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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND MAGNETIC CIRCULAR DICHROISM: A STUDY OF THE "PALLADIUM(II)–THIOETHER PEPTIDE" COMPLEXES*

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SUMMARY

By using ion-pair reversed-phase chromatography with cetyltrimethylammonium or trioctylmethylammonium as the counter ion, two classes of Pd(II) complexes with completely different properties were distinguished, depending on whether the thioether group is inside a linear moiety (L-methionine and related peptides, S-ethyl-L-cysteine) or intracyclic (L-thiazolidine-4-carboxylic acid and related peptides). The retention times clearly indicate that in the complexes these ligands interact differently with the metal through their sulphur atom. Fixation of the palladium(II)–S-ethyl-L-cysteine complex on the stationary phase is useful for the characterization of D- and L-methionine. Magnetic circular dichroism studies indicated the existence of a charge-transfer band arising from the interaction of the metal with the ligand, the intensity of which varies as a function of the chemical structure of the ligand, either cyclic or linear, and its sequence. There is a good correlation between the intensity of the charge-transfer band and the retention time of the complexes.

INTRODUCTION

Complexes of palladium(II) and platinum(II) possess antiviral properties which have been extensively studied[1]. Our laboratory has been primarily interested in the optical, *e.g.*, circular dichroism, properties of the complexes of palladium(II) with disulphide and thioether groups of amino acids and peptides[2,3] and in their chromatographic properties. This work briefly describes some correlated high-performance liquid chromatographic (HPLC) and magnetic circular dichroism (MCD) results obtained with complexes of palladium(II) with two types of thioether groups in peptides: linear thioethers (L-methionine and L-methionyl peptides) and cyclic thioethers (L-thiazolidine-4-carboxylic acid and related dipeptides). Additional results suggest

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that fixation of the palladium(II)–S-ethyl-L-cysteine complex on the stationary phase should allow the characterization of D- and L-methionine.

EXPERIMENTAL

The materials and methods of detection have been described earlier[4]. Measurements were carried out at room temperature.

L-Methionine (Sigma), D-methionine (Protein Research Foundation), D,L-methionine (Prolabo), L-methionyl-L-alanine · $0.5H_2O$, L-alanyl-L-methionine, L-methionyl-L-methionyl-L-alanine (Schwarz/Mann), L-thiazolidine-4-carboxylic acid (Fluka), S-ethyl-L-cysteine (Calbiochem) and L-proline (Sigma) were used without further purification. Glycyl-L-proline, cycloglycyl-L-proline, glycyl-L-thiazolidine-4-carboxylic acid and cycloglycyl-L-thiazolidine-4-carboxylic acid were synthesized and purified according to conventional methods.

The column for the characterization of D,L-methionine was prepared as follows: a μBondapak C_{18} (10 μm) stainless-steel column (30 cm × 4 mm I.D.) was equilibrated with the mobile phase [2 m*M* cetyltrimethylammonium bromide (CTAB) in methanol–water (40:60), pH adjusted to 3 by with orthophosphoric acid. The preformed stoichiometry palladium(II)–S-ethyl-L-cysteine complex (up to 20 μ*M* in 2 ml at pH 2.3) was injected into the column where it was fully retained; washing with 120 ml of mobile phase did not lead to any detectable absorbance at 380 nm (Fig. 3). For the elution of the complex, 10 m*M* trichloroacetic acid as mobile phase is needed. Binding of this complex to the C_{18} stationary phase is assumed to be homogeneous on the basis of previous findings by Davankov *et al.*[5] and our laboratory[4]. Whereas free $PdCl_4^{2-}$ ions in the mobile phase may attack steel columns and tubes, the palladium(II) complexes do not[4]. We checked this fact by searching for iron in the eluate by atomic-absorption spectroscopy. No iron at concentrations above 0.1 μg/ml was detectable. The column in which the reversed phase is converted into a chiral phase is now ready for the characterization of D,L-methionine.

