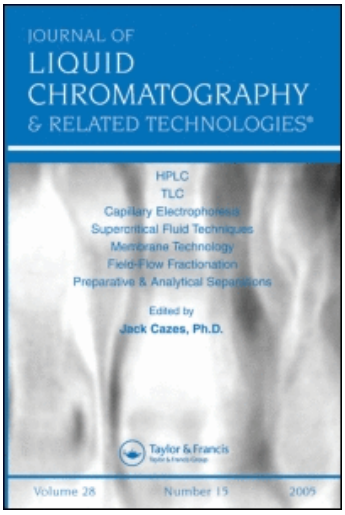

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REVERSED-PHASE, ION-PAIR SEPARATION OF L-METHIONINE AND L-METHIONINE DIPEPTIDES COMPLEXED WITH PALLADIUM (II)

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ABSTRACT

A high performance liquid chromatographic (HPLC) method is described for separation of L-methionine and L- methionine dipeptides complexed with palladium (II). Under isocratic conditions, at room temperatures, with the appropriate selection of counter-ion (cetyltrimethylammonium or trioctylmethylammonium), it was possible by ion-pairing reversed phase chromatography to resolve the palladium (II) complexes studied. Stainless steel and polythylene columns were used. The chromatograms from both the two different materials made columns indicate about the same ratio of capacity factor of the palladium (II) complexes.

INTRODUCTION

Reversed-phase HPLC using the metallic cations Cd(II) and Zn(II) in mobile phase has allowed a good separation of amino-acids and dipeptides (I). The same is true for ion-pair reversed-phase HPLC using anionic and cationic reagents (2, 3). However, no example of the ion-pair formation method for metallic complexes of amino-acids and dipeptides has yet been reported in the literature.

In previous circular dichroism studies (4, 5) of complexes between palladium (II) and sulfur of S-S-group containing amino-acids and peptides, we showed that strong interaction occurred between the sulfur and the metal and we described the different kinds of complexes which were formed via these interactions. The present work is part of an extensive HPLC:CD

study on complexes between Pd(II) and the thioether group (6) and describes how chromatographic conditions have been found in order to achieve good separation of complexes of Pd(II) with L-methionine, L-methionyl-L-alanine and L-alanyl-L-methionine in acidic aqueous medium.

MATERIALS AND METHODS

High performance liquid chromatography equipment is composed of a M6000 A solvent delivery system (Waters Assoc., USA), a U 6K universal liquid chromatograph injector, a SF-770 UV-Vis variable wavelength monitor (Schoeffel, USA) coupled a chart recorder (Meci, France). The stainless steel μ Bondapak-C_{18} (10 μm, 30 cm x 4 mm I.D.) column and polyethylene μ Bondapak-C_{18} column (C_{18}-radial Pak and RCM 100) or radially compressed system of separation (RCSS) were obtained from Waters Assoc. Counter-ions used are : cetyltrimethylammonium bromide, formulated by CH_3-$(CH_2)_{15}$-$N(CH_3)_3$ Br or CTAB (Eastman-Kodak, USA), trioctylmethylammonium chloride, formulated by $(CH_3$-$(CH_2)_7$-$)_3$ NCH_3Cl or Adogen 464 (Serva, W. Germany). All solvents are of analytical grade, pure water obtained from Milli Q system (Millipore, USA). Solvent systems were filtrated on Millipore 0.45 μm and degazed by ultra-sound, 15 min., before use. L-methionine or (M) (Merck, W. Germany), L-methionyl-L-alanine or (MA) and L-alanyl-L-methionine or (AM) (Schwarz-Mann, USA) were dissolved in water (10 mM). Palladium (II) was used as sodium tetrachloropalladate Na_2PdCl_4, 35 % Pd (Prolabo, France) in aqueous solution (100 mM). Fresh stoechiometric complexes were preformed before their injection in the column : 10 μM Pd (II) was added to 1 ml water containing 10 μM of ligand, pH was adjusted to 2.0 by HCl(N). 10 μl was injected at once with precision-syringe (Hamilton, Switzerland). Palladium (II) chelates were detected by absorbance at 380 nm, maximum wavelength of the lowest energy d-d band of the complexed metals ; the non-complexed metal has a weak molar extinction coefficient. The chromatography was performed in isocratic conditions : 20°C, 40 % methanol in water in presence of 2 to 25 mM of counter-ion. Solvent systems were adjusted to pH 3 concentrated H_3PO_4 before use.

