ELSEVIER

Journal of Chromatography A, 786 (1997) 249–257

JOURNAL OF CHROMATOGRAPHY A

Role of alkyl and aryl substituents in chiral ligand exchange chromatography of amino acids
Study using porous graphitic carbon coated with N-substituted-L-proline selectors

Qian-Hong Wan[1], P. Nicholas Shaw, Martyn C. Davies, David A. Barrett*

Department of Pharmaceutical Sciences, University of Nottingham, Nottingham NG7 2RD, UK

Received 8 April 1997; received in revised form 21 May 1997; accepted 26 May 1997

Abstract

Five chiral stationary phases were prepared by coating the surface of porous graphitic carbon with a series of N-substituted L-proline chiral selectors. The N-substituents served as anchor molecules for immobilization of chiral selectors on the support material. The effect of the alkyl (C_7, C_9, C_{12}) and aryl (naphthylmethyl, anthrylmethyl) anchor molecules on retention and enantioselectivity were studied using thirty-six amino acid enantiomers as probe compounds. The surface concentrations of the chiral selectors, determined using the breakthrough method, were found to be in the range 0.39–0.68 μmol m^{-2}. The coated PGC phases all showed appreciable enantioselectivity for both non-polar and acidic amino acids with the naphthylmethyl-L-proline showing the greatest overall values. The N-substituents were shown to have strong influence on retention and enantioselectivity. An increase in the chain length of the alkyl N-substituents resulted in improved enantioselectivity whereas the retention decreased. However, with the aryl N-substituents, both the retention and enantioselectivity decreased with an increase in the ring number of the aryl substituent. The retention order for the majority of the amino acids examined was the same on all the columns, that is D>L. These behaviours were interpreted in terms of the involvement of the N-substituent in intramolecular hydrophobic interactions responsible for the observed chiral recognition.

Keywords: Chiral stationary phases, LC; Amino acids

1. Introduction

The separation of amino acid enantiomers by chiral ligand exchange chromatography may be accomplished by the formation of transient ternary copper complexes with an optically active amino acid derivative either covalently bonded or permanently adsorbed to a solid support [1]. Recent work has indicated that porous graphitic carbon (PGC) is a suitable support matrix for chiral ligand exchange chromatography when the chiral selector is attached to the surface via a hydrophobic anchor (or spacer) molecule [2,3].

Previous studies using silica supports have demonstrated the profound effect of the alkyl spacer length on enantioselectivity [4–7]. The involvement of

*Corresponding author.

[1]Present address Department of Chemistry, Dalhousie University, Halifax, Canada.

0021-9673/97/$17.00
PII S0021-9673(97)00601-8

hydrophobic interactions between the spacer molecule and the analyte in enantioselectivity was confirmed in an investigation of the structure–enantioselectivity relationships using a series of N-substituted L-phenylalanines as a chiral selectors on PGC support [3]. The role of the alkyl and aryl N-substituents in chiral recognition was clearly demonstrated, with the retention order of a pair of amino acid enantiomers being L>D on the alkyl-L-phenylalanine phases but the reverse retention order (D>L) being observed on the aryl substituted L-phenylalanine phases. An analysis of the structure of the transient ternary complexes was consequently undertaken which revealed that the competition of the N-substituent with the phenyl group in L-phenylalanine in the interaction with the side chain of the amino acid analyte may be the key factor determining the observed retention order [3].

To verify this hypothesis, we have carried out a further investigation using chiral selectors having the same series of substituents but with L-proline as the central chiral moiety instead of L-phenylalanine. Since the L-proline selector contains no aromatic element, the competitive interactions as postulated for the L-phenylalanine selector are no longer feasible. Thus it would be predicted that a uniform retention order of amino acid enantiomers should be observed regardless of whether the N-substituent anchor molecule is an alkyl or aryl group. In this investigation, five chiral stationary phases for the separation of amino acid enantiomers were prepared by coating the surface of porous graphitic carbon with N-substituted L-proline. The effects on retention and enantioselectivity of a series of different aryl and alkyl anchor molecules were studied using amino acid enantiomers as probe compounds.

2. Experimental

2.1. Apparatus

The chromatographic system consisted of a Gilson 305 pump (Villiers le Bel, France), a Gilson 805 manometric module, a Gilson 231 XL sampling injector, a Gilson 401 diluter and an ABI 759A absorbance detector (Foster City, CA, USA) connected to a Gilson HPLC 715 system controller via a Gilson 506B interface. Porous graphitic carbon or 'Hypercarb' columns (50×3.0 mm I.D. and 100×4.6 mm I.D., particle diameter 7 μm, specific surface area 119 m^2/g, batch number 123/R3) were supplied by Hypersil (Runcorn, UK). The same batch of the packing material was used throughout.