MCD measurements were performed on a modified Jouan-Roussel (II) dichrograph in which the magnetic field of 66,600 gauss was produced in a supra coil[6]. The magnetic molar ellipticity $(\theta)_M$, expressed in degrees · cm^2 · $dmol^{-1}$ · $gauss^{-1}$, was calculated from the difference between the ellipticity in the presence and absence of the magnetic field and normalized to 1 gauss.

Titrisol solutions from Merck (Darmstadt, G.F.R.) were used as buffers in which amino acids and peptides were diluted to 1 m*M*.

RESULTS AND DISCUSSION

We have reported previously[4] that optimal separation of L-methionine, L-methionyl-L-alanine and L-alanyl-L-methionine complexed with Pd(II) is obtained on a stainless-steel column according to a dynamic ion-exchange mechanism. Such a separation has also been achieved with a polyethylene μBondapak C_{18} (RCSS; Waters) column (Figs. 1 and 2), where the retention times are about double those obtained on stainless-steel columns. Such effects can be explained by differences in both the number of theoretical plates and the volume of the stationary phase. Whereas we might expect a retention time for L-methionyl-L-methionyl-L-alanine of at most twice that of L-methionine, it is found that 10 m*M* trichloroacetic acid is

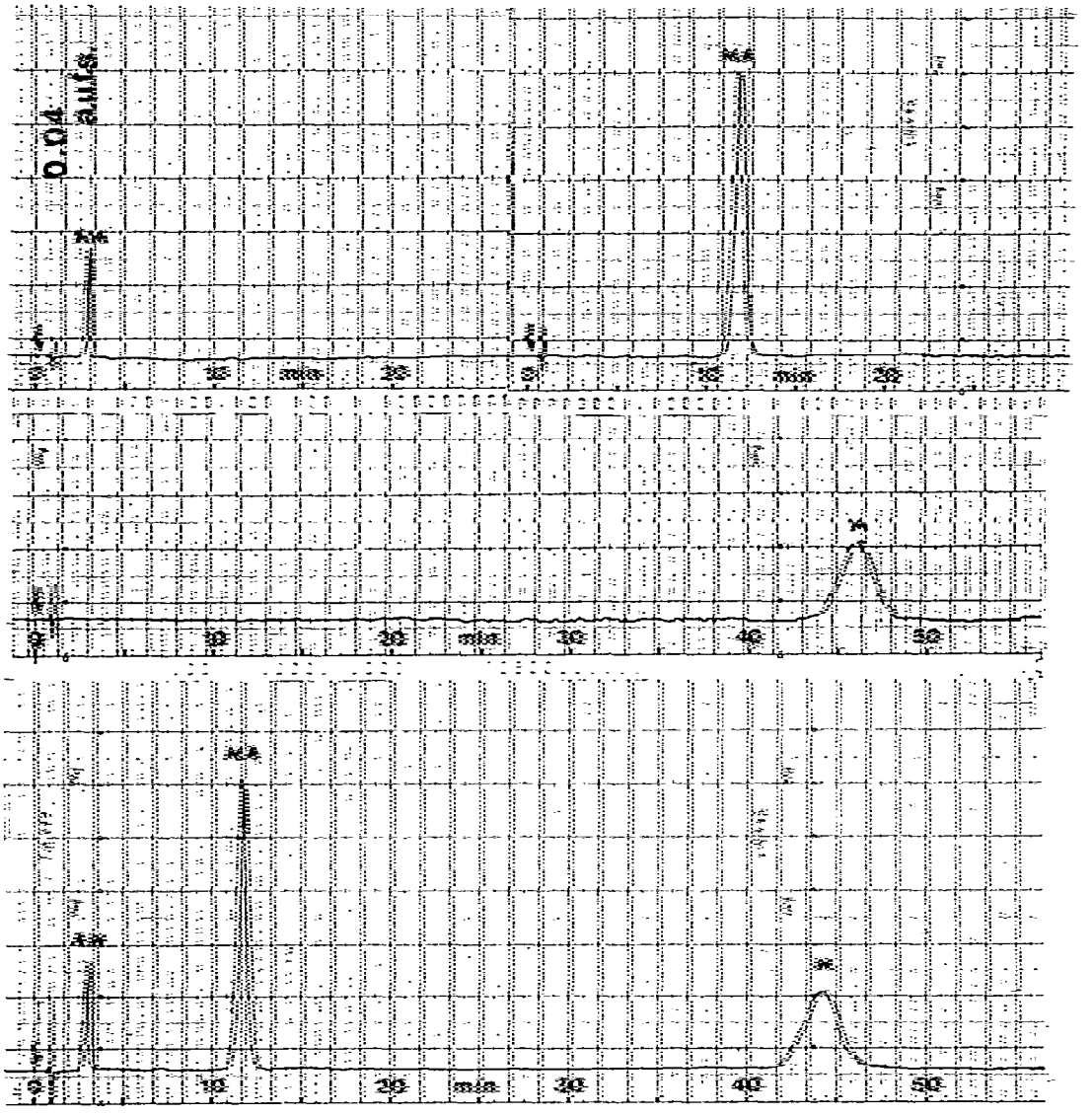

Fig. 1. Ion-pair separation by reversed-phase chromatography of L-methionine (M), L-methionyl-L-alanine (MA) and L-alanyl-L-methionine complexes with $PdCl_4^{2-}$ or Pd(II). RCSS polyethylene μBondapak C_{18} column; flow-rate, 4 ml/min; temperature, 20°C; eluent, methanol–water (40:60)–2 m*M* CTAB; pH, adjusted to 3 with orthophosphoric acid. Absorbance units full-scale (a.u.f.s.) at 380 nm, 0.040. Volume of complexes injected, 10 μl or 0.1 μmol. Top, complexes analysed separately; bottom, mixture of three complexes.

needed to elute the palladium(II)–L-methionyl-L-methionyl-L-alanine complex (Fig. 3). An additional interaction of the tripeptide carboxylate group (pK = 2.35) according to the mechanism of dynamic ion exchange might occur again in this instance. On the other hand, the retention time of S-ethyl-L-cysteine is much longer than that of its chemical isomer L-methionine, and again 10 m*M* trichloroacetate is needed for its