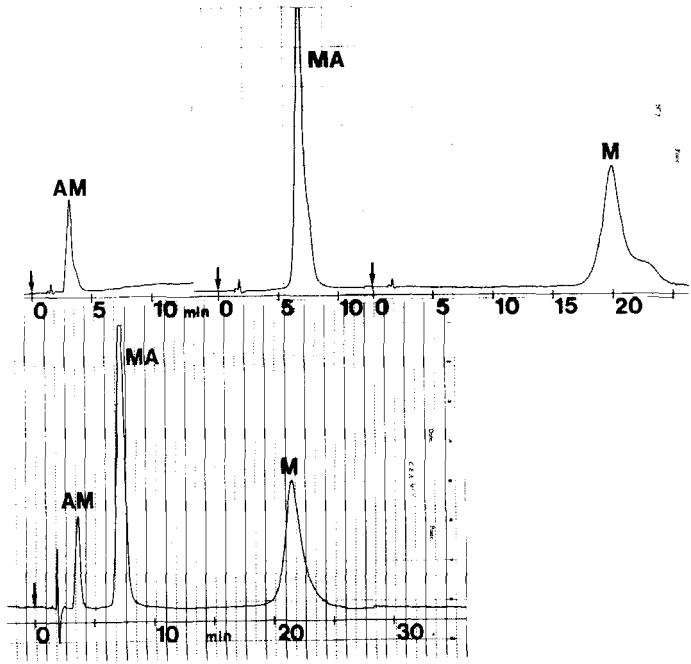

FIGURE 1

Ion-pair separation by reversed-phase chromatography of L-methionine (M), L-methionyl-L-alanine (MA) and L-analyl-L-methionine complexed with $PdCl_4^{-2}$ or Pd(II). Stainless steel column μ Bondapak-C_{18} ; flow rate 2 ml/min ; t = 20°C ; eluent 40 % methanol in water - 2 mM CTAB ; pH adjusted to 3 by concentrated H_3PO_4. Absorbance unit full scale (A.u.f.s.) at 380 nm : 0.040. 10 μl or 0.1 μM of complexes injected. Top : complexes analyzed separately, bottom : mixture of 3 complexes.

RESULTS AND DISCUSSION

Optimal separation of the L-methionine-Pd(II), L-methionyl-L-alanine-Pd(II) and L-alanyl-methionine-Pd(II) complexes was achieved by using a solvent system of 40 % methanol in water in presence of 2 mM CTAB or Adogen 464 as counter-ion acidified to pH 3 by H_3PO_4 (Figs. 1 and 2). A shoulder effect was observed on the M peak (Fig. 1, top), which however occurs only when an old solution (> 24 hours in open tubes) is used. The effect does

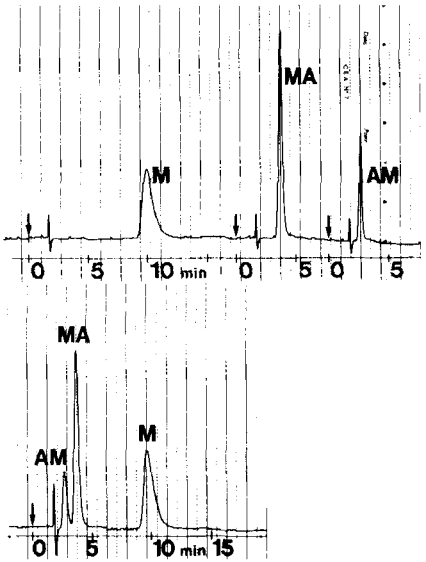

FIGURE 2

Ion pair separation by reversed phase chromatography of L-methionine (M), L-methionyl-L-alanine (MA) and L-alanyl-L-methionine complexed with $PdCl_4^{-2}$ or Pd(II). Experimental conditions as in fig. 1 except for the counter-ion used : Adogen 464.

not exist for freshly performed complexes (Figs. 1 bottom and 2) which indicates that the tailing is caused by the presence of L-methionine sulfoxyde in the medium, arising from the tendency of L-methionine to be oxidized by air even in the absence of metallic cations (7).

