Cu(II) and Ni(II) Stereocomplexes Involved in the Separation of α-Amino Acid Enantiomers by Ligand Exchange Chromatography: Stability Constants and Structural Investigations

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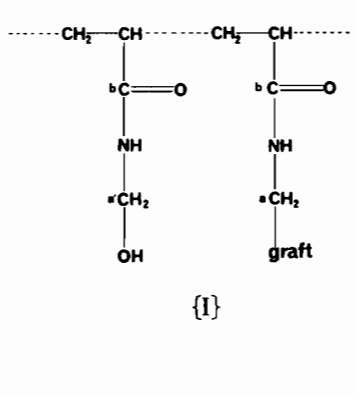
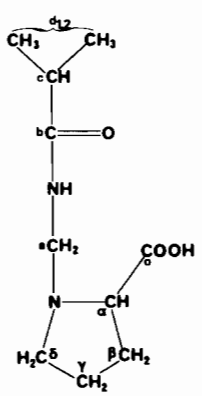
Received October 31, 1981

In order to explain the separation mechanism of α-amino acid enantiomers by ligand exchange chromatography on chiral, metal complexed stationary phases, soluble models of this packing have been prepared by reaction of isobutyramide and polyacrylamide with L-proline. Metallic ternary complexes of these ligands with L or D-valine or proline have been investigated through potentiometry and NMR spectroscopy. The results allows us to propose the coordination complex structures involved in the stereoselection.



{I}



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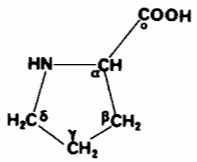

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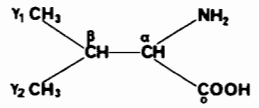

VALINE

Introduction

The efficiency of column liquid chromatography for the direct resolution of α-amino acid enantiomers is now well known [1, 2]. Ligand exchange chromatography, first proposed by Davankov *et al.* [3], appears as one of the most promising techniques in this field: the packing is prepared by grafting an inorganic substrate or an organic gel with a chiral ligand (generally an L α-amino acid) complexed by a transition metal ion.

In a previous study in our laboratory, we successfully performed such separations with macroporous hydrophilic gels, based on acrylamide and grafted with different L amino acids and complexed with various metal ions [4]. High efficiency can be observed; however, no general rule could be set up from these results in respect of the prediction of retention data, selectivity or resolution. A convenient choice of gel, ion or graft cannot be done without information on the nature and molecular structure of the ternary complexes: stationary phase/metal/(L or D) solute.

Thus, the aim of the present work is the study of the complexes involved in the chromatographic separations through potentiometry and NMR spectroscopy. The easiest way of using these techniques in solution led us to prepare polymeric {I} and monomeric {II} soluble models of the stationary phase in which L-proline was the grafted species.

Copper(II) or nickel(II) were chosen as transition metal ions and proline or valine as solutes. By potentiometry, stability constants of mixed-ligand complexes were determined and compared with each other. In addition, the effect of the paramagnetic Cu^{2+} ion on NMR spectra of the ligands gave structural or kinetic information.

Thus we propose geometrical structures for the stereocomplexes which are consistent with our chromatographic results.

