Induced Conformational States of Amphipathic Peptides in Aqueous/Lipid Environments

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ABSTRACT Specific conformational effects have been reported for amphipathic model peptides upon binding of defined hydrophobic domains to nonpolar stationary phases during reversed-phase high performance liquid chromatography (RP-HPLC). Such induced conformations are found to be especially pronounced for peptides that are amphipathic in an α-helical conformation. Such induced amphipathic conformations resulted in substantially later elution than predicted using amino acid-based retention coefficients. In the present study, the induced conformational behavior of model peptides observed during RP-HPLC was correlated with their secondary structure as determined by circular dichroism (CD) spectroscopy in both aqueous solution and C_{18}-mimetic environments. The experimental retention times of the peptides studied were found to correlate with their CD spectra in the presence of lipids, whereas a poor correlation was observed with their CD spectra in the presence of trifluoroethanol. A new approach was developed to evaluate the induction of secondary structure in peptides due to interactions at aqueous/lipid interfaces, which involves the measurement of the CD ellipticities of peptides bound to a set of C_{18}-coated quartz plates. An excellent correlation was found in this environment between the RP-HPLC retention times and CD ellipticities of the bound peptides.

INTRODUCTION

Amphipathic α-helices represent the most commonly found secondary structure in proteins and are known to be intimately involved in protein folding (Dyson and Wright, 1993). Amphipathic helices are, in turn, used in the *de novo* design of small proteins (Stewart, 1993). By their nature, amphipathic α-helices interact with lipid surfaces and have been found to play an important role in the binding of peptides to biological receptors (Kaiser and Kézdy, 1987). During the binding of a peptide at an aqueous/lipid interface, conformational changes are induced to yield a peptide/lipid complex having the lowest possible energy state.

Reversed-phase high performance liquid chromatography (RP-HPLC) has recently emerged as a straightforward tool for the study of the secondary structure of peptides (Hearn and Aguilar, 1987; Houghten, 1987; Houghten and Ostresh, 1987; Regnier, 1987; Zhou et al., 1990; Ostresh et al., 1991; Blondelle et al., 1992; Büttner et al., 1992a, b). During RP-HPLC of model amphipathic peptides, variations in retention behavior strongly indicate the occurrence of specific conformational effects (Zhou et al., 1990; Blondelle et al., 1992; Büttner et al., 1992a). Because the basis of RP-HPLC retention is hydrophobic interactions between peptides and the nonpolar stationary phase (typically C_{18}-derivatized to silica), the induction of peptides into specific conformations is thought to be caused by the binding of hydrophobic domains of the preferred low energy state to the stationary phase. The effect of variations in mobile and stationary phases on the secondary and tertiary structures of peptides and proteins using RP-HPLC has been investigated by different groups (Meek and Rossetti, 1981; Wilce et al., 1991). More recently, model peptide analogs having identical amino acid compositions but different primary sequences have been used to show that one can use elution behavior to predict the presence of induced α-helical structures (Ostresh et al., 1991). In earlier studies, we found that the biological activities of closely related amphipathic α-helical peptides correlate well with their retention times during RP-HPLC (Blondelle and Houghten, 1992; Blondelle et al., 1993). These results prompted the investigation of the extent to which a stationary phase used during RP-HPLC can be related to such biological receptors and, in turn, to what extent RP-HPLC can be used as a technique in the *de novo* design of new, biologically active peptides.

It can be anticipated that the folding of a given peptide from an initial random coil in solution to a low energy, highly ordered conformation would be dependent on the environment (Zhong and Johnson, 1992). One approach that correlates the conformation as determined by circular dichroism (CD) spectroscopy to biological processes involves the use of lipid bilayers (McLean et al., 1991; Pérez-Payá et al., 1994). Although such analyses are carried out in solution, artificial lipid bilayers provide a hydrophilic/hydrophobic interface that mimics those present in cell membranes. In the present work, the CD spectra of a number of model amphipathic peptides in aqueous solution in both the presence and absence of lipids were compared with their deviation from predicted retention times. These comparative studies were initiated to investigate our earlier findings that highly

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Abbreviations used: RP-HPLC, reversed-phase high performance liquid chromatography; CD, circular dichroism; TFE, trifluoroethanol; t-Boc, tertiary-Butyloxycarbonyl; TFA, trifluoroacetic acid; ACN, acetonitrile; lpc, lysophosphatidylcholine; SDS, sodium dodecyl sulfate; MOPS, 3-[*N*-Morpholino]propane-sulfonic acid; RT, retention time.

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ordered, specific conformations are induced during the RP-HPLC process, and also that the retention data obtained during RP-HPLC is a straightforward and rapid means for the prediction of such induced biologically active conformations. Three sets of model amphipathic peptides were used in these comparative studies: two sets of peptides composed solely of lysine and leucine residues, either varying in length or representing different structural motifs, and a set of tryptophan substitution analogs of melittin.

