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LIGAND-EXCHANGE CHROMATOGRAPHY OF RACEMATES

IV*. INFLUENCE OF STATIONARY-COMPLEX STRUCTURE ON THE MECHANISM OF LIGAND EXCHANGE

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SUMMARY

The structure of stationary complexes in chelating resins has a strong impact on the course of the sorption and chromatography of mobile complex-forming compounds. The formation constants of stationary complexes of Cu^{2+} and of sorption complexes of Cu^{2+} with L-proline as the mobile ligand have been calculated under static conditions for two sorbents with iminodiacetic acid groups (I) and L-proline groups (II) as the stationary ligands. For sorbent II, there is an appreciable dependence of the sorption affinity for both the Cu^{2+} and the mobile ligand on the copper content of the system, whereas for sorbent I the affinity for Cu^{2+} is relatively constant. This difference in the behaviour of the two sorbents is directly associated with their efficiency in the ligand-exchange chromatographic process.

INTRODUCTION

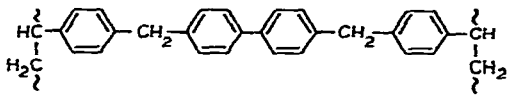
The ability of a reversible ligand-exchange reaction to transform the stationary complex of the sorbent into a sorption complex is the principle distinguishing feature of ligand-exchange chromatography. In order to understand the chromatographic separation of different complex-forming compounds, as well as the impact of such parameters as temperature, the nature of the mobile phase and of the eluting agent, knowledge of the main features of the mechanism of the ligand-exchange reaction is required.

The stoichiometry and mechanism of the formation of the sorption complex are determined, primarily, by the structure of the stationary and mobile ligands and by the nature of the complex-forming metal ion. The present paper deals with the peculiarities of sorption and chromatography of the L-isomer of the amino acid proline (Pro) on sorbents with groups of iminodiacetic acid ($\bar{R}$IDAA) and L-proline ($\bar{R}$Pro) in a cross-linked polystyrene matrix. In both cases, copper(II) ions were used as the complex-forming metal ions.

* For parts I, II and III see refs. 2, 3 and 4.

EXPERIMENTAL

The sorbents were synthesized from a macronet isoporous styrene polymer containing 5 mole percent of the cross-linking bridges of the structure



The chloromethylated copolymer was treated with the L-proline methyl ester or the dimethyl ester of iminodiacetic acid, followed by alkaline hydrolysis of the ester groups[1–4]: The asymmetric resin contained 1.68 mmole of $\bar{R}$Pro per gram; the capacity of the sorbent after treatment with the iminodiacetic acid ester was 2.94 mmole of $\bar{R}$IDAA per gram. The particle diameter ranged from 0.2 to 0.4 mm.

The formation constants of the stationary and sorption complexes were calculated from the data for the distribution of the copper and L-proline between the solution and the resin phase under static conditions. For this purpose, batches of resin containing 2.00 mmole of $\bar{R}$Pro or 1.54 mmole of $\bar{R}$IDAA were suspended in 30 ml of a solution containing 4.0 mmole of L-Pro, 0.20–2.5 mmole of $Cu(NO_3)_2$, and sufficient NaOH to maintain the pH within the range of 10.0–11.0 at equilibrium. After being shaken for 70 h in order to attain equilibrium, the resin was separated from the solution and then treated with 1.0 *N* HCl until complete extraction of the sorbed metal and Pro had been achieved. The copper content, both in the extracts and in the equilibrium solutions, was determined photometrically using sodium N,N-diethyldithiocarbamate, while the proline content was determined polarimetrically. A value of $[\alpha]_{436}^{20} = 123°$ (1.0 *N* HCl) was taken for optically pure Pro.

Chromatographic experiments with L-Pro were carried out in a column 20.0 cm long and 0.9 cm I.D.; the eluent flow-rate was up to 10 ml/h. The elution curves were obtained by use of a spectropolarimeter at 436 nm.

The time required for establishment of the ligand-exchange equilibrium was measured in a thermostatted flask with constant mixing. A batch of swollen resin in the Cu^{2+} form was flooded with an aqueous solution of L-Pro, and the optical density at 650 nm was measured on a photometer at definite intervals.

