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Study of tryptophan enantiomer binding to a teicoplanin-based stationary phase using the perturbation technique Investigation of the role of sodium perchlorate in solute retention and enantioselectivity

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Abstract

The retention of D,L-tryptophan enantiomers on an immobilized teicoplanin column was investigated in relation to the mobile phase sodium perchlorate concentration using the perturbation method to determine the solute distribution isotherms. From the experimental data, it appeared that the bi-Langmuir model was able to describe D- and L-enantiomer retention on the immobilized selector over the salt concentration range. An increase in the apparent enantioselectivity with an increase in sodium perchlorate concentration was observed. The chiral recognition enhancement was governed by (i) an increase in the difference of the adsorption constants for binding to the high-affinity site (aglycone pocket) between the two enantiomers and (ii) enhancement of the number of aglycone chiral regions interacting with D-tryptophan. It is suggested that an ion-pair formation mechanism between perchlorate and solute and/or selector is responsible for this behavior. In addition, this work shows that additional secondary sites on the teicoplanin surface are involved in the apparent enantioselectivity at low sodium perchlorate concentrations.

Keywords: Enantiomer separation; Stationary phases, LC; Retention; Tryptophan; Teicoplanin; Sodium perchlorate; Amino acids

1. Introduction

In recent years several chiral stationary phases (CSPs) have been used to separate enantiomers. The classification of CSPs is based on the type of interactions involved in the chiral discrimination process. Different types include π–π phases, chiral ligand-exchange phases, immobilized proteins, and CSPs containing inclusion cavities. CSPs for which chiral discrimination is based on an inclusion process are mainly cellulose derivatives and macrocyclic compounds such as crown ethers and cyclodextrins. The idea of using macrocyclic glycopeptides as chiral stationary phases for liquid chromatography

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was first introduced by Armstrong in 1994. Chiral stationary phases based on macrocyclic antibiotics have attracted much attention because of their broad applicability and ability to separate enantiomers of amino acids, and acidic and basic drugs [1]. Four commercially available macrocyclic antibiotics were bound to silica and used as chiral selectors: vancomycin, teicoplanin, avoparcin, and ristocetin. The high-performance liquid teicoplanin chromatography column is based on the covalent binding of teicoplanin, an amphoteric glycopeptide, to spherical silica gel. It was obtained by multiple covalent linkages while retaining the essential components for chiral recognition. This phase shows 20 chiral centers surrounding four cavities. Hydrogen-donor and -acceptor sites are available, close to seven aromatic rings, leading to the formation of a diastereomeric complex. This type of arrangement is highly favorable for a great number of enantiomeric separations. The complexity of the selector structure is responsible for several mechanisms of chiral recognition with various potential interactions. Some studies have been conducted on the aspects of enantioselective interactions for teicoplanin as a stationary phase. For example, the temperature effects on retention and enantioselectivity have been examined [2,3]. The changes in enthalpy and entropy associated with the transfer of the solute can be extracted from the linear van 't Hoff plots and analyzed in order to obtain information about the driving forces implied in the association process. Another approach for studying the interactions involved in solute binding to teicoplanin is to vary the mobile phase composition [4–10]. Investigations have been carried out by varying the proportion of organic modifier [4–6], pH [7,8] or the ionic strength [9,10] of the mobile phase. In previous studies of the salt dependence on the amino acid derivative binding to immobilized teicoplanin [9] or vancomycin-based stationary phases [11], it was found that the negatively charged solute retention factor increased with increasing concentration of the additive (at a salt concentration <100 mM). Analysis by the extended Wyman relations indicated that this retention increase was dependent on an ion-pairing mechanism [11]. In addition, it has been observed that this phenomenon is associated with an enhancement in the apparent enantioselectivity of immobilized teicoplanin [9]. In these studies, analysis of the retention data was carried out under linear chromatographic conditions, i.e. corresponding to an investigation of the solute retention factor (overall retention parameter) variation with change in the mobile phase salt concentration. A more valuable approach for analyzing solute–CSP binding involves the determination of the distribution isotherms. The binding of a chiral compound to a CSP classically involves two kinds of sites at the surface of the selector: one class of binding site (type 1) with higher affinity (generally enantioselective) and another class of binding site (type 2) with low affinity (generally non-enantioselective). Methods based on frontal analysis [12–16] or the perturbation technique [17,18] have been successfully used to determine the solute adsorption isotherms and examine the relative contributions of type 1 and 2 sites to the overall retention and enantioselectivity for various CSPs [12,13,15,17].