Variation in the molecular organization of the hydrophobic part of the lipids is thought to cause changes in the induced conformation of a peptide (Hanson et al., 1993). It should be noted that the molecular organization of the lipids in aqueous solution (i.e., of micelles and liposomes) differs from that of the RP-HPLC stationary phase. To analyze more directly the conformation of peptides induced during RP-HPLC, an approach that utilizes CD spectroscopy has been developed. This approach involves the binding of peptides on C_{18}-coated quartz plates and enables the measurement of the CD spectra of the peptides of interest in RP-HPLC solvents while they are bound to C_{18} groups. Although related methods have been reported by other groups for the study of induced conformations at air/lipid interfaces (Taylor, 1990) or for the study of the potential denaturation of α-helices upon absorption to hydrophobic surfaces (Smith and Clark, 1992), the present work evaluates the conformation of peptides induced at aqueous/lipid interfaces in a manner closely analogous to that which occur during RP-HPLC.

MATERIALS AND METHODS

Peptide synthesis

Peptides were prepared by simultaneous multiple peptide synthesis methodology using t-Boc chemistry as described previously (Houghten, 1985). Final cleavage and deprotection were carried out using a "low-high" hydrogen fluoride procedure (Houghten et al., 1986).

RP-HPLC analysis and purification

Preparative purification of the peptides was performed on a Waters Delta-Pak C_{18}-300 Å column using a Deltaprep 3000 HPLC combined with a Foxy fraction collector (Millipore, Waters Division, San Francisco, CA). Laser desorption time-of-flight mass spectroscopy (Kratos Kompact Maldi-Tof mass spectrometer, Ramsey, NJ) and analytical RP-HPLC were used to determined the identity and purity of the fractions. Relative retention times were determined using a Beckman System Gold gradient HPLC (Beckman Instruments, Fullerton, CA). Samples (20 μl, 0.5 mg/ml in H_2O) were analyzed on a Vydac 218TP54 C_{18}-column (Alltech Associates, Los Altos, CA) (4.6 mm ID $\times$ 250 mm, 5μ). Peptide elution was monitored at 215 nm. Solvent A consisted of 0.05% trifluoroacetic acid (TFA) in water, and solvent B consisted of 0.05% TFA in acetonitrile (ACN). The peptides were analyzed using a 1% per min increasing gradient. Variations in retention times of $\pm$0.05 min were observed between multiple analyses.

Preparation of C_{18}-coated plates

Quartz plates were purchased from Hellma Cells (Jamaica, NY) and modified with C_{18}-alkyl chains as described by Brock and Enser (1987). In brief, each plate was washed with concentrated sulfuric acid, rinsed with water and acetone, and dried under vacuum. The plates were then treated with 1 M octadecyltrichlorosilane (Aldrich, Milwaukee, WI) in anhydrous heptane for 24 h at room temperature in an evacuated desiccator over potassium hydroxide. After incubation at 60°C with octadecan-1-ol for 24 h to eliminate unreacted chloro groups, the plates were washed with anhydrous heptane at 60°C. The heptane was removed by repeated washes and sonication with methanol, and the plates dried under vacuum.

CD measurements

All measurements were carried out on a Jasco J-720 CD spectropolarimeter (Eaton, MD) at 25°C, using a Neslab RTE 110 waterbath and temperature controller (Dublin, CA). Lysophosphatidylcholine (lpc) and sodium dodecyl sulfate (SDS) were purchased from Sigma Chemical Co. (St Louis, MO). The instrument was routinely calibrated with an aqueous solution of *d*-10-camphorsulfonic acid. CD spectra were acquired at a scan speed of 20 nm/min, and results were obtained by averaging 3–6 scans. The concentrations of the peptides containing solely lysine and leucine residues were determined by quantitative amino acid analyses (Biotechnology Resource Laboratory, New Haven, CT). For the melittin analogs containing one or two tryptophans, the final concentrations were determined by UV spectrophotometry at 280 nm (extinction coefficients: ϵ_{280} = 5570 M^{-1} cm^{-1} (Quay and Condie, 1983) and ϵ_{280} = 11140 M^{-1} cm^{-1} (Pérez-Payá et al., 1994), respectively) using a Hewlett Packard 8452A diode array UV spectrophotometer (Palo Alto, CA). The mean residue ellipticities ([Θ] – deg cm^2 $dmol^{-1}$) were calculated using the equation $[\Theta] = 100\Theta/cnl$, where Θ is the ellipticity (mdeg), c is the peptide concentration (mM), n is the number of residues, and l is the path length (cm).

The CD spectra of the peptides while bound to the C_{18}-coated plates were measured as follows: each peptide was bound to a set of nine plates by soaking the plates for 1 h in the cell at room temperature in a solution of peptide (20 μM) in 5 mM MOPS buffer or H_2O/5% ACN/0.05% TFA, followed by washing with water to remove excess peptide solution. The CD spectra of this set of peptide-bound plates were measured while submerged in H_2O/5% ACN/0.05% TFA (CD spectra were the average of 10 scans). A blank spectrum obtained in the same way for the set of plates before the binding of peptides was subtracted. After CD measurement, the bound peptides were removed from the plates by sonication in 100% ACN/0.05% TFA for 5 min.