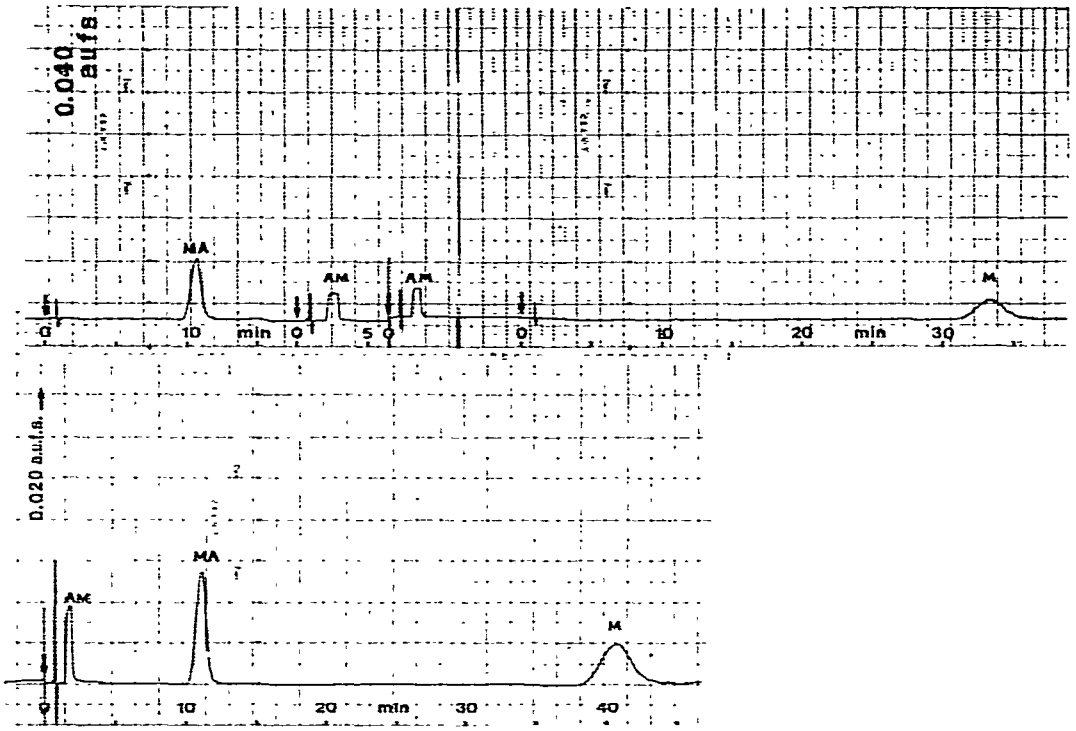

Fig. 2. Ion-pair separation by reversed-phase chromatography of L-methionine (M), L-methionyl-L-alanine (MA) and L-alanyl-L-methionine complexes with $PdCl_3^-$ or Pd(II). Experimental conditions as in Fig. 1 except that the counter ion used was trioctylmethylammonium (Adogen 464).

elution (Fig. 3). This difference might result from two conjugate effects: (i) the uncomplexed carboxylate group [the pK_{COOH} value of S-ethyl-L-cysteine is lower (2.03)[7] than that of L-methionine (2.28)[8]] interacting with the stationary phase through an ion pairing mechanism, and (ii) the stability of the chelate ring in the two complexes: a five membered ring is observed in the S-methyl-L-cysteine–palladium(II) crystal, and a six membered ring in the L-methionine–palladium(II) crystal[9,10].

Cyclic thioether derivatives

Palladium(II) complexes of L-thiazolidine-4-carboxylic acid, glycyl-L-thiazolidine-4-carboxylic acid and cycloglycyl-L-thiazolidine-4-carboxylic acid were chromatographed by ion-pair formation with CTAB in the mobile phase as with the linear thioethers (Fig. 3). Comparison of the retention times of L-thiazolidine-4-carboxylic acid (3 min) and L-proline (1.5 min) suggests that ionic and sulphur–palladium(II) interactions contribute together to the effects observed for the sulphur-containing compound. As CD experiments[11] indicate that the carboxylate group is not involved in the complex with palladium(II), this group undoubtedly contributes to the ionic interaction with the quaternary ammonium of the counter ion. On the other hand, the sulphur–metal interaction in this soft metal ion–soft base system[12] would explain the differences observed between L-proline and L-thiazolidine-4-