RESULTS AND DISCUSSION

Processes of formation of the stationary and sorption complexes in the chelate-forming sorbent phase

During investigations of ligand-exchange chromatography of racemates on asymmetric complex-forming ion exchangers in the presence of transition-metal ions[1–4], we found two types of sorbent behaviour in that stationary complexes were either formed by coordination of one stationary ligand to one transition-metal ion (1:1 complex) or by coordination of two stationary ligands to one metal ion (2:1 complex). A good model of the first type of behaviour is sorbent I which possesses iminodiacetic groups $\bar{R}$IDAA (Dowex A-1) and for which the formation of 1:1 stationary complexes according to eqn. 1 (the component charges are omitted) has been demonstrated by a number of independent methods[5–7]:

$$\bar{R}IDAA + Cu \overset{K_I}{\rightleftarrows} \bar{R}IDAA\text{–}Cu \tag{1}$$

An example of the second type of behaviour is the asymmetric sorbent II which possesses L-proline groups $\bar{R}Pro$. Investigation of the stoichiometry of the metal-sorption process and the electronic spectra of the final resin in the Cu^{2+} form made it possible to establish[3] stationary complex formation according to:

$$2\bar{R}Pro + Cu \overset{K_{II}}{\rightleftarrows} \bar{R}Pro\text{–}Cu\text{–}Pro\bar{R} \tag{2}$$

The difference in the composition of the stationary complexes formed by the sorbents I and II influences the course of the sorption of mobile ligands (L). Sorption of bidentate ligands of the amino-acid type on sorbent I generally takes place (3) without the breakdown of the stationary complex (except for the displacement of coordinated water molecules). In the case of sorbent II, the mobile ligand has to force out one of the firmly bound stationary ligands (4).

$$\bar{R}IDAA\text{–}Cu + L \overset{K_I'}{\rightleftarrows} \bar{R}IDAA\text{–}Cu\text{–}L \tag{3}$$

$$\bar{R}Pro\text{–}Cu\text{–}Pro\bar{R} + L \overset{K_{II}'}{\rightleftarrows} \bar{R}Pro\text{–}Cu\text{–}L + \bar{R}Pro \tag{4}$$

Determination of distribution of the copper ions and the mobile ligands between the solution and the sorbent phase allows calculation of the formation constants both for the stationary and the sorption complexes. We made such calculations for the sorption of L-proline (L = L-Pro) at pH 10–11, when both the mobile and stationary ligands are almost completely in an anion form available for complex formation. In Table I are presented the constants calculated in accordance with the equations:

$$K_{\bar{R}IDAA\text{–}Cu} = K_I = \frac{[\bar{R}IDAA\text{–}Cu]}{[\bar{R}IDAA]\,[Cu]} = \frac{[\bar{R}IDAA\text{–}Cu]\,[Pro]^2}{[\bar{R}IDAA]\,[Cu(Pro)_2]} \cdot K_{Cu(Pro)_2}$$

$$(mole/l)^{-1} \tag{5}$$

$$K_{\bar{R}Pro\text{–}Cu\text{–}Pro\bar{R}} = K_{II} = \frac{[\bar{R}Pro\text{–}Cu\text{–}Pro\bar{R}]}{[Cu]\,[\bar{R}Pro]^2} = \frac{[\bar{R}Pro\text{–}Cu\text{–}Pro\bar{R}]\,[Pro]^2}{[\bar{R}Pro]^2\,[Cu(Pro)_2]} \cdot K_{Cu(Pro)_2}$$

$$(mole/l)^{-2} \tag{6}$$

$$K_{\bar{R}IDAA\text{–}Cu\text{–}Pro} = K_I K_I' = \frac{[\bar{R}IDAA\text{–}Cu\text{–}Pro]}{[\bar{R}IDAA]\,[Pro]\,[Cu]} = \frac{[\bar{R}IDAA\text{–}Cu\text{–}Pro]\,[Pro]}{[\bar{R}IDAA]\,[Cu(Pro)_2]} \cdot$$

$$\cdot K_{Cu(Pro)_2}\,(mole/l)^{-2} \tag{7}$$

$$K_{\bar{R}Pro\text{–}Cu\text{–}Pro} = K_{II} K_{II}' = \frac{[\bar{R}Pro\text{–}Cu\text{–}Pro]}{[\bar{R}Pro]\,[Pro]\,[Cu]} = \frac{[\bar{R}Pro\text{–}Cu\text{–}Pro]\,[Pro]}{[\bar{R}Pro]\,[Cu(Pro)_2]} \cdot K_{Cu(Pro)_2}$$

$$(mole/l)^{-2} \tag{8}$$

$$K_{Cu(Pro)_2} = \frac{[Cu(Pro)_2]}{[Cu]\,[Pro]^2}\,(mole/l)^{-2};\ \log K_{Cu(Pro)_2} = 16.58 \text{ at } 20° \text{ (ref. 8)} \tag{9}$$