In this study, salt effects on the retention and enantioselectivity of zwitterionic species (D,L-tryptophan enantiomers) on immobilized teicoplanin were examined in order to further investigate the role of the ion-pairing mechanism in the enantiomer–macrocyclic antibiotic interaction. The choice of sodium perchlorate salt as mobile phase additive was dictated by the ability to form ion-pairs with both cationic [19,20] and zwitterionic [21–24] species, mainly due to its chaotropic nature. Also, Langmuir and bi-Langmuir isotherms were derived from D,L-tryptophan enantiomer retention data using the perturbation technique. From the adsorption isotherms, the retention contributions corresponding to the two kinds of sites were analyzed in relation to the salt concentration in the mobile phase. Apparent and true enantioselectivities were calculated from the retention data to obtain information about salt effects on the enantioselective binding of D,L-tryptophan to immobilized teicoplanin.

2. Theory

Distribution isotherms can be determined using the perturbation technique. This consists of measuring the retention times of small amounts of sample injected onto a column equilibrated with sample solutions at different concentrations [17,18]. For the

determination of isotherms, the column is equilibrated with an eluent which contains a single compound. Perturbation is then triggered by the injection of a small sample volume containing a higher concentration of the same compound. Initially, the retention time of the analyte was evaluated under elution conditions. Then, subsequent concentration steps were introduced to saturate the column at higher concentrations. The decrease of the retention times for higher liquid-phase concentrations is related to the nonlinear character of the assumed isotherm. A theory for non-linear chromatography is required. Various adsorption isotherm equations have been proposed and validated to describe the distribution of sample components between the stationary and the mobile phases. The most common and successful equation is the classical competitive Langmuir equation:

$$Q = \frac{q_s K c}{(1 + Kc)} \tag{1}$$

where Q is the concentration of the sample compound in the stationary phase and c that in the mobile phase, q_s (mol/L) is the column saturation capacity and K the adsorption constant between the solute and the ligand. In the case of a single-component equilibrium of a compound, the distribution isotherm depends only on the concentration of a single solute. The experimental retention factor of the solute is directly proportional to the first derivation (slope) of the adsorption isotherm of the compound. In the case of a Langmuir isotherm, the following relation can be obtained as previously described [17,18]:

$$k = \frac{t_R}{t_0} - 1 = \phi \frac{dQ}{dc} = \phi \frac{q_s K}{(1 + Kc)^2} \tag{2}$$

where t_R is the retention time of the solute, t_0 the column hold-up time, i.e. the elution time of a non-retained compound, and ϕ the phase ratio, V_S/V_M (V_s is the volume of the stationary phase in the column and V_M the void volume). Plotting the retention factor versus the analyte concentration, it is possible to determine parameters q_s and K.

In the case of the interaction between an enantiomer and a CSP, the Langmuir model is often not able to fit the experimental data well and more complex isotherms should be used. If a compound can be adsorbed on two different adsorption sites (1 and 2), the distribution can be adequately described by the bi-Langmuir model with different adsorption constants K_1 and K_2, and saturation capacities of the two adsorption centers 1 and 2, q_{s1} and q_{s2}:

$$Q = \frac{q_{s1} K_1 c}{(1 + K_1 c)} + \frac{q_{s2} K_2 c}{(1 + K_2 c)} \tag{3}$$

The model assumes that the individual binding regions on the receptor have constant and independent affinities for the solute, i.e. the value of K_1 is not affected by binding of the solute to the second binding regions on the receptor. In the case of a solute injected onto a column containing a receptor with two kinds of binding sites R_1 and R_2, the following relationship can be used:

$$k = \frac{t_R}{t_0} - 1 = \phi \frac{dQ}{dc} = \phi \left(\frac{q_{s1} K_1}{(1 + K_1 c)^2} + \frac{q_{s2} K_2}{(1 + K_2 c)^2} \right) \tag{4}$$

