH ~ 6.0. The best model for simulating this spectrum also considers the coordination of one nitrogen, but the cooper hyperfine coupling constant is higher and the g_0 value is slightly lower than those for the species ML. Compared with the potentiometric results, this spectrum may be attributed to MLH_{-1}. The copper hyperfine coupling constants of mixed hydroxo species are usually smaller than those of the corresponding parent complexes [19]. Consequently, the EPR data suggest that the complex MLH_{-1} of FRU–GLY is not a hydroxo mixed complex. The ligand is coordinated to the copper(II) by a carboxylate, an amino and one deprotonated hydroxy group of the sugar moiety, corroborating the CD spectroscopic results. This hydroxy group is probably situated in an equatorial position since EPR would not be sensitive to axial coordination. The structure suggested on the basis of the EPR and CD measurements for the species $CuLH_{-1}$ is shown in Fig. 6(a). The structure is supported by the decreased linewidth in the spectra of the species MLH_{-1} compared with those of the ML complexes. According to the anisotropic magnetic relaxation theory in solution [29], the linewidth is porportional to the correlation time τ_R (the lifetime of a given orientation

of the molecule). Equation (5) shows that the correlation time is also proportional to the molecular radius of

$$\tau_R = 4\pi\eta r^3/3kT \quad (5)$$

the equivalent rotating sphere (where η is the coefficient of viscosity). The line broadening effect depends on the nuclear quantum number (M), and increases from $M = -3/2$ to $M = 3/2$ in copper(II) complexes. Therefore, we assume that the species MLH_{-1} must have a more compact structure than that of ML, indicating the coordination of a deprotonated alcoholic hydroxy group.

In solutions containing ligand and metal in a ratio of 5:1, formation of the species ML was already observed at pH ~ 2. At pH ~ 3.6, this is the predominant species, with the same EPR parameters as in the equimolar ligand–metal mixture, but a new species is also observed. On increase of the pH to 5.8, the latter complex becomes the predominant one. The calculated EPR parameters are $A_0 = 68.1$ G, $A_0(N) = 10.5$ G and $g_0 = 2.129$, assuming two nitrogen donor atoms around the copper(II) on the basis of trial and error experiments. The increased coupling constant also supports this model. An increasing number of nitrogen donor atoms in the copper(II) coordination sphere is expected to cause such an effect. Goodman and McPhail [30] found that the complexes ML_2 of amino acids may exist in *cis* and *trans* form, and also determined their EPR parameters as $A_0 \sim 60$ G, $A_0(N) \sim 9$ G for *cis* and $A_0 \sim 70$ G, $A_0(N) \sim 10$ G for *trans*. On comparison of our data with these values, we may state that the bis FRU–GLY complex of copper(II) is the *trans* isomer with $A_0 = 68.1$ G and $A_0(N) = 10.5$ G. The extremely large $\sigma_{3/2}$ linewidth data for the complexes ML_2 show that the molecular radius is increased as far as possible. This also suggests the *trans* form for the complexes ML_2, as shown in Fig. 6(b). Its exclusive formation may be attributed to the steric effect of the sugar residue. The spectra at intermediate pH values (3–5) can be well simulated as those of various mixtures of the species ML_2 and ML.

The behaviour of FRU–β-ALA is similar to that of FRU–GLY in the equimolar mixture. However, below pH ~ 3.5 no complex formation is observed. In the pH region 3.5–4.5, MLH_{-1} is formed, only a small part of the ligand remaining in the form ML, with a hyperfine coupling constant higher than that obtained for the species ML_2 of FRU–GLY. The same spectrum, i.e. the presence of the species MLH_{-1}, was observed even in 5:1 L:M mixtures, indicating the high stability of this complex.

In the pH region 4.5–6.5, fructose–amino acids with amino acids bearing a side-chain form complexes MLH_{-1} in solutions containing ligand and metal in equimolar ratio, while increase of this ratio results in the formation of various mixtures of the complexes ML_2 and MLH_{-1}.

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