TABLE I

FORMATION CONSTANTS OF STATIONARY AND SORPTION COMPLEXES AT 22°

Sorbent R̄IDAA (I)			*Sorbent R̄Pro (II)*		
Degree of saturation with Cu (%)	*log* $K_{\bar{R}IDAA-Cu}$	*log* $K_{\bar{R}IDAA-Cu-Pro}$	*Degree of saturation with Cu (%)*	*log* $K_{\bar{R}Pro-Cu-Pro\bar{R}}$	*log* $K_{\bar{R}Pro-Cu-Pro}$
13.7	18.29	19.09	14.4	16.33	16.73
27.3	18.25	19.19	24.8	16.15	16.58
41.3	18.36	19.47	33.9	16.07	16.52
55.0	18.39	19.52	39.1	15.88	16.39
68.0	18.47	19.68	47.7	15.87	16.41
83.2	18.57	19.83	52.3	15.73	16.32
94.2	18.47	19.92	57.1	15.60	16.26
			61.1	15.45	16.18
			68.7	15.41	16.17
			79.3	15.39	16.23
			92.9	15.07	16.12

Unlike the remaining constants, it is necessary to take into account the ratio of the volumes of water contained in the resin phase and in the liquid phase when calculating the value of $K_{\bar{R}Pro-Cu-Pro\bar{R}}$.

For comparison we give the formation constants of the model complexes of copper with N-benzyl derivatives of iminodiacetic acid and L-proline which may be regarded as monomeric analogues of the stationary ligands R̄Pro and R̄IDAA:

$\log K_{BzlIDAA-Cu} = 10.29$ at 25° (ref. 9)

$\log K_{BzlPro-Cu-ProBzl} = 12.39$ at 25° (ref. 10)

$\log K_{BzlPro-Cu-Pro} = 14.93$ at 25° (ref. 10)

It appears that the values of the formation constants given in Table I are substantially too large. This is probably due to the fact that the true concentration of non-coordinated Cu^{2+} in the negatively charged resin is significantly higher than the concentration in the equilibrium solution, whereas the concentration of the proline anions is higher in the external solution than in the resin phase. For the same reason, the absolute value of the constants should be dependent on the properties of the resin and the experimental conditions. (Loewenschuss and Schmuckler[5] found $\log K_{\bar{R}IDAA-Cu} = 16.9$ at 23–27° when investigating Dowex A-1 resin.)

Nevertheless, the constants obtained allow one to understand the difference in the chromatographic behaviour of the two sorbents under investigation. The formation constant of the stationary complex R̄IDAA–Cu on resin I is independent of the concentration of metal ion present in the system. It is considerably lower than the formation constants of the sorption complex R̄IDAA–Cu–Pro. This means that sorbent I possesses a high and constant affinity for copper ions, while the latter readily accept the second ligand, *i.e.*, the proline anion.

In the case of sorbent II we observe a quite different situation. The average value of the formation constant of the stationary complex R̄Pro–Cu–ProR̄ decreases almost 50-fold with increasing content of copper in the resin. This means that the formation constants of the individual complexes arising at low and at high contents

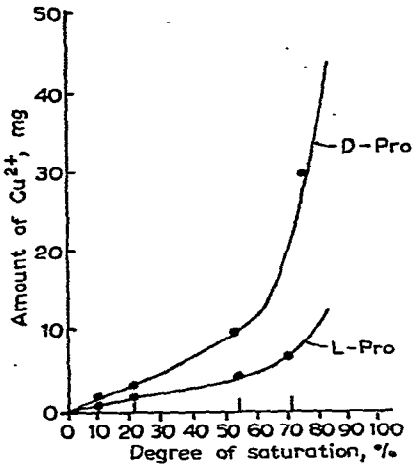


Fig. 1. Washing out the copper(II) ions with the separated proline antipodes from the chromatographic columns. Sample injected, 0.1 g of D,L-proline. Degree of saturation of the asymmetric resin with Cu^{2+}: 10.1, 21.3, 54.4 and 71.0%. Ammonium concentration in the eluent: 0, 0, 0.1 and 0.2 *M* for L-Pro; 0, 1.0, 1.0 and 1.5 *M* for D-Pro. Column, 20 × 0.9 cm. Room temperature. Flow-rate 6 ml/h.