Plotting the retention factor versus the analyte concentration, it is possible to determinate the adsorption constants K_1 and K_2, the saturation capacities of the two adsorption centers 1 and 2, and then the retention factors $k_1 = \phi q_{s1} K_1$, $k_2 = \phi q_{s2} K_2$ (corresponding to the retention contributions of the two kinds of sites, under linear conditions) and $k_T = k_1 + k_2$.

For a solute interacting with the two binding regions of a selector, the apparent enantioselectivity, α_{app}, can be defined. In the general case where the two classes of binding sites (types I and II) are enantioselective and $k_D > k_L$, α_{app} can be described as follows:

$$\alpha_{app} = \frac{k_D}{k_L} = \frac{k_{1D} + k_{2D}}{k_{1L} + k_{2L}} \tag{5}$$

As previously demonstrated, this global parameter does not describe adequately the chiral discrimination mechanisms of the selector at the microscopic level [12,13,15]. A more detailed parameter is related to the true enantioselectivity defined for the two kinds of sites (with $k_{1D} > k_{1L}$ and $k_{2D} > k_{2L}$) as follows:

$$\alpha_1 = \frac{k_{1D}}{k_{1L}} = \frac{q_{s1D}}{q_{s1L}} \cdot \frac{K_{1D}}{K_{1L}} \quad (6)$$

and

$$\alpha_2 = \frac{k_{2D}}{k_{2L}} \quad (7)$$

Using Eqs. (6) and (7), it is possible to investigate the relative enantioselective contributions of the two types of binding sites to the overall apparent enantioselectivity. Also, the K_1 and q_{s1} ratios can be determined to obtain additional information about the mechanism of the true enantioselectivity variations at the type I site.

3. Experimental

3.1. Reagents

D-Tryptophan and L-tryptophan ($C_{11}H_{12}N_2O_2$) were purchased from Sigma–Aldrich (Saint-Quentin-Fallavier, France). Sodium phosphate monobasic and sodium phosphate dibasic were supplied by Merck (Fontenay-sous-Bois, France) and anhydrous sodium perchlorate by Acros (Saint-Quentin-Fallavier, France). Water was obtained from an Elgastat Option water-purification system (Odil, Talant, France) fitted with a reverse osmosis cartridge.

3.2. Chromatography

Experiments were carried out using Shimadzu LC-10AD VP pumps interfaced to a Shimadzu SPD-10A VP UV–Vis detector (wavelength fixed at 310 nm for the detection of tryptophan). The liquid chromatograph was equipped with a Shimadzu SIL-10D VP autoinjector and monitored by means of a SCL-10A VP system controller. Data acquisition was performed using Shimadzu Class-VP software (version 5.032). The chromatographic separations were achieved on a 150 mm×4.6 mm I.D. Astec Chirobiotic T HPLC column packed with a stationary phase produced by chemically bonding the macrocyclic glycopeptide teicoplanin to 5-μm silica gel. The mobile phase was 5 m*M* phosphate buffer (pH 7.0). The concentration of sodium perchlorate salt was varied from 10 to 75 m*M*. The mobile phase flow-rate was 1 mL min^{-1}. The column temperature was set at 25 °C by means of an Igloocil oven (Interchim, Montluçon, France). The void volume was determined experimentally using sodium nitrate. This volume was not modified by the eluent salt concentration.

3.3. Operating conditions

To determine the solute adsorption isotherms, column equilibration was carried out with various D- or L-enantiomer solutions (*c*, 0 to 6 m*M*) until a stable detector response was observed. Five microliters of the most concentrated D- or L-enantiomer sample was injected in triplicate onto the column and the retention times were measured.

3.4. Non-linear regression analysis of retention data

The model equation was fitted to the retention factor of the enantiomers by a non-linear regression using the software Table curve 2D (SPSS Science Software, Erkrath, Germany).