RESULTS AND DISCUSSION

Design of amphipathic α-helical model peptides

In earlier studies (Blondelle et al., 1992; Büttner et al., 1992a; Ostresh et al., 1991), model peptides having the same amino acid compositions (comprised solely of leucine and lysine residues) but different primary sequences representing several structural motifs were found to elute over a range of 20 min during RP-HPLC. In particular, the peptides that were designed to be induced into an amphipathic α-helical conformation had the longest retention times (19 min later than predicted using standard retention coefficients (Houghten, 1987; Ostresh et al., 1991)), whereas those peptides designed to adopt a β-structure eluted the earliest (3 min earlier than predicted). Specific conformations were considered to be induced upon the interaction of a preferred hydrophobic domain of the peptides with the stationary phase of the RP-HPLC. To investigate further the effect of this interactive hydrophobic "footprint," another set of model peptides composed solely of leucine and lysine residues was designed that represented the amphipathic α-helical motif and varied in length from 14 to 21 residues. All had periodic distributions of hydrophobic and hydrophilic residues with periods of 3.6

residues (Table 1). All of the peptides eluted, as anticipated from our earlier studies, 14–22 min later than predicted using standard retention coefficients (Table 1). These results suggest that all of the peptides are induced into an amphipathic α-helix during the RP-HPLC process. It should be noted that the retention coefficients are based solely on the hydrophobicity of each residue (Meek and Rossetti, 1981; Sasagawa et al., 1982; Houghten, 1987; Ostresh et al., 1991; Büttner et al., 1992b). Thus, to be able to compare the retention times of peptides having different compositions, the difference between experimental and predicted retention times (ΔRT) was used in these comparative studies. The variation in ΔRT between closely related peptides is therefore directly related to the conformational effects induced at aqueous/C_{18} group interfaces.

Although the 19-, 20-, and 21-residue peptides have the same number of leucine residues and, therefore, the same induced interactive hydrophobic "footprint," their ΔRT varied by as much as 3 min (Table 1). These differences in ΔRT are either due to the number of lysine residues or to the relative position of the interactive hydrophobic "footprint" to the N- and C-terminal functional groups from one sequence to the other. However, the effect of the number of lysine residues can be precluded because such an effect is considered in the calculation of the predicted retention times and, therefore, eliminated when only considering ΔRT. The relative position of the footprint is expected to affect the peptides' binding affinity and, therefore, their retention behavior. Similar effects are seen for the 16- and 17-residue peptides, both of which contain eight leucine residues.

CD spectra in solution

The RP-HPLC process can be divided into two components: a mobile phase composed of an organic solvent/water mixture, and a stationary phase that can generally be considered a lipid surface. The solution conformation of each model peptide was therefore characterized by CD spectroscopy under various conditions expected to mimic either of the two components. The CD spectra measured under the typical conditions used during RP-HPLC injection (i.e., H_2O/5% ACN/0.05% TFA—20 μM in peptide) indicated that a random coil conformation existed for all of the peptides being studied (data not shown).

A decrease in the polarity of aqueous medium caused by the increase of the organic component of the mobile phase during the elution process of RP-HPLC is known to increase the α-helical content of peptides (Brooks and Nilsson, 1993). As shown in Fig. 1, all of the peptides adopted the expected α-helical conformation in 60% TFE (mean residue ellipticities at 222 nm ranging from -27670 to -32290 deg cm^2 dmol^{-1}, representing an approximately >90% α-helix (Yang et al., 1986)). Similar results were found in 80% ACN, the highest ACN concentration used in our RP-HPLC studies (Fig. 1). Although TFE and ACN have been used to mimic the hydrophobicity of the reversed-phase column (Zhou et al., 1990; Krause et al., 1994), no clear correlation can be seen between the mean residue ellipticities in either TFE or ACN and the ΔRT of the peptides studied here (Fig. 1).

The conformational behavior of this set of model peptides was next studied in a stationary phase mimetic environment using lpc micelles. The lpc micelles can be compared with the stationary phase of the RP-HPLC because of their ordered structure and the aliphatic nature of their chains. As shown in Fig. 1, the peptides were also induced into an α-helical conformation under these conditions with a better correlation between the mean residue ellipticities and the retention times ($r = 0.737$) than in the presence of the organic solvents. Titration studies (Pérez-Payá et al., 1994) of each peptide with various concentrations of lpc micelles showed that the variation in mean residue ellipticities observed between each peptide can be related to its binding affinities to the micelles (data not shown). Under the experimental conditions used here, the mean residue ellipticities plotted in Fig. 1 correspond to the maximum ellipticities obtained from these titration studies. These results support our premise that the retention times of peptides during RP-HPLC are driven by the binding affinity of peptides to lipid surfaces, resulting, in turn, in specific induced conformations. Because an α-helical conformation was also observed for these peptides in high concentrations of ACN, one can envision a partition equilibrium process described in terms of $(\text{conformation})_a \rightleftharpoons (\text{conformation})_b$, where a and b represent the lipid and organic solvent-induced conformation. The partitioning occurs

TABLE 1 Variations in experimental versus predicted retention times upon increasing the length of model amphipathic α-helical peptides