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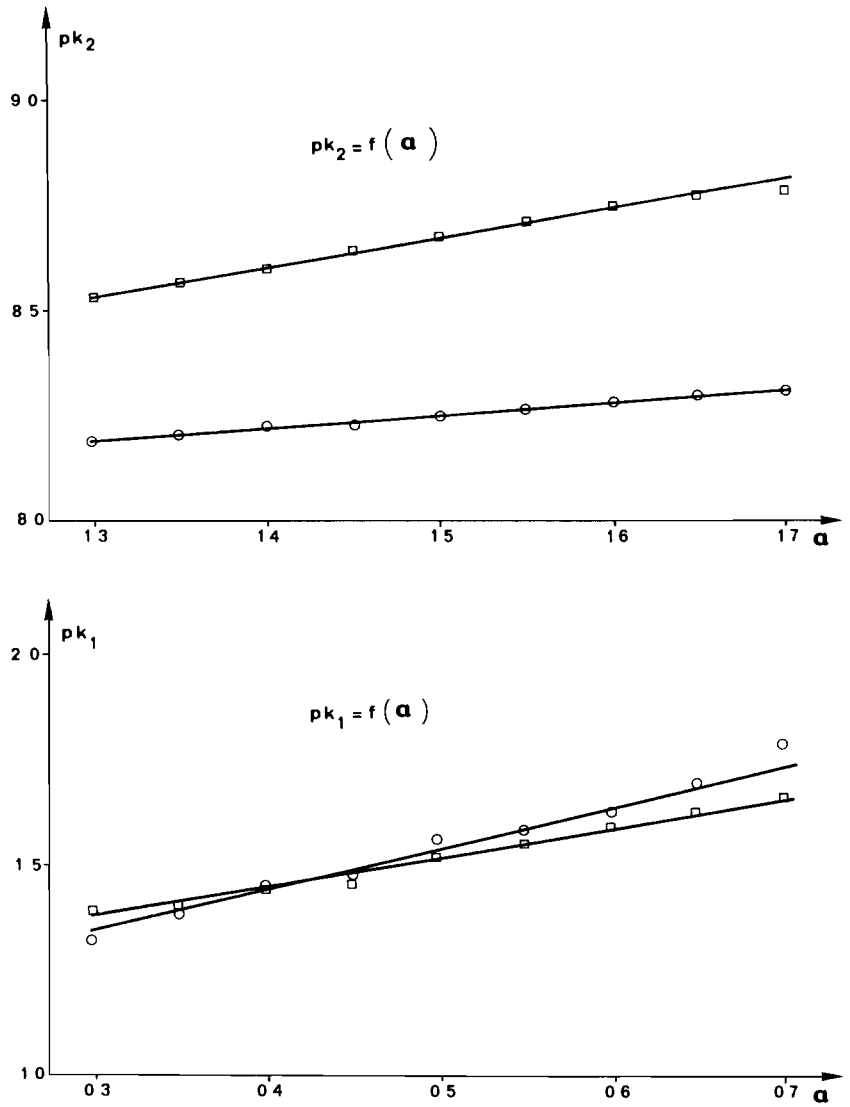

Fig. 1. Variation of the polymer dissociation constants pk_1 and pk_2 with the degree of neutralization α. ○ = {Ia} □ = {Ic}.

Experimental

Materials

Amino acids and salts were of analytical grade. The method for obtaining hydrosoluble polymethylolacrylamide grafted with L-proline {I} was described earlier [5]. Three different rates of grafting were prepared (expressed in capacity of proline): 0.7 meq/g {Ia}, 0.86 meq/g {Ib} and 3.83 meq/g {Ic}.

A simple model {II} of the complexing sequences of the latter copolymers was also obtained from the reaction of methylolisobutyramide [6] with L-proline: 0.05 mol of methylolisobutyramide in 15 ml of a saturated barium hydroxide solution and 0.05 mol of L-proline are shaked together 24 h at room temperature. Complementary addition of barium hydroxide is necessary to maintain the pH higher than 12. Barium carbonate is then precipitated and filtered. Evaporation of the aqueous solution gives a white powder from which unreacted methylolisobutyramide and proline are extracted twice with 80 ml chloroform once at 60 °C and then at 40 °C. The remaining solid phase is dissolved in water and freeze-dried. Purity controls were made by liquid liquid reverse phase chromatography (octadecyl bonded phase, water as eluent), infrared and ^1H NMR spectra. *Anal.* Calc. C% 56.0, H% 8.5, N% 13.1, O% 22.4. Exp C% 56.05, H% 8.7, N% 13.0, 0% 22.4.

The stability of the grafting group was tested by chromatography. At room temperature, after 24 h in aqueous solution, 6% free proline appeared for pH =

TABLE I. Proton dissociation constants of aminoacids and models: 25 °C, I = 0.1 M KNO_3 (data of Ref. [10] are given for I = 0.5 M).