4. Results and discussion

4.1. Adsorption isotherms and determination of the retention parameters

The retention factor values for D,L-tryptophan on immobilized teicoplanin were determined in relation to the respective analyte concentration in the mobile phase (0 to 6 m*M*) at various concentrations of sodium perchlorate in the mobile phase (*c*, 10 to 75 m*M*). The column temperature was held at 25 °C. The coefficients of variation of the *k* values were $<1.20\%$, indicating good reproducibility for the chromatographic system. The *k* values were plotted against *c*. Figs. 1 and 2 show *k* vs. *c* plots of D- and L-tryptophan enantiomers for all sodium perchlorate concentrations in the mobile phase. Theoretical binding curves were fitted to the experimental data using Eqs. (2) and (4). The non-linear regression coefficient R^2, the *F* value (from the Fisher test with the confidence level at 95%) and the equation

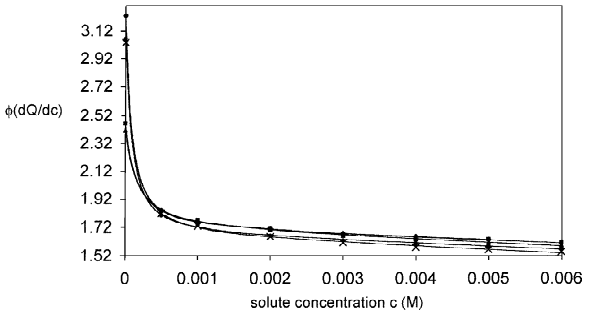

Fig. 1. Local isotherm slope, $\phi(dQ/dc)$, versus eluent solute concentration, c (M), for the D-tryptophan enantiomer. Mobile phase, phosphate buffer pH 7.0; sodium perchlorate concentration, 10 mM (■), 25 mM (▲), 37.5 mM (●), 50 mM (×), 75 mM (♦); stationary phase, immobilized teicoplanin; column temperature, 25 °C; solid lines, bi-Langmuir model (Eq. (4)).

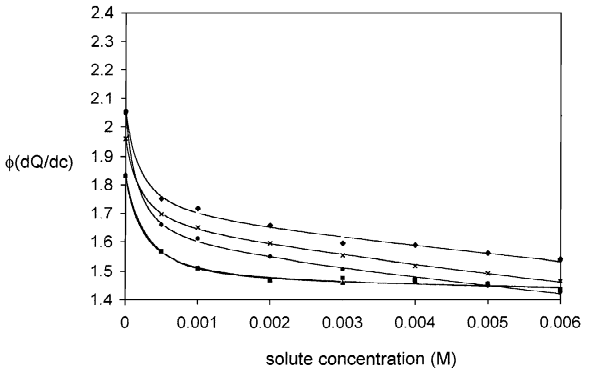

Fig. 2. Local isotherm slope, $\phi(dQ/dc)$, versus eluent solute concentration, c (M), for the L-tryptophan enantiomer. Mobile phase, phosphate buffer pH 7.0; sodium perchlorate concentration, 10 mM (■), 25 mM (▲), 37.5 mM (●), 50 mM (×), 75 mM (♦); stationary phase, immobilized teicoplanin; column temperature, 25 °C; solid lines, bi-Langmuir model (Eq. (4)).

parameters were determined. These are shown in Tables 1 and 2. The F value constitutes a more discriminating parameter than the R^2 value when assessing the significance of the model equations. This statistical parameter is greater for the bi-Langmuir model than for the langmuir model in all cases. Therefore, it appears that the bi-Langmuir model describes adequately the retention behavior of both enantiomers. This means that the interaction of the two enantiomers with the chiral selector involves one site of high affinity (type 1) and a number of additional sites of lower affinity (type 2). This is consistent with previous studies of solute retention on numerous CSPs such as proteins, cellulose derivatives or macrocyclic antibiotics [12,13,15,17]. From the parameters of Eq. (4), k_T, k_1 and k_2 were calculated for the D- and L-tryptophan enantiomers. These values were plotted against the sodium perchlorate concentration in the mobile phase, as shown in Figs. 3 and 4. The overall retention factor increases significantly for the D-enantiomer with increasing salt concentration, while k_{TL} increases slightly over the additive concentration range (Fig. 3). The variation of k_{TD} with c can be analyzed by taking into account the interaction with the two kinds of sites. The increase of the overall retention factor is dependent on the pronounced increase in the k_{1D} value since k_{2D} remains roughly constant with increasing c (Fig. 4). It can be seen that this effect is due to two concomitant phenomena: (i) enhancement of the adsorption constant with the type I site and (ii) the increase in the binding capacity for the site of high affinity (Table 2). In the case of the L-enantiomer, the weak k_{TL} variation with increasing c is attributed to the slight increase in k_{2L} with c since