Peptide sequence	Length	*RT* found*	RT predicted‡	ΔRT§
Ac-KKLLKKLKKLLKKL-NH_2	14	21.92	7.97	13.95
Ac-LKKLLKKLKKLLKKL-NH_2	15	26.98	11.00	15.98
Ac-LLKKLLKKLKKLLKKL-NH_2	16	32.01	14.03	17.98
Ac-KLLKKLLKKLKKLLKKL-NH_2	17	29.82	13.11	16.71
Ac-LKLLKKLLKKLKKLLKKL-NH_2	18	35.11	16.14	18.97
Ac-LLKLLKKLLKKLKKLLKKL-NH_2	19	40.72	19.17	21.55
Ac-KLLKLLKKLLKKLKKLLKKL-NH_2	20	36.85	18.26	18.59
Ac-KKLLKLLKKLLKKLKKLLKKL-NH_2	21	36.40	17.34	19.06

*The retention times were determined using a 1% gradient starting at 20% B.

‡The predicted retention times were calculated using a set of retention coefficients derived from the retention times of over 1,000 different peptides (Houghten, 1987; Ostresh et al., 1991).

§ΔRT = *RT* found − RT predicted.

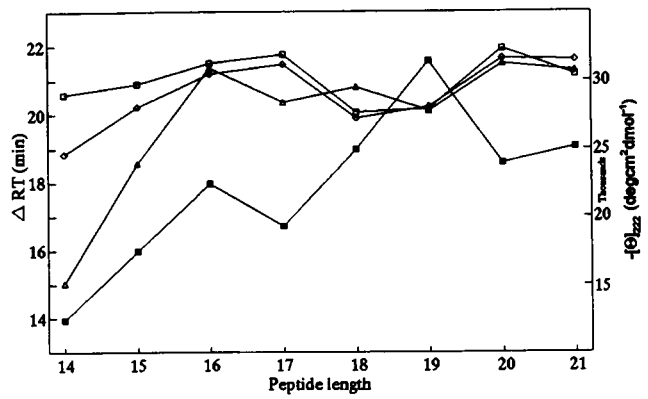

FIGURE 1 Relationships between ΔRT and CD ellipticities of model amphipathic peptides in solution. The ΔRT (■) and mean residue ellipticities at 222 nm (*open symbols*) are plotted as a function of the peptide length (see Table 1). The ΔRT were determined as described in Table 1. The CD spectra of each peptide at 20 μM were carried out in 60% TFE (□), 80% ACN (◇), or in the presence of 2 mM lpc micelles (△).

when the energy states of the two conformations are approximately equal. Elution of the peptide is expected to occur when the mobile phase conformation is in a lower energy state than the stationary phase conformation.

CD spectra of model amphipathic peptides at aqueous/lipid interfaces

Although the various conditions examined above can be considered RP-HPLC mimetic environments, they are dissimilar to RP-HPLC in that they are all studied in solution. Therefore, these environments cannot be absolutely correlated to the phenomena that occurred during the RP-HPLC process involving aqueous/restrained lipid interfaces. Furthermore, the adsorption and induced conformational preference of peptides at such interfaces have important consequences in the peptides' biological behavior. Multi-plate CD cells were designed that could be modified with C_{18}-alkyl groups for peptide adsorption. Fig. 2 illustrates the cell used in these studies. The cell is a 2 cm long quartz cell in which up to nine quartz plates can be inserted. Each plate was modified with

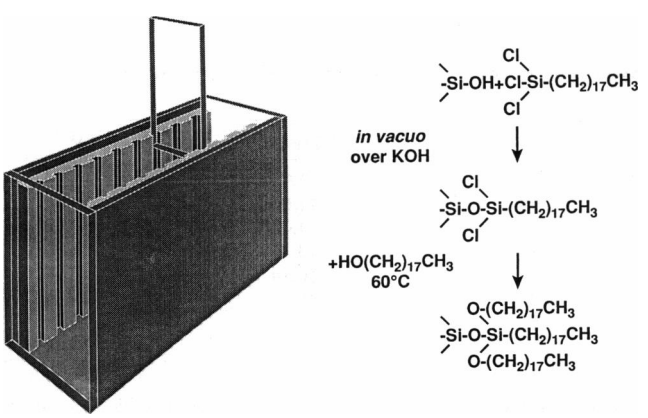

FIGURE 2 Multi-plate CD cell and coating reaction.

C_{18}-alkyl groups using standard protocols (Fig. 2 (Brock and Enser, 1987)) without further modification. To mimic an aqueous/lipid interface, and in turn to correlate CD spectra with the RP-HPLC process, the CD spectra were recorded while the plates in the cell were submerged in water. Under these conditions, in contrast to the rigidity of peptides at air/lipid interfaces, the bound peptides are not constrained in a specific plane (Hanson et al., 1993). As mentioned above, all of the peptides when dissolved in aqueous solution were found to exhibit a random conformational state under the conditions used for binding to the plates (i.e., at 20 μM in H_2O/5% ACN/0.05% TFA). Fig. 3 illustrates the CD spectra of two of the model peptides while bound to the plates: the 16-mer and 19-mer peptides (spectra A). Both peptides were found to adopt an α-helical conformation in the bound state. As expected from the titration studies with the lpc micelles described above, the binding affinity of these peptides to the octadecyl groups on the plates was sufficient to measure their CD spectra while bound to the plates. No modification of the standard coating protocol, therefore, was necessary to study the conformation of these peptides at aqueous/lipid interfaces. As in the case during the RP-HPLC process, the peptides were removed from the plates by washes and sonication with ACN containing 0.05% TFA. The CD spectra of the plates after removal of the peptides are shown in Fig. 3 (spectra B).