Ligand	pk_1	pk_2
DL-Valine	2.10 (lit. 2.24 [9])	9.70 (lit. 9.65 [9], 9.71 [10])
L-Proline	1.80 (lit. 1.80 [9])	10.85 (lit. 10.63 [9], 10.69 [10])
II	1.45	8.45
Ia	1 75	8 25
Ib	1.90	8.25
Ic	1.70	8.68

TABLE II. Stability Constants of Cu(II) and Ni(II) Complexes with the Various Ligands 25 °C, I = 0.1 M (data of Ref. [10] are given with I = 0.5 M).

Ligand	Copper(II)		Nickel(II)	
	$\log \beta_1$	$\log \beta_2$	$\log \beta_1$	$\log \beta_2$
DL-Valine	8.20	15.10	5 37	9.68
	lit 8.19 [9]	lit. 15.19 [9]		lit. 9.71 [11]
	8.14 [10]	14.99 [10]	lit. 5.47 [11, 12]	9.72 [12]
L-Proline	9.00	16.69	5.83	11.00
	lit. 8.92 [9]	lit. 16.58 [9]	lit. 5.95 [13]	lit. 10.90 [13]
	8.99 [10]	16.29 [10]		
II	7 87	14.49	5.60	10.77
Ia	7.76	14.73	5.14	11.28
Ib	7.71	14 76	5.15	11.29
Ic	8.97	17.77	6.35	12.76

6 and 14% for pH = 2. In the following experiments we carefully avoided systematic errors due to this hydrolysis.

Potentiometric Titrations

The ionic strength (I) was kept constant with 0.1 M KNO_3. Titration curves were recorded with a TACUSSEL TAT 5 automatic apparatus standardized against 2 buffers (pH = 4.66 and pH = 8) and equipped with a glass electrode TACUSSEL TBHA.

Ligand to metal ion ratios (C_L/C_M) were 2/1 for parent complexes. Initial concentrations were then 4 × 10^{-3} M for polymeric ligands {I} and 10^{-2} M for (II) and other amino acids. In mixed-ligand (A and B) complexes, $C_A = C_B = C_M$, and initial concentrations were 2 × 10^{-2} M if A is (I), 5 × 10^{-2} M in the other cases. Polyelectrolytic effects were neglected since the dissociation constant of polymer models does not vary significantly with the degree of neutralization α (Fig. 1).

Our purpose was chiefly a comparative study of the complexing power of each optical isomer involved in a heterocomplex including the same ligand and the same ion. We could then reasonably assume that activity coefficients and polyelectrolytic effects have the same magnitude in the compared systems. Conversely, great care was required for the rigorous reproducibility of experimental conditions since the observed differences were not expected to be large. The following causes of systematic error were investigated and minimized: electrode ageing and calibration, temperature stability, digitization errors in pH measurements from titration curves.

Determination of stability constants were made by using the SCOGS computer program [7] which offers two advantages: taking into account the whole titration curve and calculating the standard deviation σ. Many determinations were made with each system and the reproducibility was better than 0.1 pk unit.

NMR

Half-width of HOD peak was measured at room temperature with a Varian T 60 NMR spectrometer.

^{13}C T_1 measurements were performed at 27 °C with the classical inversion–recovery pulse sequence (180°, t, 90°). Spectrometers were a Varian XL 100 or a Jeol PS 100 (25 MHZ) and a Varian CFT 20 for 20 MHZ determinations. Values of T_1 were calculated from exponential regressions. Samples consisted of 0.5 M or 1 M solutions of various ligands in D_2O in which small amounts of a concentrated copper nitrate solution were added with a microsyringe. pH

TABLE III. Values of log k_{MAB} (−pk) and their standard deviations (σ) for the various mixed ligand complexes of copper(II)

α-aminoacid other ligand	Valine (α' = 1.9)*			Proline (α' = 0.65)*		
	L − $pk_L(\sigma_L)$	D − $pk_D(\sigma_D)$	$\Delta pk = pk_D - pk_L$	L − $pk_L(\sigma_L)$	D − pk_D (σ_D)	$\Delta pk = pk_D - pk_L$
L Proline	15.72(0.19)	15.72(0.17)	–	–		
L Valine	–	–		15.72(0.19)	15.73(0.17)	
II	15.36(0 10)	14.95(0.13)	0 41	15 82(0.07)	16.05(0.05)	–0.23
Ia	14.87(0.05)	14.30(0.15)	0.57	–	–	
Ib	14.84(0.06)	14.66(0.08)	0.18	15..99(0.04)	16.08(0.04)	–0 09
Ic	16.50(0.05)	16.37(0.08)	0.13	17.41(0.05)	17.46(0.05)	–0.05

*α' values are relative to a cross-linked gel grafted with L proline (2meq/g) [4].