2.2. Chemicals

The enantiomers and racemates of, alanine, norvaline, norleucine, valine, leucine, isoleucine, methionine, proline, phenylalanine, serine, threonine, asparagine, glutamine, ornithine, lysine, arginine, aspartic acid and glutamic acid were purchased from Sigma (Poole, Dorset, UK). Sodium cyanoborohydride, copper (II) acetate (analytical grade), heptaldehyde, nonyl aldehyde, dodecyl aldehyde, 2-naphthaldehyde and 9-anthraldehyde were purchased from Aldrich Chemical Company (Gillingham, Dorset, UK). Methanol (HPLC grade) was obtained from Fisons (Loughborough, UK). All solvents used as reaction media were of HPLC or analytical grade.

2.3. Synthesis of chiral selectors

Five chiral selectors derived from L-proline were synthesized by a one-step reductive N-alkylation of the amino acid, as described by Ohfune et al. [8] (Fig. 1). The typical experimental procedure is briefly described here. To a solution of 10 mmol L-proline (1.65 g) and 7 mmol $NaBH_3CN$ (0.44 g) in methanol (15 ml) was added the appropriate aldehyde (11 mmol) over a 15-min period at room temperature. The reaction mixture was stirred for 18 h at room temperature. The solvent was evaporated under reduced pressure and the resulting residue was crystallised from 80% ethanol. The product was dried in vacuo at 60°C for 16 h.

Uncorrected melting points were determined using a Gallenkamp melting point apparatus and the data reported were the average of at least three measurements. Optical rotation measurements were made using a NPL automatic polarimeter 143C and the results are given in 10^{-1} deg cm^{-2} g^{-1}. Elemental analyses (CHN) were obtained using a Perkin Elmer PE 240B CHN Elemental Analyzer, at Nottingham University. ^1H NMR spectra were recorded where necessary on a Bruker AC 250 at 250 MHz. The

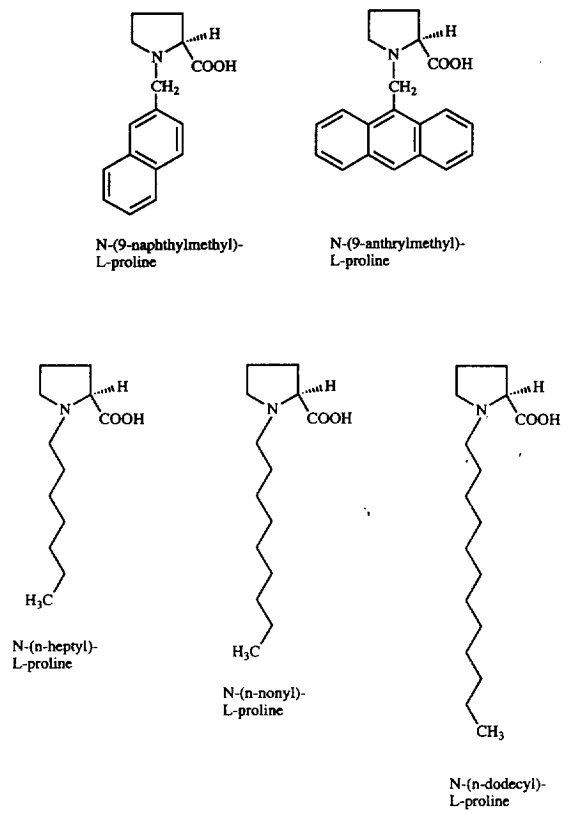

Fig. 1. Chemical structures of the N-substituted L-proline chiral selectors.

sample solution was prepared by dissolving appropriate amount of N-substituted amino acid sample in 0.5 ml of dimethyl sulfoxide-d_6. Chemical shifts are reported in parts per million (δ).

N-(*n*-Heptyl)-L-proline (C_7-L-Pro): m.p. 140°C; $[\alpha]_D$ +168.0° (*c*=0.5, 50% methanol–1 *M* NaOH). ^{1}H NMR (DMSO-d_6) δ 0.85 (m, 3H), 1.3 (m, 8H), 1.55 (m, 2H), 1.80–2.10 (m, 3H), 2.20–2.30 (m, 1H), 2.90 (m, 1H), 3.16 (m, 1H), 3.55 (m, 1H), 3.88 (m, 2H). $C_{12}H_{23}NO_2$ (213.32) requires: C, 67.75%; H, 10.89%; N, 6.57%; found: C, 67.35%; H, 10.82%; N, 6.5%.