All other peptides studied in this set were also found to adopt an α-helical conformation upon binding to the coated plates. As shown in Fig. 4, an excellent correlation (r = 0.975) was found between the CD intensities of each peptide at 222 nm and their ΔRT. These results show that the system of multiple C_{18}-bound plates is a useful means to study induced conformation at aqueous/lipid interfaces and also to

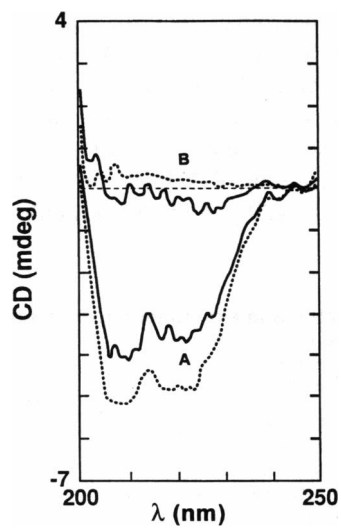

FIGURE 3 CD spectra of model amphipathic peptides bound to C_{18}-coated plates. (*A*) The CD spectra of the 16-mer (——) and 19-mer (- - - -) were recorded in water when the peptides are bound to the plates. The peptide sequences are shown in Table 1. (*B*) The CD spectra of the plates were recorded after ACN washes of the 16-mer bound plates (——) and of the 19-mer bound plates (- - - -).

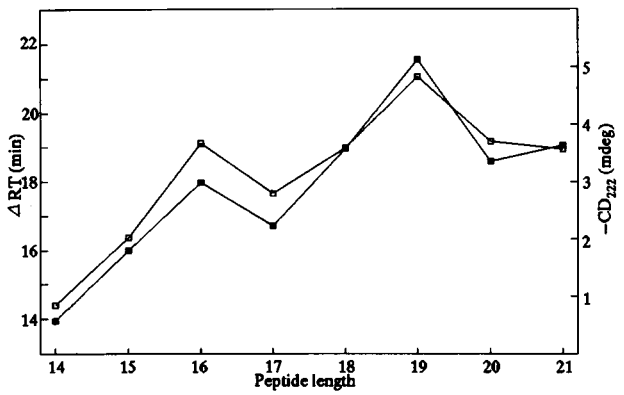

FIGURE 4 Relationships between ΔRT and CD intensities of model amphipathic peptides when bound to C_{18}-coated plates. The ΔRT (■) and CD intensities at 222 nm (□) are plotted as a function of the peptide length. The ΔRT were determined as described in Table 1. The CD spectra of each peptide when bound to the plates were measured as described in Materials and Methods.

evaluate the elution behavior of peptides during RP-HPLC. Furthermore, these results confirm that specific conformations are induced during the RP-HPLC process that affect the retention times of peptides.

CD spectra of model peptides representing different structural motifs

As shown above, although those peptides that were designed to be inducible into an amphipathic α-helical conformation eluted later than predicted during RP-HPLC, those peptides that were designed to adopt a β-structure eluted earlier than predicted (Ostresh et al., 1991). To confirm that the early elution times of these peptides were due to their induced conformations, similar CD studies in solution were carried out using three model peptides composed solely of leucine and lysine residues. These three peptides were designed to represent three different structural motifs to compare their induced conformations under various environments expected to mimic the conditions found during the RP-HPLC process. Their primary sequences are shown in Table 2, as are their RP-HPLC retention times. Thus, the first peptide was designed to have a periodicity of two residues to be able to adopt an amphipathic β-sheet conformation (referred to as $[LK]^{2.0}$). This peptide was compared with two peptides that were more likely to be induced into α-helices: a peptide with a sequence that has an amphipathicity perpendicular to the induced helical axis (K_9L_9), and a peptide with a periodicity of 3.6 residues in a manner similar to the sequences described previously (18 residues in length, referred to here as $[LK]^{3.6}$).