of copper in the resin differ from each other by several orders of magnitude. [In fact, at >70% saturation of sorbent II with Cu^{2+} the amount of metal which is desorbed by Pro during chromatography drastically increases (Fig. 1). The unstable stationary complexes formed at high copper content are probably rapidly destroyed by attack of dissolved Pro.]

This, perhaps, may be explained by the fact that the polymeric sorbent matrix influences the complex-formation process. No doubt, the copper ions initially interact with pairs of stationary ligands at a distance which is comparable to that between the ligands in the final 2:1 stationary complex. After the saturation of these favourable sorption sites on the resin, the remaining pairs of stationary ligands are involved in complex formation. For this purpose it is necessary for the stationary ligands, together with the adjacent polymer-chain segments, to adjust to the required distance of approach. Hering *et al.*[11] were the first to note this complication of the formation of 2:1 complexes in polymers.

The affinity of the sorbent for the mobile ligand is characterized by the difference in the formation constants of the sorption and stationary complexes, as is seen from the sorption reaction 4. Since the formation constant of the stationary complex on sorbent II decreases rapidly with increasing content of copper ions present in the resin phase, the value of $\log K_{\bar{R}Pro-Cu-Pro} - \log K_{\bar{R}Pro-Cu-Pro\bar{R}}$ increases from 0.40 to 1.05. In this case the ability of the sorbent to absorb the mobile ligands is enhanced correspondingly. We took advantage of this property during the chromatographic resolution of racemic 2-aminopropanol-1, the sorption of which on the asymmetric sorbent is enhanced in the presence of a larger content of metal ions.

The peculiarities in the formation of sorption complexes in the resin phase limit the usefulness of formation constants of low-molecular-weight model complexes in predicting the behaviour of these or other compounds in ligand-exchange chromatography. In this respect, the situation is, perhaps, simpler in resins where the

complex-forming metal ion is bonded to the polymer phase only by means of electrostatic forces, as is the case with sulphonated resins. In the present system it may be still possible to use the literature values of the complex-formation constants in order to evaluate the chromatographic processes[12–14]. The situation becomes considerably more complicated for the carboxyl resins and sorbents of the Dowex A-1 type. If the complex-forming metal ion is coordinated to more than one stationary ligand, the peculiarities of the polymeric nature of the sorbent play a leading role, thus considerably influencing the constants of formation of the stationary and sorption complexes.

The polymeric character of the sorbent can also explain the difficulty that arises in the formation of sorption complexes with a charge of 2 (complexes having even higher charges are formed readily in solutions). For example, resin I, like Dowex A-1 (Cu^{2+} or Ni^{2+}), fails to sorb monoaminopolycarboxylic acids from alkaline solutions[15,16]. It may be that the negatively charged resin matrix exerts a rather strong electrostatic repulsion towards the amino-acid anions. The observed formation constants of the sorption complexes may, in this case, depend on the degree of neutralization of the charge on the matrix by the Cu^{2+} (see $K_{\overline{RIDAA}-Cu-Pro}$ in Table I) as well as on the pH.

Nevertheless, the selection of optimal conditions in ligand-exchange chromatography is possible on the basis of experimental data on the distribution of components under static conditions[17,18].

Efficiency of chromatography on sorbents possessing 1:1 or 2:1 stationary complexes

The larger the difference between the formation constants of sorption complexes of two mobile ligands, the higher the selectivity of the chelate-forming sorbent[4]. However, if the elution zone of the components is sufficiently narrow, *i.e.*, if the chromatographic column has a high plate number, it is no longer necessary for the sorbent to have a high selectivity in order to obtain full separation of the components. Arikawa and Toshida[19], for example, considerably reduced the time required for the amino-acid analysis by increasing the efficiency of the ligand-exchange chromatography by simply substituting Cu^{2+} in the sulphonated resin for Cd^{2+} (in this case the selectivity is slightly lowered).