Table 1
Non-linear regression coefficients obtained by fitting theoretical binding curves to the experimental retention data for the D,L-tryptophan enantiomers using Eqs. (2) (Langmuir model: Lang) and (4) (bi-Langmuir model: bi-Lang)

c (mM)	L-Enantiomer				D-Enantiomer			
	R^2		F		R^2		F	
	Lang	bi-Lang	Lang	bi-Lang	Lang	bi-Lang	Lang	bi-Lang
10	0.5247	0.9962	7	353	0.5235	0.9994	6	2370
25	0.5177	0.9975	6	524	0.5680	0.9997	8	4546
37.5	0.6629	0.9991	11	1600	0.4466	0.9998	5	6240
50	0.7537	0.9995	18	2535	0.4761	0.9998	5	5884
75	0.7008	0.9955	14	292	0.4535	0.9999	4	378 804

Table 2
Bi-Langmuir isotherm parameters (Eq. (4)) for D,L-tryptophan enantiomers at a column temperature of 25 °C. Standard deviations are in parentheses

c (mM)	Adsorption constants				Saturation capacities			
	K_1 (mM^{-1})		K_2 (M^{-1})		q_{s1} (mM)		q_{s2} (mM)	
	D	L	D	L	D	L	D	L
10	3.102	2.031	7	2	0.21	0.23	229	171
	(0.012)	(0.203)	(0.2)	(0.2)	(0.01)	(0.02)	(<1)	(21)
25	2.894	1.814	8	2	0.21	0.18	201	225
	(0.041)	(0.177)	(<0.1)	(0.2)	(<0.01)	(0.02)	(3)	(20)
37.5	4.939	2.786	7	10	0.25	0.15	224	147
	(0.276)	(0.139)	(0.6)	(0.2)	(0.01)	(<0.01)	(12)	(7)
50	4.190	2.364	8	11	0.29	0.12	195	138
	(0.188)	(0.274)	(<0.1)	(0.4)	(<0.01)	(0.01)	(9)	(16)
75	5.347	2.366	6	9	0.33	0.14	324	174
	(0.011)	(0.203)	(<0.1)	(0.4)	(<0.01)	(0.01)	(19)	(14)

k_{1L} is roughly constant over the salt concentration range (Fig. 4).

It has been shown previously by NMR and modeling studies that ligands such as *N*-acetyl-D-alanine are specifically bound in a 1:1 stoichiometry to the pocket of the aglycone of macrocyclic antibiotics [25]. This can be explained by the fact that this antibiotic acts on bacteria by binding to cell wall mucopeptide precursors terminating in D-alanine. Recently, using a displacement study, Slama et al. [26] demonstrated that both D- and L-enantiomers of various dansyl amino acids bind to the high-affinity aglycone site of macrocyclic antibiotic such as vancomycin. Therefore, it is strongly expected that the high-affinity site, which binds both D- and L-tryptophan enantiomers, corresponds to the aglycone pocket of immobilized teicoplanin. Moreover, the K_1 values obtained in the present study are of the same order of magnitude as the association constant value reported for *N*-acetyl-D-alanine binding to the macrocycle aglycone pocket, i.e. 1.3 mM^{-1} at 23 °C [25].