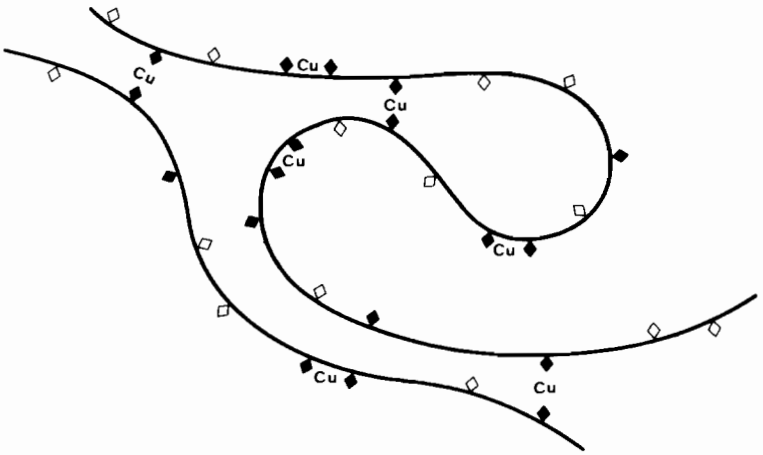

Fig. 2. The different kinds of copper complexes involved in the grafted polymer chains. ♦ L-Proline graft. ◇ Ungrafted site.

was adjusted to 7 as in chromatographic separations with a concentrated NaOD solution. In such samples, no trace of hydrolysis of {II} occurred up to 3 days on standing at room temperature. However, no T_1 investigation could be done at temperatures higher than 50 °C, the rate of the hydrolysis being too much important during the time required for a measurement. Because of the low sensitivity of the corresponding signals, values of T_1 for C = O groups were estimated*. The straight-lines $(T_{1P})^{-1} = f([Cu^{2+}]/[L])$ were drawn from twelve points, the correlation being better than 98% with a least squares fit.

Results

Potentiometry

Proton dissociation constants (k_1, k_2) of various ligands are given in Table I. From these data, it is obvious that the amine function acidity of the L-proline moiety is enhanced in the different models if compared to proline. This was already explained by the presence of the amide group in the β position [8].

ML_2 Complexes

When the ligand (L)/metal (M) ratio is 2/1, the equilibria corresponding to the overall formation of each complex may be written as follows:

$$M + L \leftrightarrows ML \text{ with } \beta_1 = \frac{[ML]}{[M][L]}$$

$$M + 2L \leftrightarrows ML_2 \text{ with } \beta_2 = \frac{[ML_2]}{[M][L]^2}$$

Table II gives $\log\beta_1$ and $\log\beta_2$ values for the various ligands with Cu(II) and Ni(II).

The distribution species curves of the various systems were determined [14]. In neutral medium used for both chromatography and NMR practically all the metal ions are involved in ML_2 complex type, except if a very large excess of metal ion is used. Polymer {Ic} gives stronger complexes than {Ia}, {Ib} or the low molecular weight model {II}. As {Ic} corresponds to a 75% substitution of the initial poly-

*In the other cases the precision was better than 10%.

TABLE IV. Values of log K_{MAB} and their standard deviations (σ) for the various mixed ligand complexes of Nickel(II).