N-(*n*-Nonyl)-L-proline (C_9-L-Pro): m.p. 150°C; $[\alpha]_D$ +69.0° (*c*=0.5, 50% methanol–1 *M* NaOH). ^{1}H NMR (DMSO-d_6) δ 0.86 (m, 3H), 1.25 (m, 12H), 1.55 (m, 2H), 1.85–2.10 (m, 3H), 2.20–2.30 (m, 1H), 2.90 (m, 1H), 3.16 (m, 1H), 3.60 (m, 1H), 3.90 (m, 2H). $C_{14}H_{27}NO_2$ (241.37) requires: C, 69.67%; H, 11.30%; N, 5.80%; found: C, 69.57%; H, 11.04%; N, 5.71%.

N-(*n*-Dodecyl)-L-proline (C_{12}-L-Pro): m.p. 148°C; $[\alpha]_D$ −40.2° (*c*=0.5, 50% methanol–1 *M* NaOH). ^{1}H NMR (DMSO-d_6) δ 0.86 (m, 3H), 1.25 (m, 18H), 1.55 (m, 2H), 1.85–2.10 (m, 3H), 2.20–2.30 (m, 1H), 2.90 (m, 1H), 3.16 (m, 1H), 3.60 (m, 1H), 3.90 (m, 2H). $C_{17}H_{33}NO_2$ (283.45) requires: C, 71.97%; H, 11.64%; N, 4.94%; found: C, 71.91%; H, 11.55%; N, 4.88%.

N-(2-Naphthylmethyl)-L-proline (NA-L-Pro): m.p. 228°C; $[\alpha]_D$ −80.0° (*c*=0.5, 50% methanol–1 *M* NaOH). ^{1}H NMR (DMSO-d_6) δ 1.78–1.97 (m, 3H), 2.27 (m, 1H), 3.01 (m, 1H), 3.30 (m, 1H), 3.90 (m, 1H), 4.33 (d, d, 2H, *J*=12.8, 57.4 Hz), 7.55 (m, 3H), 7.95 (m, 4H). $C_{16}H_{17}NO_2$ (255.31) requires: C, 75.27%; H, 6.71%; N, 5.48%; found: C, 74.99%; H, 6.49%; N, 5.33%.

N-(9-Anthrylmethyl)-L-proline (AN-L-Pro): m.p. 171°C; $[\alpha]_D$ −63.0° (*c*=0.5, 50% methanol–1 *M* NaOH). ^{1}H NMR (DMSO-d_6) δ 1.65–2.02 (m, 3H), 2.40 (m, 1H), 2.77 (m, 1H), 3.10 (m, 1H), 4.23 (m, 1H), 5.25 (d, d, 2H, *J*=13.3, 38.7 Hz), 7.60 (m, 4H), 8.17 (d, 2H, J+8.0 Hz), 8.73–8.76 (m, 3H). $C_{20}H_{19}NO_2$ (305.37) requires: C, 78.66%; H, 6.27%; N,4.59%; found: C, 78.20%; H, 6.42%; N, 4.43%.

2.4. Coating procedures and surface coverage calculations

The coating solution was prepared by dissolving an appropriate amount of chiral selector in aqueous alkaline solution (1.0 *M* NaOH) or in a methanol–1.0 *M* aqueous NaOH mixture. To achieve a complete dissolution of the chiral selector, the concentration of the chiral selector and the proportion of methanol were varied depending upon the anticipated properties of the chiral selector. Details of the solutions used to coat the PGC columns, and the surface coverage obtained are given in Table 1.

The surface concentration of the chiral selector coated onto the PGC column from an aqueous solution was determined by the breakthrough method [9]. The PGC column was washed with analytical grade methanol before the coating procedure to ensure that the support surface was clean and the

solution contained no UV absorbing impurities. The solution containing the N-substituted L-proline was passed through the PGC column at flow-rate of 1 ml/min and any change in the absorbance of the eluent was followed by UV detection. Equilibrium in the coating process was indicated by an abrupt rise in the UV base line. The surface concentration of the substituted L-proline on the packing material was calculated using a previously described method [10].

After the passage of about 10 ml of coating solution through the column following the observation of the breakthrough curve, distilled water was used to displace the methanolic solution within the column. This was followed by an aqueous solution of 0.001 *M* copper (II) acetate. The column was then ready for sample injection once an equilibrium between the stationary and mobile phase was attained, which was normally achieved within 30 min.