The K_{1D} values increase with increasing sodium perchlorate concentration (Table 2), which suggests that an ion-pairing process is involved in the binding

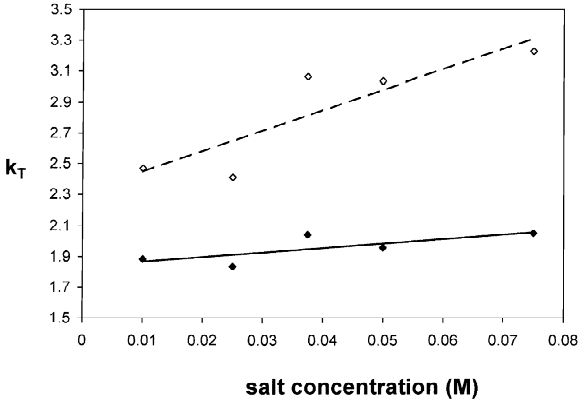

Fig. 3. Plots of k_T versus sodium perchlorate concentration (M) for the D- (◊) and L- (♦) tryptophan enantiomers. Mobile phase, phosphate buffer pH 7.0; stationary phase, immobilized teicoplanin; column temperature, 25 °C.

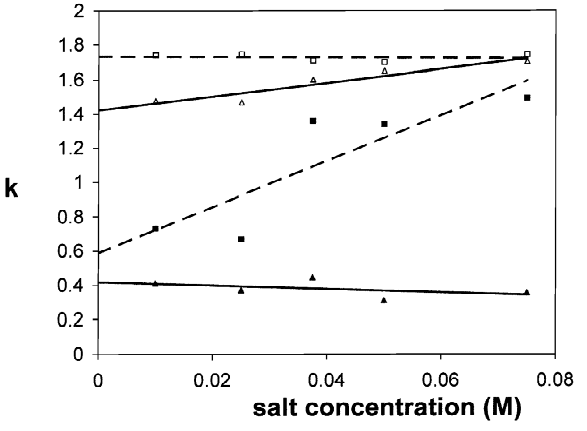

Fig. 4. Plots of k_1 (filled symbols) and k_2 (open symbols) versus sodium perchlorate concentration (M) for the D- (squares) and L- (triangles) tryptophan enantiomers. Mobile phase, phosphate buffer pH 7.0; stationary phase, immobilized teicoplanin; column temperature, 25 °C.

of D-tryptophan to this aglycone site. Such behavior can be analyzed with regard to previous results [21–24]. Despite the neutral nature of zwitterions, Okada and co-workers [21,22] showed that charged groups of zwitterionic species can interact significantly with salt ions, depending on the nature of the salt. This interaction is primarily determined by the nature of the anion. For large and poorly hydrated (hydrophobic) anions, i.e. chaotropic species such as perchlorate, it has been demonstrated that the zwitterion–salt association corresponds to an ion-pairing mechanism; the anion forms an ion-pair with the cationic groups of the zwitterion, while the cation forms an ion-pair with the anionic groups of the zwitterion [21,22]. Both tryptophan and teicoplanin are zwitterionic at the eluent pH of this study. Therefore, three hypotheses can be formulated to explain this salt dependence on solute retention. (i) The charged groups of tryptophan are expected to form ion-pairs with the mobile phase counter-ions; as perchlorate has hydrophobic character, ion-paired tryptophan is assumed to be able to interact more strongly with the uncharged regions of the aglycone pocket. (ii) The mobile phase counter-ions interact with the oppositely charged groups of teicoplanin with the formation of ion-pairs, which are responsible for the increase in the relative hydrophobicity of the selector. (iii) A combination of (i) and (ii) above. In all cases, this behavior agrees well with previous data (from NMR, molecular modeling and chromatographic studies), which have shown that non-ionic interactions such as the hydrophobic effect and hydrogen bonds are fundamental in ligand-specific binding to the aglycon pocket of teicoplanin [5,27]. Furthermore, this result suggests that the ion-pairing phenomenon plays an important role in solute binding to the high-affinity site of macrocyclic antibiotics, not only for negatively charged compounds [9,11], but also for zwitterionic species.