α-aminoacid other ligand	Valine ($\alpha' = 0.61$)*			Proline ($\alpha' < 1$)*		
	L – pk_L (σ_L)	D – pk_D (σ_D)	$\Delta pk = pk_D - pk_L$	L – pk_L (σ_L)	D – pk_D (σ_D)	$\Delta pk = pk_D - pk_L$
II	10.15(0.08)	10.33(0.07)	–0.18	11.29(0.09)	11.40(0.11)	–0.11
Ib	10.08(0.18)	10.40(0.10)	–0.32	11.37(0.10)	11.47(0.09)	–0.10
Ic	11.27(0.06)	11.32(0.08)	–0.05	12.05(0.05)	12.13(0.05)	–0.08

*α' values are relative to a cross-linked gel grafted with L proline (2meq/g) [4].

TABLE V. Values (s^{-1}) of the slopes of the straight lines $(T_{1P})^{-1} = f([Cu^{2+}]/[L])$.

Ligand	Carbon atom								
	C_o	C_α	C_β	C_γ	C_δ	C_a	C_b	C_c	C_d
Valine		3950	520	190 243					
Proline		1950	1560	430	660				
{II}	≅25	20	14	15	21	22	≅25	15	18
{II}	≅40	37	25*	32	37	40	≅40	35	36
L-Proline	≅20	22		19	22				
{II}	≅40	37	23*	35	37	44	≅40	34	35
D-Proline	≅20	19		20	19				
{II}	≅100	72	57*	34	78	80	≅90	36	25
L-Valine	≅100	77		34					
{II}	≅100	80	56*	27	79	78	≅90	33	26
D-Valine	≅100	79		28					

*Two overlapping peaks

acrylamide (10% for {Ib}), we think that in this case very stable complexes involving vicinal proline moieties [5] might also occur (Fig. 2).

Mixed-Ligand Complexes

Tables III and IV give the stability constants of the various mixed-ligand complexes, together with the corresponding standard deviations σ (expressed in pk units). In these tables we have included the corresponding chromatographic selectivity factor, α' for cross-linked acrylamide gel grafted with L proline. α' is defined from retention data: $\alpha' = K'_L/K'_D$* [15]; the D isomer is first eluted with $\alpha' > 1$ and the most retained when $\alpha' < 1$.

Poor stereoselectivity is observed for {Ic} with any ion or solute. This loss of efficiency supports our assumption of very stable complexes between vicinal substituents in great amount in {Ic}; these hinder the formation of labile ternary complexes which are required to observe stereoselectivity. Furthermore, in the case of valine, the effect of the grafting rate on the chromatographic selectivity factor α' has been more completely studied [5] and it was found that α' first increases with that rate, reaches a maximum value for about 2 meq/g, and then decreases.

Other data corroborate previous chromatographic results [4] since the most stable complexes correspond to the most retained isomers.

Nuclear Magnetic Resonance

Paramagnetic Cu(II) induced relaxation rates T_{1P}^{-1} were measured for the various ^{13}C nuclei of our ligand system:

$$T_{1P}^{-1} = T_{1obs}^{-1} - T_{1o}^{-1}$$

where T_{1obs}^{-1} is the relaxation rate of a nucleus in presence of cupric ion and T_{1o}^{-1}, the relaxation rate of

*Where K′ is the classical chromatographic capacity factor.

the same nucleus in the free ligand. The slopes of the straight lines $T_{1P}^{-1} = f([Cu^{2+}]/[L])$ are reported in Table V (in the case of mixed-ligand complexes, $[L] = C_A + C_B$).

Such slopes are known to be proportional to $(T_{1M} + \tau_M)^{-1}$ [16] where T_{1M} is the relaxation time of the ligand in the coordination sphere and τ_M the lifetime of the bound species. As cupric ions generally exhibit a relative long relaxation time for the unpaired electron [17], T_{1M} must be proportional to r^{-6} [18], where r is the distance between the electron and the observed nucleus. Strictly speaking, this last relationship is only valid for ions with an isotropic g tensor but it may be used for axial g tensors when $0.4 < g_{\parallel}/g_{\perp} < 4$ [19], which is consistent with values encountered in Cu(II)–amino acids complexes [20].

Because of their larger chemical shift range and the collapsing of multiplets with proton decoupling, ^{13}C nuclei were chosen rather than ^{1}H in our study. Furthermore, they are closer to the paramagnetic center and not much affected by intermolecular interactions.