For the chiral selectors without chromaphore at 254 nm such as C_7, C_9 and C_{12}-L-proline, an indirect method was used to determine the surface concentration. A 0.005 *M* copper (II) acetate solution was pumped through the column coated with the alkyl-L-proline until a breakthrough occurred. This method is based on the assumption that the adsorbed alkyl-L-proline molecules form 1:1 complexes with copper (II) ions at equilibrium. Thus the surface concentration of the alkyl-L-proline is equal to that of the copper (II) ions, as determined by the breakthrough method.

2.5. Chromatographic measurements

An aqueous solution of 0.001 *M* copper (II) acetate (pH 5.6) was used as the eluent in all experiments. All separations were performed at ambient temperature with a flow-rate of 1 ml/min and used UV detection at 254 nm. The solute solutions were prepared by dissolving the amino acid enantiomers and racemates in distilled water to give a concentration of 10–100 μg/ml. Injections of 1–10 μl of these analytes produced satisfactory chromatographic peaks. The retention order of a pair of enantiomers was determined by injecting the enantiomer individually. The mobile phase hold-up time, t_M, was taken as the time from injection to the occurrence of the first solvent disturbance peak. The mean retention factors, k_D and k_L for D and L-isomers, and separation factors, α, defined as the ratio k_D/k_L, were calculated from multiple injections of the racemates.

3. Results and discussion

3.1. Surface concentration of the substituted L-proline selectors

The surface concentration of the chiral selector coated on porous graphitic carbon was determined by the breakthrough method and found to be in the range of 0.39–0.68 μmol m^{-2} for all the phases (Table 1). As expected, the surface concentrations of the aryl-L-prolines were higher than those of alkyl-L-prolines, confirming an earlier observation that aryl compounds serve well as anchor molecules on the surface of graphitic carbon [3]. Previous work with N-(2-naphthalene)sulphonic acid [3] and 17*H*-tetrabenzo[*a*,*c*,*g*,*i*]fluorene [11] as anchor molecules linked to chiral selectors indicated that the monolayer surface coverages of these compounds on porous graphite carbon were approximately 1.4 μmol m^{-2} and 0.4 μmol m^{-2} respectively. The latter

Table 1
Details of coating solutions and surface coverage

Chiral selector	Concentration used for coating[a]	Surface coverage (μmol m^{-2})
C_7-L-Pro	0.0133 *M*	0.39
C_9-L-Pro	0.010 *M*	0.48
C_{12}-L-Pro	0.010 *M*	0.48
NA-L-Pro	0.004 *M*[b]	0.59
AN-L-Pro	0.010 *M*[c]	0.68

[a] In aqueous 1.0 *M* NaOH.
[b] In water–methanol (1:1, v/v).
[c] In methanol.

Table 2
Retention and enantioselectivity of amino acids on N-substituted L-proline coated porous graphitic carbon