4.2. Enantioselectivity variation with the eluent perchlorate concentration

From the k_1 and k_2 values, apparent and true enantioselectivities were calculated according to Eqs. (5)–(7). α_{app}, α_1 and α_2 were plotted against the sodium sulfate concentration (c) as shown in Fig. 5. Apparent enantioselectivity increased with increasing

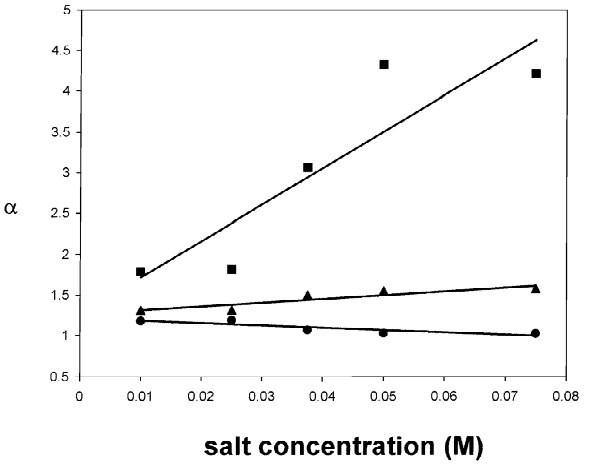

Fig. 5. Plots of α_{app} (▲), α_1 (■) and α_2 (●) versus sodium perchlorate concentration (M). Mobile phase, phosphate buffer pH 7.0; stationary phase, immobilized teicoplanin; column temperature, 25 °C.

salt concentration. It ranged from 1.31 at $c = 10$ mM to 1.57 at $c = 75$ mM. Chromatograms illustrating the effect of sodium perchlorate on the retention and separation of tryptophan enantiomers are shown in Fig. 6.

This behavior was governed by the very strong increase in the α_1 value (from 1.78 to 4.21 over the salt concentration range), which corresponds to the solute enantioselective association with the aglycone pocket. In order to further investigate the mechanistic aspects of this enantioselective behavior with increasing sodium perchlorate concentration, both the K_1 ratio (K_{1D}/K_{1L}) and the q_{s1} ratio (q_{s1D}/q_{s1L}) were determined and plotted against c (Fig. 7). The increase in the α_1 value is governed by an increase in both the K_1 ratio (from 1.53 to 2.26) and the q_{s1} ratio (from 0.92 to 2.40). This indicates that the addition of sodium perchlorate salt to the mobile phase enhances the difference in interaction energies with the aglycone pocket. The number of available type I sites increases with increasing sodium perchlorate concentration for the D-enantiomer, but decreases for the L-enantiomer (Table 2). Although both enantiomers are expected to bind to the same type I site, this difference in binding abilities supports the model in which the D-tryptophan enantiomer is able to interact with additional regions at the binding pocket when the salt concentration increases. A difference in site

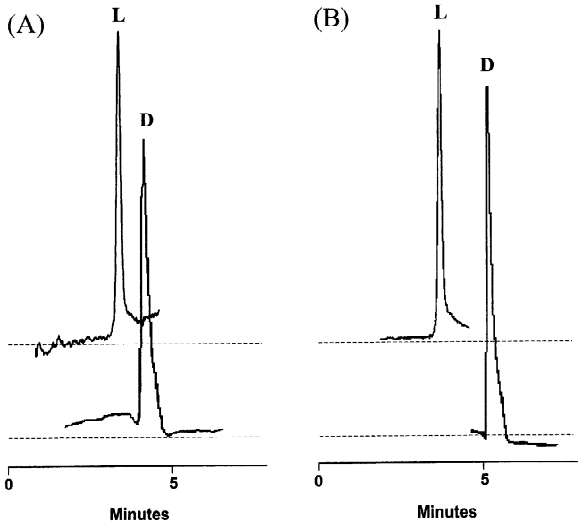

Fig. 6. (A) Chromatograms at a sodium perchlorate concentration of 10 m*M* (retention time of L-enantiomer 3.36 min and D-enantiomer 4.17 min) and (B) at a sodium perchlorate concentration of 75 m*M* (retention time of L-enantiomer 3.65 min and D-enantiomer 5.08 min). $c = 0$ *M*. Mobile phase, phosphate buffer pH 7.0; stationary phase, immobilized teicoplanin; column temperature, 25 °C.