As we worked at a constant pH of 7, we could reasonably assume that the predominant species involved were of the ML_2 type even in the NMR concentration ratios [21].

ML_2 Complexes

Proline and valine samples show a great selectivity of the induced rates T_{1p}^{-1} between their various carbons. As a result, rapid exchange ($T_{1M} \gg \tau_M$) must occur in this case. Moreover, the relative distances calculated from the data of Table V are in good agreement with those determined from molecular models [14] or by X-ray diffraction [22], which confirms the validity of our assumptions. Such a selectivity was not found for {II} and this is characteristic of slow exchange. It may also be further proof of the amide group coordination, since such an additional binding increases the stability of the ML_2 complex but decreases its exchange rate [23]. On the other hand, in presence of an equivalent amount of diamagnetic Zn(II) ion, the $^{13}C{=}O$ amide peak of (II) was shifted by 2.9 ppm. Such a shift is also characteristic of the amide function coordination [8]. Unfortunately, no structural information can be obtained in the slow exchange limit and the weak chemical stability of the models did not allow us to operate at temperatures high enough to get them.

Mixed Ligand Complexes

An analogous situation obviously occurs for the mixed ligand complexes involving (II). As a result, no structural information could be obtained from the systems with L and D proline. However, when the second ligand is L or D valine, those carbon atoms which are far from the paramagnetic center are a little

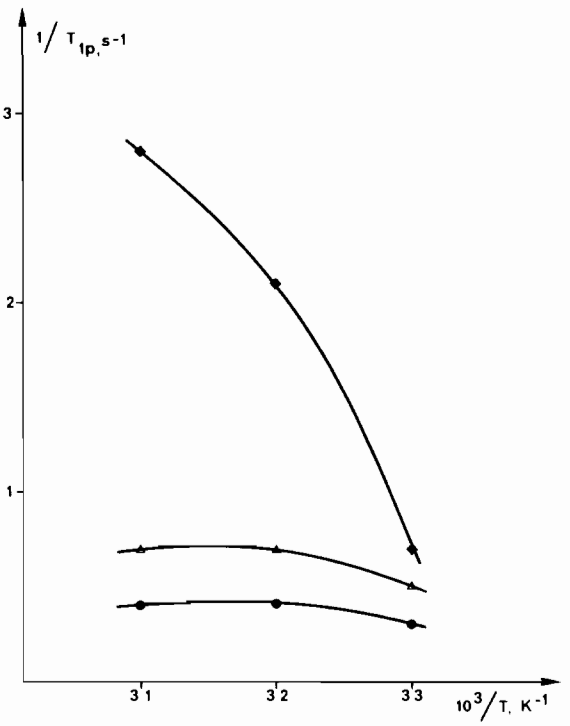

Fig 3. Temperature variation of the T_{1P}^{-1} relaxation rates for the different kinds of carbon atoms in the system II/Cu^{2+}/L-Valine. ♦ Nitrogen bonded carbon atoms of II and valine • Methyl groups of valine △ Others.

less affected by the addition of Cu^{2+} than the nearest ones. As T_{1p} values were not frequency dependent (measurements at 20 MHz), the temperature effect (see Fig. 3) shows that slow exchange indeed occurs for the nearest carbon atoms ($\tau_M \gg T_M$) but when r increases T_M (evolution in r^{-6}) becomes of the same order of magnitude (if not longer) than τ_M and the exchange is then intermediate. Therefore, it was still impossible to reach the complete fast exchange region.

In any case, from T_{1p} values the methyl groups of valine in the D isomer complex appear to be farther from the cupric ion than in the L one, this could be explained by a larger steric hindrance at this level. Besides, it must be noticed that the amide carbonyl group is as much disturbed by Cu^{2+} as the carboxyl carbon atoms. This result confirms the Cu^{2+} coordination by the amide group, because molecular models show that otherwise it would be rather remote from the copper ion.