Analyte	R-CH(NH_2)COOH	C_7-L-Pro			C_9-L-Pro			C_{12}-L-Pro			NA-L-Pro			AN-L-Pro		
	R-...	k_L	k_D	α	k_L	k_D	α	k_L	k_D	α	k_L	k_D	α	k_L	k_D	α
Non-polar																
Ala	CH_3	–	–	–	1.05	1.15	1.09	0.21	0.21	1.00	0.73	2.18	2.99	1.36	1.46	1.08
N-Val	$CH_3(CH_2)_2$–	5.42	9.46	1.74	3.31	5.79	1.79	0.69	1.85	2.68	6.39	29.54	4.62	5.71	7.51	1.31
N-Leu	$CH_3(CH_2)_3$–	15.19	27.15	1.79	7.56	14.91	1.97	1.56	5.46	3.50	14.01	61.08	4.36	9.21	12.97	1.41
Val	$(CH_3)_2CH$–	4.54	7.45	1.68	2.62	4.30	1.64	0.35	1.50	4.28	2.24	9.71	4.33	1.96	1.46	0.74
Leu	$(CH_3)_2CH(CH_3)$–	10.79	24.07	2.23	6.01	10.66	1.77	1.29	3.47	2.69	6.14	32.57	5.30	7.39	11.86	1.61
Ileu	$CH_3CH_2CH(CH_3)$–	10.70	21.34	1.99	5.85	9.64	1.65	0.84	3.37	4.02	5.26	25.62	4.87	–	–	–
Met	$CH_3S(CH_2)_2$–	13.86	19.50	1.41	7.20	9.97	1.38	1.24	2.65	2.14	–	–	–	18.46	45.43	2.46
Pro	–$(CH_2)_3$–	2.61	4.36	1.67	1.68	3.91	2.27	0.22	1.61	7.17	1.13	14.07	12.45	1.61	2.72	1.69
Phe	$PhCH_2$–	76.89	93.86	1.22	33.41	45.79	1.37	4.35	11.69	2.69	–	–	–	–	–	–
Polar																
Ser	$HOCH_2$–	–	–	–	1.21	1.21	1.00	0.20	0.20	1.00	0.80	1.52	1.90	0.61	1.51	2.48
Thr	$CH_3CH(OH)$–	–1	–	–	1.24	1.24	1.00	0.27	0.27	1.00	1.61	2.09	1.30	0.46	1.46	3.18
Asn	H_2NCOCH_2–	2.51	2.51	1.00	1.17	1.17	1.00	0.16	0.16	1.00	0.61	1.91	3.14	1.41	12.95	9.18
Gln	$H_2NCO(CH_2)_2$–	–	–	–	2.06	2.52	1.22	0.50	1.06	2.12	1.35	1.94	1.43	–	–	–
Ionizable																
Orn	$H_2N(CH_2)_3$–	–	–	–	0.65	1.05	1.61	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00
Lys	$H_2N(CH_2)_4$–	–	–	–	0.71	0.71	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00
Arg	$H_2NC(NH)NH(CH_2)_3$–	–	–	6.19	7.52	1.21	0.14	0.14	1.00	0.00	0.00	1.00	0.47	0.47	1.00	
Asp	$HOOCCH_2$–	6.12	5.77	0.94	12.67	14.22	1.12	0.87	0.87	1.00	10.35	12.57	1.21	10.11	13.02	1.29
Glu	$HOOC(CH_2)_2$–	12.61	16.32	1.27	11.26	12.90	1.14	1.10	1.62	1.48	16.14	38.28	2.37	–	–	–

Conditions: column, PGC coated with N-substituted L-proline (a) 50×3.0 mm I.D; (b) 100×4.6 mm I.D.; eluent, 0.001 *M* $Cu(OAc)_2$ aqueous solution; flow-rate, 1 ml/min; detection, UV 254 nm.

compound has a significantly larger molecular size than naphthalene and hence would be expected to have a lower monolayer surface coverage. The results reported here indicate that the alkyl-L-Pro compounds, with surface coverages of less than 1.0 $\mu mol\ m^{-2}$, are unlikely to have reached monolayer coverage under the conditions used for coating. The values of surface coverage of the alkyl and aryl L-proline selectors are low compared to those for corresponding N-substituted L-phenylalanines [3], reflecting the hydrophilic nature of the L-proline compared to the relatively hydrophobic L-phenylalanine.

3.2. Effect of N-substituents on retention order

Table 2 shows the retention and separation factors of the amino acid enantiomers obtained on the columns coated with C_7-L-Pro, C_9-L-Pro, C_{12}-L-Pro, NA-L-Pro and AN-L-Pro. The amino acid analytes were classified according to the nature of their side chains as non-polar, polar and ionizable. All the chiral phases showed appreciable enantioselectivity for both non-polar and acidic amino acids examined here, with selectivity factors ranging between 1 and 12. However, basic amino acids were predominantly unretained. Typical examples of the chiral separation of D- and L-norvaline on each of the coated phases are shown in Fig. 2.

The involvement of hydrophobic interactions of the analyte side-chain with the non-polar surface of the chromatography support have been implicated in the mechanism of chiral separation on alkyl-bonded silica materials [12,13]. However, the specific roles of intra- and inter-molecular factors in chiral separations of amino acids on coated stationary phases have not been established. In a previous study using N-substituted L-phenylalanine phases [3] we provided evidence that the competitive hydrophobic interactions between the N-substituent and the side chains of the amino acid chiral selector may play a major role in influencing retention and enantioselectivity in chiral ligand exchange chromatography on porous graphitic carbon. With the N-substituted L-proline selectors used in the present study, the difference in side chains compared with the N-substituted L-phenylalanine selectors should lead to a difference in chromatographic behaviour. A detailed comparison of the retention behaviours of selected