The CD spectra of the three peptides were initially measured both in mobile phase mimetic environments (TFE and ACN) and in the presence of lpc micelles, all in a manner similar to that described above for the previous series of amphipathic α-helical model peptides. The three peptides were found to be in a random conformational state under the solvent conditions used for RP-HPLC injection (Fig. 5 *A*). All, however, adopted an α-helical conformation with similar mean residue ellipticities in high concentrations of the two organic solvents studied here (Fig. 5, *B* and *C*). The similarity in the CD spectra indicate that the variation in retention times found for these three peptides during RP-HPLC was not due to a different conformation induced by the elution concentration of the mobile phase of the RP-HPLC. In contrast, in the presence of lpc micelles, which were used to mimic the stationary phase of the RP-HPLC, different CD spectra were observed for the three peptides (Fig. 5 D). Thus, the $[LK]^{2.0}$ peptide appeared to be primarily random coil under these conditions, whereas the K_9L_9 and $[LK]^{3.6}$ peptides were partially and fully induced, respectively, into an α-helix with different mean residue ellipticities. These results indicate that K_9L_9 has a lower binding affinity to the lpc micelles than $[LK]^{3.6}$. These results also agree with the differences in retention times found for these two peptides (Table 2). Surprisingly, no β-structure was observed for the $[LK]^{2.0}$ peptide under these conditions. This may be due to the low binding affinity of this peptide to the lpc micelles as determined by titration studies (data not shown). We believe that this prevents an ordered conformation from being induced. This also correlates with the early retention time found for this peptide during RP-HPLC (Table 2).

SDS was then used as a lipid model system for CD studies of the $[LK]^{2.0}$ peptide. In addition to aliphatic side chains, SDS has a negatively charged character. SDS should therefore provide a hydrophobic environment and is expected to enhance the binding affinity of $[LK]^{2.0}$ to the aliphatic chains by providing initial electrostatic interactions. Such interactions should allow the peptides to be in closer contact to the lipid chains, and in turn promote induction into an ordered conformation. As shown in Fig. 6, although the two peptides K_9L_9 and $[LK]^{3.6}$ were again induced into an α-helix (Fig. 6, *B* and *C*), the $[LK]^{2.0}$ peptide was induced into the expected β-sheet conformation in the presence of 1.64 mM SDS, with a plateau reached at 2.47 mM SDS (Fig. 6 *A*). This latter SDS concentration also corresponded to the α-helical plateau observed for the two other peptides. It should be noted that

TABLE 2 Retention times of model peptides representing three different structural motifs

Peptide sequence	Motif	Name	*RT* found*	*RT* predicted‡	ΔRT§
Ac-KLKLKLKLKLKLKLKLKL-NH_2	β	$[LK]^{2.0}$	13.16	16.14	2.98
Ac-KKKKKKKKLLLLLLLLL-NH_2	α	K_9L_9	28.65	16.14	12.51
Ac-LKLLKKLLKKLKKLLKKL-NH_2	α	$[LK]^{3.6}$	35.11	16.14	18.97

*The retention times were determined using a 1% gradient starting at 20% B.

‡ The predicted retention times were calculated as described in Table 1.

§ ΔRT = *RT* found − RT predicted.

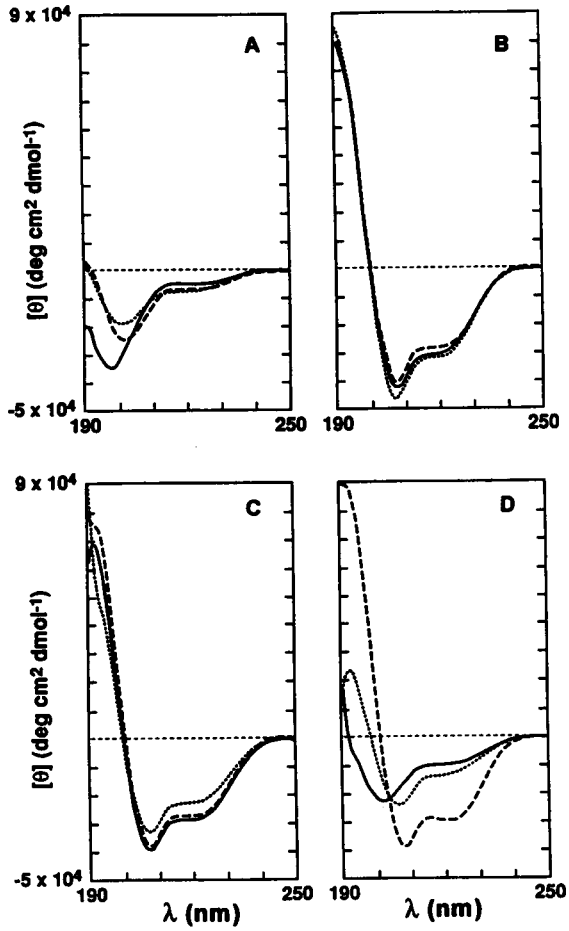

FIGURE 5 CD spectra of model peptides representing different structural motifs. The CD spectra were recorded (*A*) in H_2O/5% ACN/0.05% TFA, (*B*) in 60% TFE, (*C*) in 80% ACN, and (*D*) in the presence of 2 mM lpc micelles. The CD spectra are represented for the three peptides $[LK]^{2.0}$ (——), K_9L_9 (· · · · ·), and $[LK]^{3.6}$ (- - - - -).