Acetonitrile as solvent in the mobile phase was not inert towards $PdCl_4^{-2}$ since a slight precipitate was observed probably corresponding to a complex $Pd(CH_3CN)_2Cl_2$ (8). Stainless steel tubing and column are not altered by several injections of 0.1 µM of the complexes but by 2 mM $PdCl_4^{-2}$ alone in the mobile phase, as might be expected from the electromotive series.

To check the first observation, separation of preformed complexes were achieved on the C_{18}-polyethylene column of the RCSS-Waters system. The chromatograms obtained from the stainless steel column and the polyethylene column (not shown) indicate about the same ratio of capacity factor of the complexes for the two solvent systems used. Therefore, the metal (if any) from the stainless steel column does not interfere with palladium (II) in the complexation process between Pd(II) and M, MA or AM. On the other hand, the anionic reagent, sodium dodecyl sulfate (SdS) did not yield separation of Pd(II) chelates. The result clearly indicates that Pd(II) chelates and SdS adsorbed on the stationary phase do not form ion-pairs (9), and suggests that in these conditions both palladium (II) complexes and SdS were charged negatively.

pH effects corroborate the role of the sign of the electrical charges on the separation of complexes. In the solvent system consisting of 40 % methanol in water and 2 mM CTAB at pH 5.6, under the dynamic conditions of ionization in the column, palladium (II) chelates should have their carboxylic groups completely deprotonated and therefore should be retained. When H_3PO_4 is added to the mobile phase for a decrease in pH to 3, the observed retention time of complexes is now compatible with a good separation (Figs. 1 and 2). This result could be explained by the partial ionization at pH 3 of the carboxylic group (X-ray studies on the L-methionine-Pd(II) complex show that the carboxylic group is free of all interaction) (10), by the minimal interaction of $H_2PO_4^{-2}$ with the stationary phase and the hydrophilic mechanism of ion-pairing of $H_2PO_4^{-2}$ with palladium (II) chelates (11).

Chromatographically, we can demonstrate the complexation effect of palladium (II) with L-methionine, L-methionyl-L-alanine and L-alanyl-methionine. Injection of $PdCl_4^{-2}$ alone in the (40 % methanol in water, 2 mM CTAB, pH 3), solvent system leads to elution which does not show any absorbance at 380 nm. $PdCl_4^{-2}$ is completely retained and can be eluted only when 10 mM trichloroacetate was used instead of 2 mM CTAB in the mobile phase (6). In contrast, successive injections of $PdCl_4^{-2}$ alone and then of ligand L-methionine or L-methionyl-L-alanine

or L-analyl-L-methionine yield a chromatogram similar to the one of the preformed complex. Ammonium counter-ion e.g. CTAB with a long alkyl chain is known to be adsorbed on the C_{18}-stationary phase (11), a dynamic ion-exchange mechanism should be proposed according to the previous observation :

$$2\ CTAB^{+}_{stat.phase} + PdCl_4^{-2} \rightleftarrows (2\ CTAB^{+} - PdCl_4^{-2})_{stat.phase}$$

$$(2\ CTAB^{+} - PdCl_4^{-2})_{stat.phase} + M \rightleftarrows 2\ CTAB^{+} + (M\text{-}PdCl_4^{-2})_{mob.phase}$$

Experiments were also carried out with different concentrations of cationic hydrophobic ion-pairing reagents. A plot