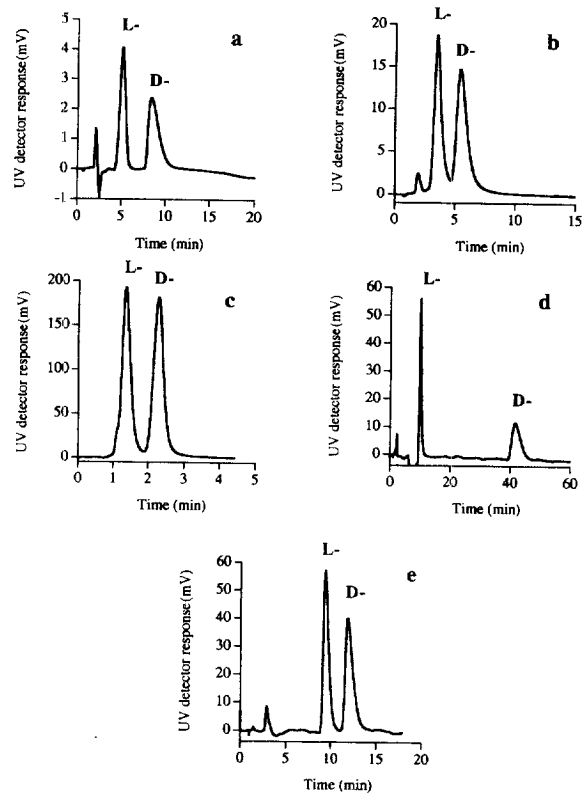

Fig. 2. Chromatograms of norvaline enantiomers separated on porous graphitic carbon coated with N-substituted-L-proline (a), C_9-L-Pro (b), C_7-L-Pro (c), C_{12}-L-Pro (d) NA-L-Pro, and (e) AN-L-Pro. Chromatographic conditions were as indicated in Section 2.

amino acids on these two types of columns revealed several significant differences in behaviour:

(1) The order of retention of the D- and L-amino acid enantiomers was essentially the same on both the alkyl and aryl N-substituted L-proline phases (D>L) compared to the reversal of order previously observed on the alkyl and aryl N-substituted L-phenylalanine coated PGC phases.

(2) The enantioselectivity was observed to increase as the length of the alkyl substituent increased from C_7 to C_9 to C_{12}, whereas the retention was observed to decrease. On the N-substituted L-phenylalanine phases, the retention was only slightly decreased but the enantioselectivity remained unchanged.

(3) Both the retention and enantioselectivity decreased as the aryl substituent changed from naph-

thyl to anthryl on the N-substituted L-proline phases. The same trends were not observed with the naphthyl and anthryl N-substituted L-phenylalanine phases.

The factors responsible for these differences in behaviour between the L-proline and L-phenylalanine coated phases are discussed below.

The retention order of the D- and L-enantiomers is essentially the same on all the N-substituted L-proline phases, i.e. the L-enantiomer is always eluted before the D-enantiomer. This is in contrast to what was observed on the N-substituted L-phenylalanine phases where two opposite retention orders occur corresponding to the alkyl and aryl substituents. This reversal in retention order of a pair of D- and L-enantiomers may be understood in terms of the structure of the transient diasteriomeric complex formed between the chiral selector and the analyte. The retention order of a pair of D- and L-enantiomers in chiral chromatography contains useful information on the structure of the transient diasteriomeric complex formed between the chiral selector and analyte. Unlike the N-substituted L-phenylalanine, the molecular structure of the copper complexes with the N-substituted L-proline has been well established. X-ray diffraction studies of bis-(N-benzylprolinato) copper (II) complexes by Aleksandrov et al. [14] show that the N-substituent and the propyl group (within the pyrrolidine) of proline are on opposite sides of the main coordination plane. Incorporating this structural evidence with our chromatographic observations, we depict, in Fig. 3, the most probable structures of the ternary copper (II) complexes formed between the alkyl and aryl N-substituted L-proline and amino acid enantiomer. In the complexes with D- or L-enantiomer, the side chain R of the amino acid analyte and the N-substituent of the chiral selector will be on the same side of the coordination plane, thus permitting a stronger interaction between them. The D-enantiomer shows stronger retention relative to the L-enantiomers probably because the *trans* (N,O) isomers are observed to be more stable than the *cis* (N,O) isomers [15]. Since the propyl group (within the pyrrolidine) of proline cannot compete with the N-substituent for hydrophobic interactions with the side chain of the analyte only one retention order is expected to occur. And this is indeed the case as the majority of amino acids examined in this work show the retention order D>L. Thus, the unchanged order of enantiomer retention