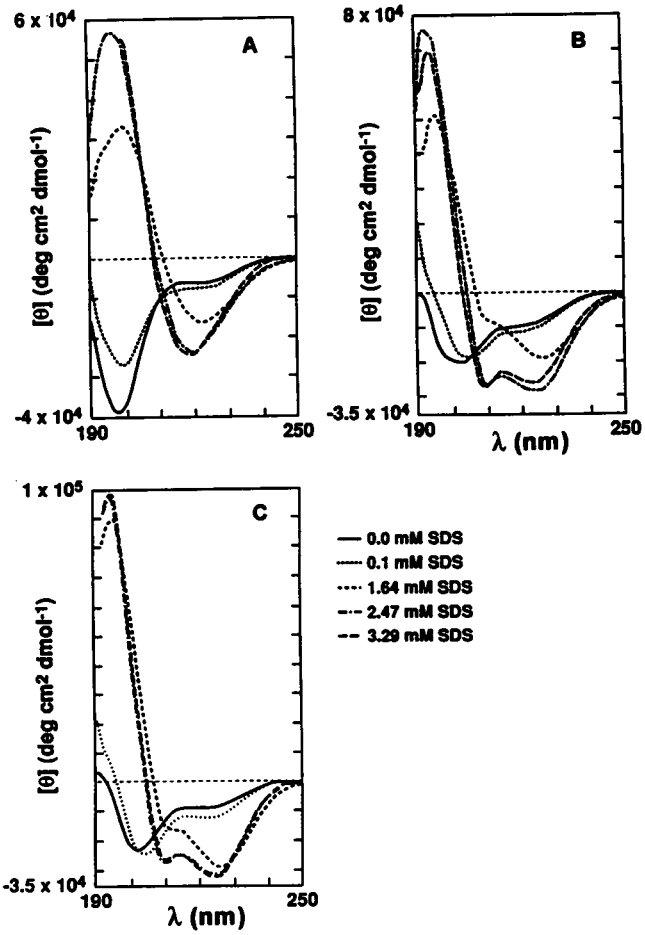

FIGURE 6 CD spectra of model peptides in the presence of SDS. The CD spectra were recorded for (*A*) $[LK]^{2.0}$, (*B*) K_9L_9, and (*C*) $[LK]^{3.6}$ in 5 mM MOPS buffer and in the presence of SDS varying from 0.1 to 3.29 mM.

under the experimental conditions used (SDS concentration <3.5 mM), SDS is monomeric and does not form micelles, in contrast to the previously used lpc micelles (Wu et al., 1981; Wu and Yang, 1981). β-sheets are generally thought to form upon peptide aggregation due to the formation of hydrogen bonds that stabilize such a conformation. In this case, the induction into a β-sheet structure observed in the presence of SDS is more likely due to hydrophobic interactions between monomeric SDS and a single peptide molecule. In agreement with our results, Zhong and Johnson (1992) have found that a protein fragment was induced into an α-helical conformation in TFE, whereas the β-structure present in the native protein was found when using similar concentrations in SDS. It was concluded that the hydrophobic tail of the SDS interacts with the protein fragment and mimics the environment found in the hydrophobic core of the native protein.

As expected from these results, the binding of $[LK]^{2.0}$ to the C_{18}-coated plates was too low to be able to detect a CD signal using the standard protocol described above. In an attempt to increase the binding of $[LK]^{2.0}$, the initial peptide concentration of the solution used for the binding of peptide to the plates was increased to 4.3 mM (vs. 20 μM), the highest concentration in which this peptide is still random in solution, and the peptide was allowed to bind to the plates overnight at room temperature (vs. 1 h). Under these conditions, the shape of the CD spectra appeared to be similar to the shape of a β-sheet structure with a minimum around 215 nm, although the signal observed was low in intensity (data not shown). No minimum was found at 222 nm, which tends to preclude the possibility of its induction into an α-helical conformation. These preliminary results support the expectation that SDS is a useful lipid system to study the RP-HPLC process of peptides with low binding affinity to lipid surfaces. To determine the induced conformational state of peptides having early retention times and/or low binding affinity at aqueous/lipid interfaces, studies are ongoing to determine the best conditions for these peptides binding to the C_{18}-coated plates.

Although $[LK]^{2.0}$ was found to adopt the predicted β-structure in the presence of SDS, and upon binding to the C_{18}-coated plates, it was found to adopt an α-helical conformation in solution in the presence of organic solvents such TFE and ACN. Because the RP-HPLC process involves in-

teraction of the peptide with both the lipid surfaces and organic solvent, these contrasting results prompted the study of the conformation of the $[LK]^{2.0}$ peptide in the presence of both environments by CD spectroscopy. Such studies were expected to provide insight into conformational changes during the elution process of peptides during RP-HPLC.

$[LK]^{2.0}$ was induced into a β-sheet by the presence of 2.47 mM SDS and conformational changes were followed by increasing concentrations of ACN. Although no change appeared upon the addition of up to 40% ACN, the peptide was found to be partially α-helical in 45% ACN, and fully α-helical in 50% ACN (Fig. 7). Although the shapes of the CD spectra were clearly defined, the ellipticity values were slightly decreased because of a small degree of turbidity that occurred upon increasing the ACN concentration. Although this peptide eluted with 30% ACN during RP-HPLC, it is known that the actual ACN concentration in contact with the C_{18}-chains is higher than the nominal ACN concentration in the mobile phase (Hearn and Aguilar, 1987). These results suggest that a continuum of conformations is induced during the elution process.