Analysis of the literature data and the results of our experiments, lead us to conclude that sulphonated resins employed in ligand-exchange chromatography are distinguished by a sufficient rate of establishment of equilibrium between the phases and, hence, we observe here a higher efficiency; sorbents possessing stationary complexes of composition 1:1 are less efficient, and sorbents possessing 2:1 complexes are least efficient. In fact, the efficiency of chromatography of L-Pro on sorbent I which possesses iminodiacetic groups is higher than that on sorbent II. In order to compare directly the efficiencies of the two resins, we selected chromatographic conditions where the retention times of the proline on two columns were approximately equal (Fig. 2). For this purpose we had to use an eluent containing a high ammonia concentration for sorbent I, since on this resin L-Pro forms very stable sorption complexes. Measurement of the optical activity of the eluate (after its acidification) allows one to easily detect the amino-acid concentration in the presence of ammonia.

Hering and Heilmann[20] used an other sorbent in ligand-exchange chromatography with an α-amino-acid type stationary ligand. This resin contained sarcosine

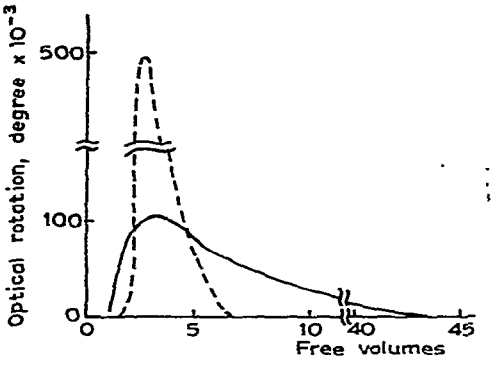


Fig. 2. Chromatography of L-Pro (50 mg) on the asymmetric sorbent II (———) (degree of saturation with Cu^{2+}, 50.0%; column, 25 × 0.9 cm; eluent, 0.045 *M* NH_4OH; flow-rate, 10 ml/h) and on sorbent I (– – –) (degree of saturation, 50.0%; column, 7.3 × 0.9 cm; eluent, 7.8 *M* NH_4OH; flow-rate, 10 ml/h).

groups on a cross-linked polystyrene matrix and in the Cu^{2+} or Ni^{2+} form showed an extremely low efficiency for the separation of α-amino acids.

We suggest that the low efficiency of ligand-exchange chromatography[4] on sorbents possessing 2:1 stationary complexes is due to two basic reasons. The first reason is the difference in the binding energy of the mobile ligand at different sorbent sites, *i.e.*, the energetic heterogeneity of the sorption complexes. The formation of sorption complexes takes place as a result of ligand exchange with stationary complexes. The energy of the latters varies, however, in a very broad range. The difference in the sorption energy of the mobile ligand at different resin sites corresponds to a marked non-linearity of the sorption isotherm. The second reason is of kinetic character. It is known that the rate of the ligand exchange in the labile complexes of copper with amino acids is very high. However, measurement of the rates of establishment of the inter-phase equilibrium under static conditions have shown that for sorbent I in the Cu^{2+} form the equilibrium with an aqueous proline solution is established in 10–15 min, whereas for sorbent II under identical conditions the process takes *ca.* 10 h.

It should be pointed out that both sorbents are synthesized from the same polystyrene matrix. Nevertheless, the swelling of sorbent I is on average five times higher than that of sorbent II. This large difference is associated not only with the difference in the exchange capacities of the sorbents and the amounts of the ionizable carboxyl groups, but to a great extent also with the fact that the stationary complexes $\bar{R}$Pro–Cu–Pro$\bar{R}$ on sorbent II form additional cross-linking bridges. These bridges are distinguished by the fact that they are disrupted on formation of the sorption complexes. The rupture of such cross-linking bridges changes the energy of the neighbouring stationary complexes, and, naturally, their ability to participate in the exchange reaction with the mobile ligands. Therefore, the diffusion of the mobile ligands inside the granules of sorbent II is accompanied by a whole series of ligand-exchange reactions in which the energy of the individual complexes as well as the conformation of whole segments of the polymer chains is changing continuously. It is quite evident

that such a transformation of the whole system requires some time to attain equilibrium, thus leading to substantial "tailing".

Nevertheless, it should be noted that, for the separation of a small number of components, the application of chelate-forming sorbents which form 2:1 stationary complexes is fully justified in spite of their low efficiency. In previous papers[1–4] we have reported the successful application of sorbent II to the resolution of racemic compounds.

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