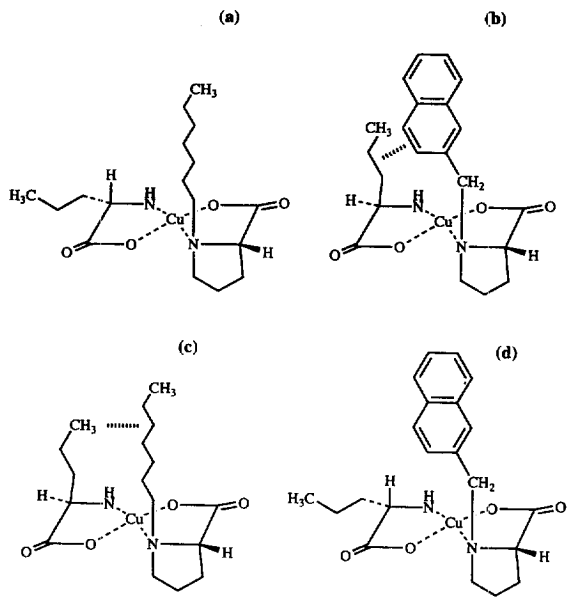

Fig. 3. Proposed copper complexes formed between: (a) L-norvaline (analyte) and N-alkyl L-proline (selector), (b) D-norvaline (analyte) and N-aryl L-proline (selector), (c) D-norvaline (analyte) and N-alkyl L-proline (selector), (d) L-norvaline (analyte) and N-aryl L-proline (selector). Dotted lines indicate potential hydrophobic interactions which stabilise the complexes and thereby influence enantioselectivity.

between the alkyl and aryl anchor molecules can be explained by the lack of hydrophobic interactions between the side-chains of the chiral selector and the analyte. These observations support our hypothesis that differences in the extent of hydrophobic interaction between the N-substituent of the chiral selector and the side chain of the amino acid analyte is a key factor in explaining the disparities in retention order of D- and L- enantiomers between the alkyl and aryl N-substituted L-Pro and L-Phe chiral selectors.

3.3. Effect of N-substituents on retention and enantioselectivity

All the alkyl N-substituted L-prolines show a substantial decrease in analyte retention as the alkyl chain length increased. This is particularly true for hydrophobic amino acids such as Val, Leu and Phe. The retention of the amino acid analyte in ligand exchange chromatography is primarily controlled by two factors: surface concentration of the chiral selector and the stability of the ternary copper

complex [3]. Since there is little difference in the measured surface concentrations of the C_7-L-Pro, C_9-L-Pro and C_{12}-L-Pro it is unlikely that the surface concentration of the chiral selector is responsible for the differences in retention behaviour. Thus the change in stability of the ternary copper complex is most likely to be the dominant factor.

It is well known that porous graphitic carbon shows greater retention relative to alkyl-bonded silica materials when comparable conditions are used [16–18]. This is because the surface of the graphitic carbon consists essentially of gigantic aromatic sheets whereas the surface of alkyl silica is composed of a highly open framework of solvated and mobile alkyl chains [16,17]. As the alkyl chain length increases from C_7 to C_{12}, the surface of the graphitic carbon can be assumed to be covered with an increasing proportion of the alkyl groups, making the surface properties similar to those of an alkyl-bonded silica. This change in surface property is likely to have a strong influence on the retention and enantioselectivity behaviour of the alkyl N-proline phases since the magnitude of intramolecular hydrophobic interactions between aliphatic chains and aromatic rings is larger than that between aliphatic chains alone. With an increasing proportion of alkyl groups on the surface of the graphitic carbon, the stability of copper complexes would be expected to decrease as a result of the corresponding reduction in the intramolecular hydrophobic interactions.

Comparison of the retention data on two aryl substituted L-proline phases showed that the retention and enantioselectivity for amino acids with non-polar side chains separated were generally reduced on the AN-L-Pro compared to the NA-L-Pro. The surface concentrations of AN-L-Pro and NA-L-Pro are very similar (Table 1), so the observed effects cannot be accounted for by differences in surface concentration. Also, the decrease in retention and selectivity cannot be accounted for by the change in intramolecular hydrophobic interactions as the anthryl substituent is expected to offer greater hydrophobic interactions compared to the naphthyl substituent. The most probable reason for the observed decrease in retention and enantioselectivity lies in the change in chelating property of the L-proline upon the substitution on its amino group. It has been suggested that the chelating ability of an amino acid is proportional to the basicity of the nitrogen group involved in the chelate [19]. Thus it may be postulated that the anthryl substituent is capable of reducing the basicity of an amino group more than the naphthyl substituent, and hence may reduce the chelating ability of the N-substituted proline to a considerable degree.