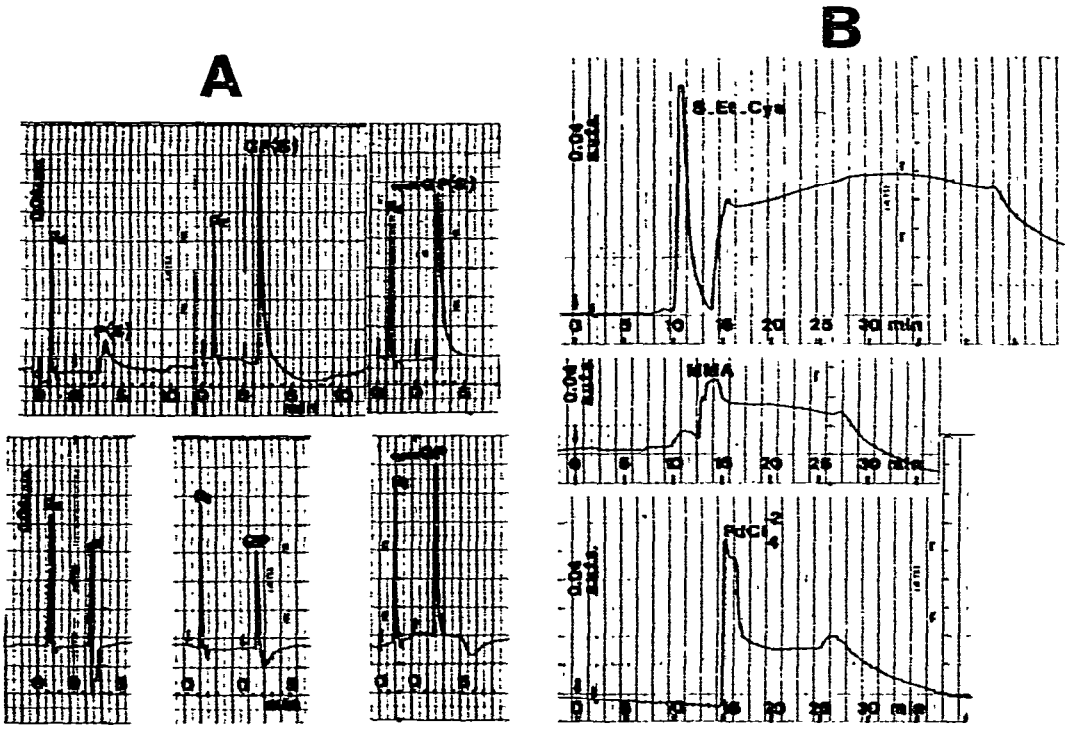

Fig. 3. (A) Ion-pair separation by reversed-phase chromatography of cyclic thioethers complexed with $PdCl_4^{2-}$ or Pd(II). P(S), L-thiazolidine-4-carboxylic acid; GP(S), glycyl-L-thiazolidine-4-carboxylic acid; cycloGP(S), cycloglycyl-L-thiazolidine-4-carboxylic acid. L-Proline (P), glycyl-L-proline (GP) and cycloglycyl-L-proline (cycloGP) complexed with Pd(II) were chromatographed as controls. Complexing was performed at pH 2 with amino acid– or peptide–Pd(II) = 1:1 at a concentration of 10^{-3} *M*. Amount of each complex injected, 100 nmol. Stainless-steel column, μBondapak C_{18}; flow-rate, 2 ml/min; temperature, 20 C; eluent, methanol–water (40:60)–2 m*M* CTAB; pH, adjusted to 3 with orthophosphoric acid. A.u.f.s. at 210 nm, 0.04. (B) Ion-pair separation by reversed-phase chromatography of S-ethyl-L-cysteine (S-Et-Cys) and L-methionyl-L-methionyl-L-alanine (MMA) complexed by palladium(II). Experimental conditions as in A. After injection of the complex elution was performed with methanol–water (40:60)–2 m*M* CTAB (pH 3) for 30 min (*i.e.*, the elution time of L-methionine). The start of the elution shown corresponds to the use of 10 m*M* trichloroacetate (pH 3). Sodium tetrachloropalladate ($PdCl_4^{2-}$) was also eluted with 10 m*M* trichloroacetate (pH 3) as a control. A.u.f.s. at 380 nm, 0.04.

carboxylic acid. The retention times of glycyl-L-thiazolidine-4-carboxylic acid and the cycloglycyl-L-thiazolidine-4-carboxylic acid complex lie between 1.5 and 2.0 min. The peak widths of sulphur-containing dipeptides are larger than those of non-sulphur-containing compounds. This probably results from a better diffusion of thioether compounds in the stationary phase.

As the S-ethyl-L-cysteine–palladium(II) complex is completely retained on the μBondapak C_{18} stationary phase under the conditions described in Fig. 3, we examined the possibility of using this new chiral support for the resolution of D,L-methionine. The stationary phase is prepared by immobilization of the chiral ligand on the solid support to create a specific solute–sorbent interaction for resolution of racemates of underivatized amino acids[13–16]. The system works under the conditions

given above unless the mobile phase is 10 m*M* trichloroacetate (TCA). Fig. 4 shows that D-methionine has a shorter retention time than L-methionine when the two compounds are chromatographed separately, in agreement with the results obtained by Lefebvre *et al.*[15] and Foucault *et al.*[16]. The retention time of D,L-methionine (24.4 min), however, lies between those of D-methionine (23.2 min) and L-methionine (27.6 min). This phenomenon could be explained by the concomitant resolution of the D- and L-peaks and the formation of a bis-ligand complex [D–Pd(II)–L] as suggested by Vicol *et al.*[17]. The role of palladium(II), uncomplexed to S-ethyl-L-cysteine but ion paired to the stationary phase, is emphasized by the strong absorbance of methionine