accessibility between the two ion-paired enantiomers could also be involved, as previously expected for the binding of *R*,*S*-warfarin with immobilized human serum albumin [28]. This observation demonstrates that the addition of perchlorate to the mobile phase is

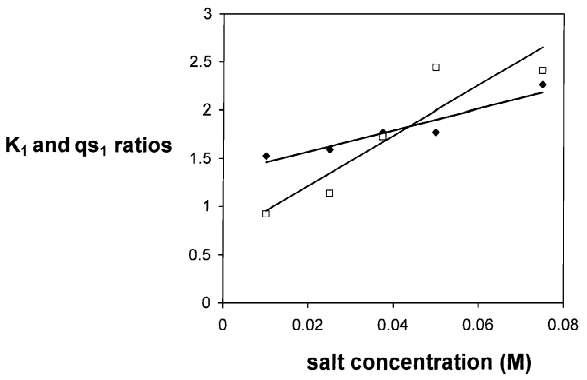

Fig. 7. Plots of K_1 (♦) and q_{s1} (□) ratios versus sodium perchlorate concentration (*M*). Mobile phase, phosphate buffer pH 7.0; stationary phase, immobilized teicoplanin; column temperature, 25 °C.

responsible, at the aglycone binding pocket, for the additional enantioselective phenomenon characterized by both an increase in the energy of the stereoselective interactions and an enhancement in the number of interacting chiral regions.

Another important phenomenon is also involved in the apparent enantioselectivity for the teicoplanin-based stationary phase. As shown in Fig. 5, the interactions of solutes with the type II sites are responsible for an additional chiral discrimination process. At low salt concentrations (10 and 25 m*M*), $\alpha_2 \sim 1.18$. However, it is lower than the α_1 value at the same concentrations (~1.80). Similar results have been reported by Slama et al. [26] for the interactions of dansyl amino acids on immobilized vancomycin. It was shown that secondary sites (excluding the active aglycone pocket contribution) are involved in chiral recognition with an enantioselectivity value varying from 1.08 to 1.14 in relation to the compound type (for a high-affinity site, the enantioselectivity varies from 1.28 to 1.47) [26]. This behavior can be explained by the fact that this type of macrocyclic antibiotic contains several accessible chiral interaction sites. Moreover, Berthod et al. [29] and Jandera et al. [17] previously demonstrated that various enantiomers can interact with some chiral environments of macrocyclic antibiotics in addition to the high-affinity aglycone site. It should be noted that these enantioselective interactions decrease when the sodium perchlorate concentration of the mobile phase increases (Fig. 5). They become nearly insignificant at a concentration of 50 and 75 m*M* ($\alpha_2 \sim 1.03$). However, a more acute analysis of this phenomenon, as presented above for the case of the type I site, would not be sufficiently accurate. The bi-Langmuir model used for the fitting procedure is based on the assumption of a two-site surface. This model is oversimplified in the case of the interaction of tryptophan with the teicoplanin-based stationary phase. As explained above, it is strongly expected that several chiral sites are involved in the secondary enantiorecognition process. Therefore, this selector heterogeneous surface would probably be better described by other models such as tri-Langmuir adsorption isotherms. However, in such a case, the number of equation coefficients would be too large and would not lead to significantly accurate estimates.

5. Conclusion

In the present work, it has been demonstrated that the increase in the apparent D,L-tryptophan enantioselectivity with increasing sodium perchlorate concentration is governed by: (i) an increase in the difference of the adsorption constants for binding to the high-affinity site (the aglycone pocket) between the two enantiomers and (ii) enhancement of the number of site I regions interacting with D-tryptophan. This work also shows that additional secondary sites at the selector surface are involved in the apparent enantioselectivity, but at lower sodium perchlorate concentrations. Finally, these results suggest that the ion-pairing mechanism plays a role in retention and chiral recognition on macrocyclic antibiotic-based stationary phases, not only for negatively charged solutes, but also for zwitterionic species.

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