4. Conclusions

Using a series of alkyl and aryl substituted prolines as a chiral selector, we have studied the effect of N-substituents on retention and enantioselectivity in chiral chromatography. Although the elution order of a pair of D- and L-enantiomers is essentially the same on both the alkyl and aryl substituted prolines, the retention and enantioselectivity are found to vary with the length of the alkyl chain or the number of the benzene rings in the aryl substituent. These investigations provide support for our previous observation [3] that there is an important role for molecular interactions involving N-substituents in determining retention and enantioselectivity in chiral ligand exchange chromatography. The structural analyses of the transient diasteriomeric complexes show that in the cases where the intramolecular interactions between the N-substituent of the chiral selector and the side-chain of the chiral analyte are dominant, the enantiomeric analyte of the same configuration as the chiral selector will be retained less than that of the opposite configuration. Since the stability of the transient complex formed between the chiral selector and analyte is sensitive to changes in the nature of the N-substituent and the basicity of amino group, the retention strength and enantioselectivity could be regulated by an appropriate choice of the N-substituent in an amino acid chiral selector.

These results lead to a new view of N-substituents in chiral ligand exchange chromatography. The N-substituents studied to date have been mainly non-polar, with hydrophobic interactions being the dominant force involved. Our data indicate that, as well as hydrophobic interactions, both polar and ionic molecular interactions are important in chiral chromatography. Thus there is a role for the N-substituent in chiral recognition which is more extensive than

previously thought. Further evidence for the importance of molecular interactions involving the N-substituent in chiral chromatography and in the wider field of molecular recognition is likely to arise from studies of new chiral ligand exchange selectors and re-examinations of earlier data.

Acknowledgements

This work was supported by a grant from the UK Biotechnology and Biochemical Research Council as part of the Process Separations Initiative. The authors thank Hypersil (Runcorn, Cheshire, UK) for the provision of materials. We are grateful to Dr. W.C. Chan for valuable assistance in the chemical synthesis.

References

[1] V.A. Davankov, J.D. Navrati, H.F. Walton, Ligand Exchange Chromatography, CRC press, Boca Raton, FL, 1988.
[2] J.H. Knox, Q.H. Wan, Chromatographia 40 (1995) 9.
[3] Q.H. Wan, D.A. Barrett, P.N. Shaw, M.C. Davies, J. Chromatogr. A 765 (1997) 187.
[4] V.A. Davankov, A.S. Bochkov, A.A. Kurganov, P. Roumeliotis, K.K. Unger, Chromatographia 13 (1980) 677.
[5] P. Roumeliotis, K.K. Unger, A.A. Kurganov, V.A. Davankov, Angew. Chem. Int. Ed. Engl. 21 (1982) 930.
[6] P. Roumeliotis, K.K. Unger, A.A. Kurganov, V.A. Davankov, J. Chromatogr. 255 (1983) 51.
[7] P. Roumeliotis, A.A. Kurganov, V.A. Davankov, J. Chromatogr. 266 (1983) 439.
[8] Y. Ohfune, N. Kurokawa, N. Higuchi, M. Saito, M. Hashimoto, T. Tanaka, Chem. Lett. (1984) 441.
[9] D.H. James, C.S.G. Phillips, J. Chem. Soc. (1954) 1066.
[10] J.H. Knox, Q.H. Wan, Chromatographia 42 (1995) 83.
[11] J.K. Dutton, J.H. Knox, X. Radisson, H.J. Ritchie, R. Ramage, J.C.C. Perkin. Trans. 1 (1995) 2581.
[12] V.A. Davankov, A.A. Kurganov, Chromatographia 17 (1983) 686.
[13] V.A. Davankov, V.R. Meyer, M. Rais, Chirality 2 (1990) 208.
[14] G.G. Aleksandrov, Yu.T. Struchkov, A.A. Kurganov, S.V. Rogozin, V.A. Davankov, J. Chem. Soc. Comm. (1972) 1328.
[15] B.W. Delf, R.D. Gillard, P. O'Brien, J. Chem. Soc., Dalton Trans. (1979) 1301.
[16] J.H. Knox, B. Kaur, G.R. Millward, J. Chromatogr. 352 (1986) 3.
[17] J.H. Knox, B. Kaur, in P.R. Brown, R.A. Hartwick (Eds.), High Performance Liquid Chromatography, Wiley, New York, 1989.
[18] Q.H. Wan, M.C. Davies, P.N. Shaw, D.A. Barrett, Anal. Chem. 68 (1996) 437.
[19] H. Sigel, Angew. Chem. Int. Ed. Engl. 14 (1975) 394.