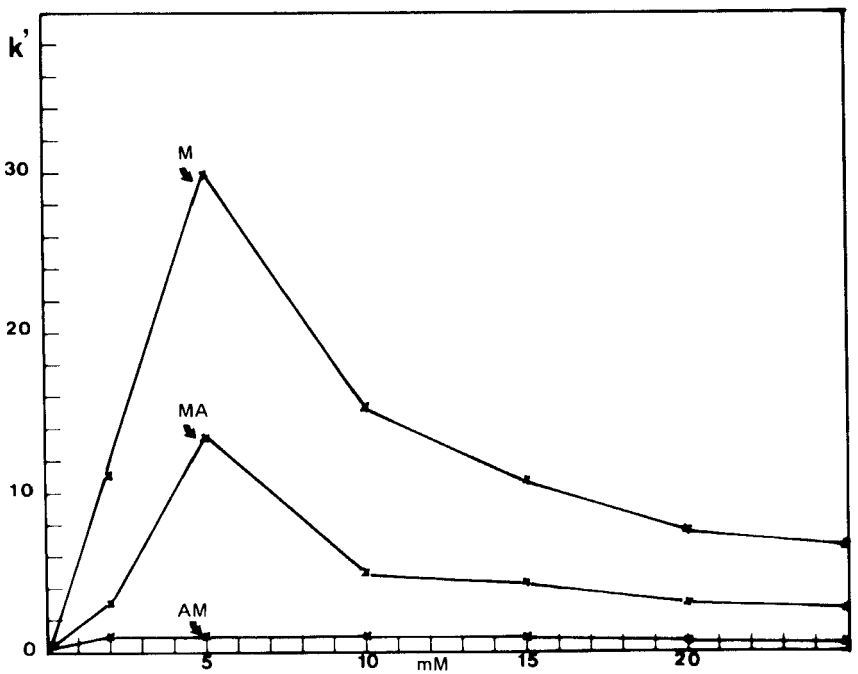

FIGURE 3

Dependence of the capacity factor L-methionine (M), L-methionyl-L-alanine (MA), L-alanyl-L-methionine (AM) complexed by Pd(II) on the concentration of CTAB in the mobile phase. Experimental conditions as in fig. 1.

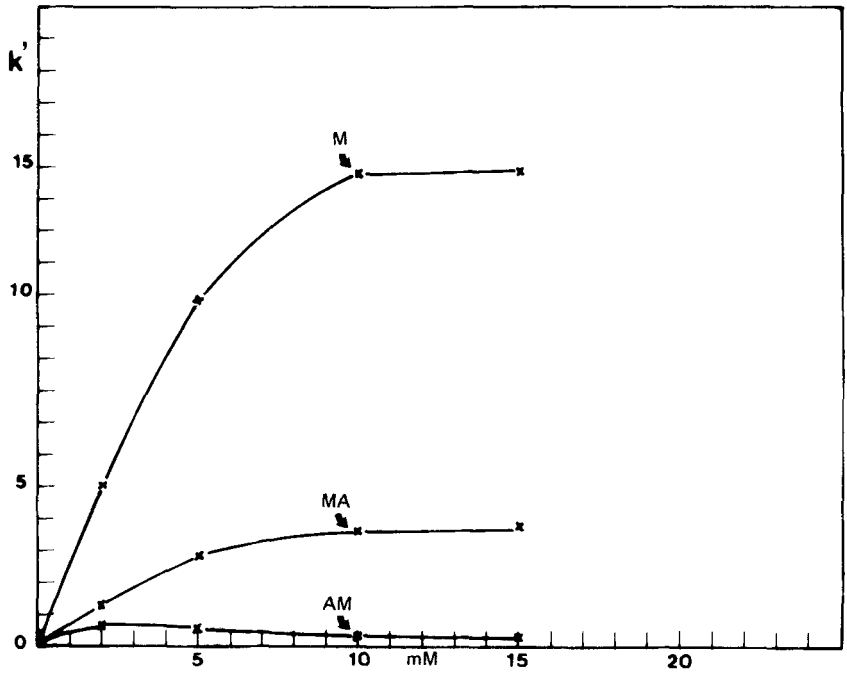

FIGURE 4

Dependence of the capacity factor of L-methionine (M), L-methionyl-L-alanine (MA), L-analyl-L-methionine (AM) complexed by Pd(II) on the concentration of Adogen 464 in the mobile phase. Experimental conditions as in fig. 1.

of the capacity factor of palladium (II) chelates versus increasing concentration of cationic reagents shows an optimum at 5 mM CTAB and 10 mM Adogen 464 (Figs. 3 and 4). The difference of retention time of complexes at the same concentration of counter-ion (2 mM) should be explained by the difference of hydrophobic area between the two counter-ions (C_{16} for CTAB and $3XC_8$ for Adogen 464) but also by the steric hindrance on the quaternary ammonium. The decrease of the capacity factor of complexes with increasing concentration of counter-ion (Fig. 3) could be interpreted, within the ion-pairing mechanism (2, 3), as being due to the lack of balance of the hydrophobic interactions :

$$(\text{counter-ion})_{\text{mob.phase}} - \text{Stat. phase}$$

$$> (\text{counter-ion-complex})_{\text{mob.phase}} - \text{Stat. phase}$$

However this cannot be true for the Adogen 464 counter-ion (Fig. 4) owing to the difference of the hydrophobic area between CTAB and Adogen 464.

We think that this method of dynamic ion-exchange is useful for rapid and efficient separation of metal chelates of amino-acids or oligopeptides in the analytical range.

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