Other attempts were also made to observe L and D valine in their mixed-ligand complexes with cupric ion and {Ia}. Such experiments were not very accurate due to the high viscosity of the medium. However, T_{1p} values were of the same order of magnitude as for {II}, and since they were again not

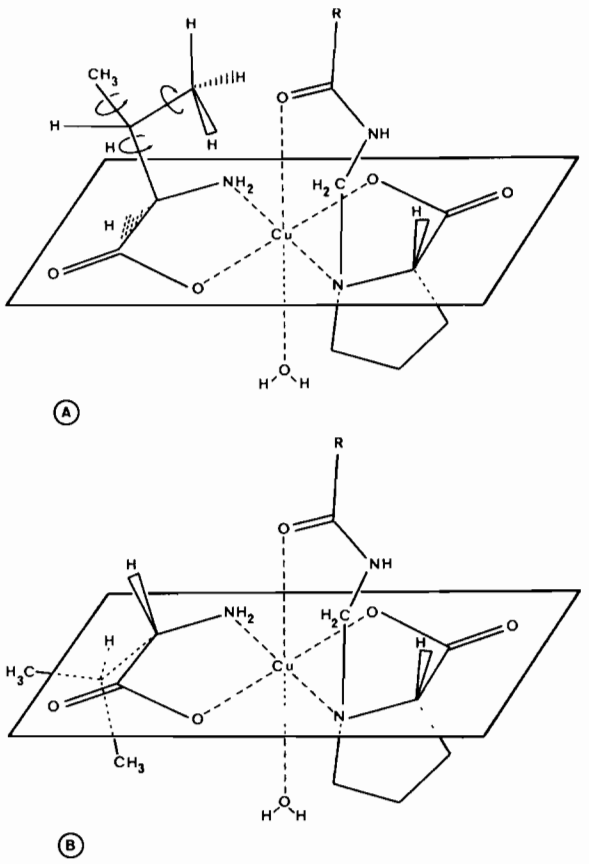

Fig. 4. Geometrical structures of the mixed ligand complexes. A: (II)/Cu^{2+}/D-Valine. B. (II)/Cu^{2+}/L-Valine.

frequency dependent intermediate exchange certainly occurred. Conversely, the mixed complex Pro/Cu^{2+}/Val exhibited fast exchange, which is consistent with the bidentate behaviour of these ligands.

Discussion

From this study it appears that metal complexes of our models can display a given stereoselectivity towards optically active α-amino acids such as valine or proline. Potentiometry and NMR spectroscopy confirm the coordination by the amide function for the various model complexes and, from the stability constants of polymeric ones, two kinds of structures can be inferred depending upon the rates of grafting. Moreover, there are reliable differences between stability constants of LD and LL mixed complexes, and they are larger for valine than for proline.

As a consequence of slow exchange, no information can be obtained from NMR results about the distances of the proline nuclei to the copper ion in the complexes. Intermediate exchange in the case of valine is more favourable and the asymmetric carbon methyl substituents of the D isomer appear to be farther from copper than the L ones. In these conditions, a steric hindrance between the isopropyl substituent of the D valine and the amide group of the stationary phase could explain why this enantiomer is eluted first; this is emphasized by the lower stability of the D-valine mixed complexes with models.

Other authors assumed a difference in the number of coordinated water molecules between LL and LD complexes to account for chromatographic results [24]. The contribution of the axial H_2O molecules to the width of the ^1H NMR water signal was evaluated for the various ligands with an excess of Cu(II): no difference could be found between LL and LD ternary complexes, either for valine, or for proline. All these results suggest geometrical structures (Fig. 4) which are consistent with our original assumptions [4].

For other α-amino acid enantiomers with low polarity substituents a similar structure of mixed complexes may be expected, for steric hindrance around the C_α–C_β axis would increase with the size of the substituent. However, for large alkyl groups, free rotation no longer takes place around the C_α–C_β axis and the most stable conformer is not necessarily the most hindered. Taking all these data into account it becomes then possible to predict the elution order of racemic mixtures [5].

For DL proline as a solute, there is no rotation around the C_α–C_β axis, for the substituent is a cyclic one. The above separation mechanism is irrelevant and we observed that the L isomer is eluted first.

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