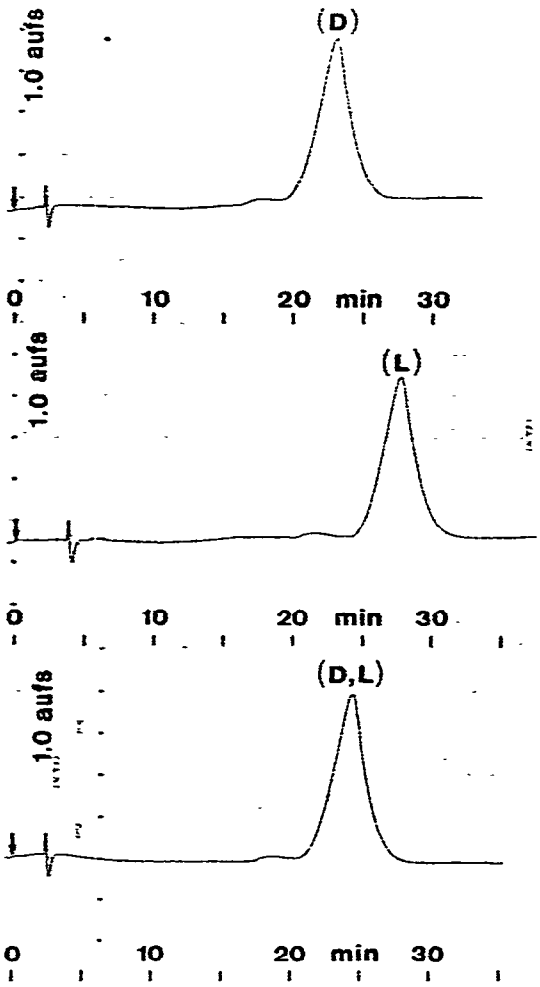

Fig. 4. Elution patterns of L-, D- and D,L-methionine from S-ethyl-L-cysteine (S-Et-Cys)–Pd(II) chiral phase. The complex is bound to the μBondapak C_{18} column (experimental conditions as in the text): Top, D-methionine (0.2 μ*M*); middle, L-methionine (0.2 μ*M*); bottom, racemate (0.2 μ*M*). Mobile phase: methanol–water (40:60)–2 m*M* CTAB; pH, adjusted to 3 with orthophosphoric acid. Flow-rate, 2 ml/min. a.u.f.s. at 210 nm, 1.0.

(0.4 absorbance unit at 210 nm for 0.2 μM injected) which is, of course, eluted in a palladium(II)-bound form as previously observed[4].

To understand better the behaviour of the thioether group in the various complexes with palladium(II), we examined the charge-transfer transition by MCD. Fig. 5 shows the Faraday effect obtained at acidic pH where the most intense effects are observed. Looking at the signals around 230 nm, which have previously been assigned to the interaction of the metal with the sulphur atom from the L-cysteine–palladium(II) complex[2,3], we find differences according to the type of compound examined, either cyclic or linear. These can be explained by the properties of the p orbital of the sulphur atom: the bond angle of the S atom (R_1–S–R_2) is 92.5° in the cyclic compound and appears strained compared with the value of 100° found in the linear compounds. Also, the shape and size of the chelates may influence the MCD properties. More specifically, for linear compounds, we find that intensities vary from one compound to another as a function of chemical structure. For instance, the magnetic ellipticity of L-methionyl-L-alanine is higher than that of L-alanyl-L-methionine, a feature which can be related to the difference in the distance existing between the donors S and NH_2 in the two compounds. We also find that the magnetic ellipticity in L-methionyl-L-methionyl-L-alanine (containing two L-methionyl residues) is about twice as strong as that in L-methionyl-L-alanine (not shown). S-Ethyl-L-cysteine, a chemical isomer of L-methionine, is an apparent exception: the Faraday effect is twice that found for L-methionine. The weaker interaction between palladium(II) and the thioether sulphur of methionine compared with S-ethyl-L-cysteine might be a consequence of the atomic coordinates: the Pd–S distance and the N–Pd–S bond angle are 2.230 Å and 86.8°, respectively, in the S-methyl-L-cysteine–palladium(II) complex[10] compared with 2.265Å and 96.9° in the L-methionine–palladium(II) com-

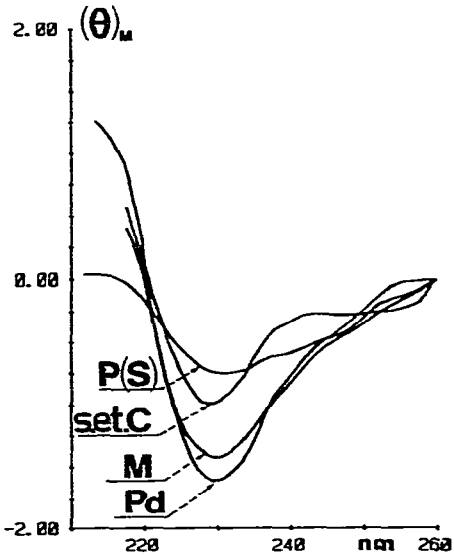

Fig. 5. MCD spectra of three thioether amino acids complexed by palladium(II) in citrate buffer at pH 2. The spectrum of sodium tetrachloropalladate ($PdCl_4^{2-}$) was recorded as a control. Amino acid–palladium(II), 1:1 at a concentration of 10^{-3} M. P(S) = L-thiazolidine-4-carboxylic acid; S-et-C = S-ethyl-L-cysteine; M = L-methionine.

plex[9]. In addition, the size of the chelate rings is different in the two complexes, as S-methyl-L-cysteine–palladium(II) is stabilized through a five-membered ring[10] and L-methionine–palladium(II) through a six-membered ring[9]. Hence the strain and the sum (or resultant) of the dielectric and magnetic dipoles are larger in the S-ethyl-L-cysteine (or S-methyl-L-cysteine) complex.

In conclusion, the palladium(II) complexes of linear and cyclic thioethers show distinctly different chromatographic and MCD properties. Retention times indicate that ligands interact with the palladium(II)-bound stationary phase through a variety of forces. A good parallelism is observed between the intensity of the charge-transfer band (MCD) of the complexes and the retention time: the larger the magnetic ellipticity, the longer is the retention time (Fig. 6).

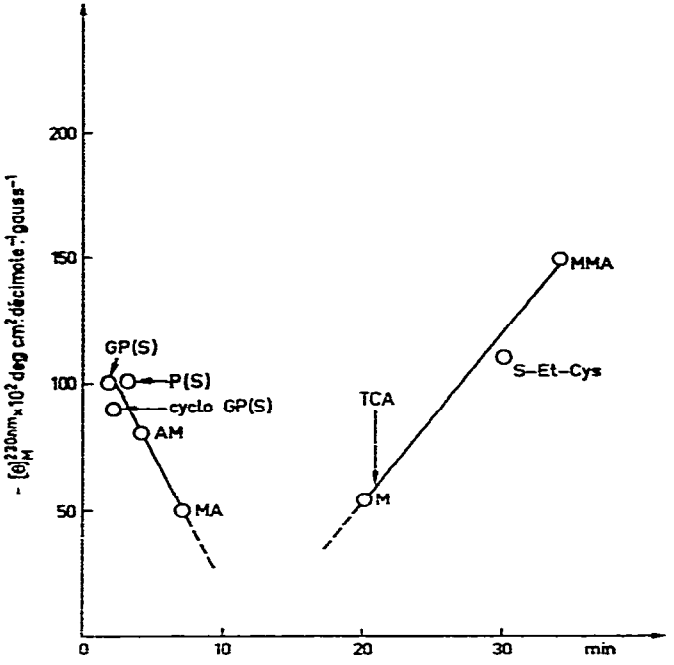

Fig. 6. Magnetic ellipticites $[\theta]_M$ plotted *versus* retention times (min) obtained in reversed-phase chromatography for the thioether–palladium(II) complexes. Trichloroacetate (TCA), 10 m*M*, pH 3.

Finally, the chiral support obtained by fixation of the S-ethyl-L-cysteine complex to μBondapak C_{18} columns seem promising for the resolution of underivatized amino acid racemates.

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