CD spectra of melittin's tryptophan substitution analogs at aqueous/lipid interfaces

Although the model peptides studied above were designed to adopt a single specific conformation in a given environment, naturally occurring peptides or proteins have commonly been found to contain several domains, each adopting a specific conformation. Melittin is a useful naturally occurring model peptide the structure that contains two α-helices joined by a hinged region (GIGAVLKVLTTGLPALISWIKRKRQQ-NH_2 (Terwilliger and Eisenberg, 1982; Terwilliger et al., 1982)). In earlier studies, the RP-HPLC retention times of several sets of melittin analogs were found to correlate with their lytic activity (Blondelle et al., 1993; Pérez-Payá et al., 1994). Comparative studies between the RP-HPLC behavior and CD spectra of the melittin's analogs were used in a manner similar to that described above for the model lysine/leucine amphipathic peptides. These studies provided further insight into the potential use of RP-HPLC for the evaluation of more complex induced conformations at aqueous/lipid interfaces, and their relation to biological activity. In particular, the hemolytic activities of selected tryptophan substitution analogs of melittin were found to correlate well with both their ΔRT and their variation in mean residue ellipticities at 222 nm in the presence of dihexadecylphosphatidylcholine liposomes (Blondelle et al., 1993) or lpc micelles (Pérez-Payá et al., 1994). Thus, melittin and 11 tryptophan substitution analogs were chosen from the complete set of single substitution analogs for the present study. The peptides selected represent variations in each domain of melittin's structure, i.e., the first α-helix (residues 1–9), the hinge (glycine-12), the second α-helix (proline-14), and the basic C-terminus (lysine-21). Each peptide exhibited high affinity when bound to the C_{18}-coated plates. The CD spectra of these peptides indicated that each one adopted an overall α-helical conformation upon binding to the plates. The CD intensities of these peptides at 222 nm followed a pattern similar to their ΔRT as shown in Fig. 8. Although the correlation between ΔRT and CD intensities was not as clear as for the model peptides described above, it does indicate that RP-HPLC can be useful for studying the induced conformations of more complex peptides at aqueous/lipid interfaces. From the results presented, the use of C_{18}-coated plates also appears to be a useful analytical tool for the study of induced conformational states of peptides at aqueous/lipid interfaces.

CONCLUSION

The work presented here focuses on the use of CD to study the binding and conformational states of peptides during the

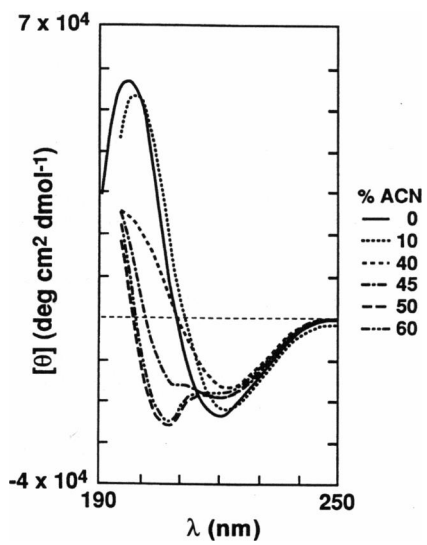

FIGURE 7 Effect of acetonitrile on the CD spectra of $[LK]^{2.0}$ in SDS. The CD spectra were recorded in the presence of 2.47 mM SDS, with ACN varying from 10 to 60%.

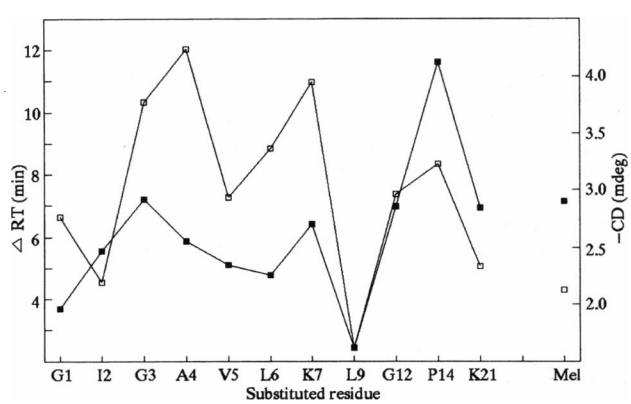

FIGURE 8 Relationships between ΔRT and CD intensities of melittin's tryptophan substitution analogs bound to C_{18}-coated plates. The ΔRT (■) and CD intensities at 222 nm (□) are plotted as a function of the residue being substituted by a tryptophan. The ΔRT were determined using a 1% gradient starting at 5% B. The CD spectra of each peptide when bound to the plates were measured as described in Materials and Methods.

RP-HPLC process. These studies confirm earlier premises that RP-HPLC can be used to study the induced conformations of peptides at aqueous/lipid interfaces. Furthermore, these studies indicate that RP-HPLC elution behavior can be correlated to membrane-mediated biological activity. In these simple model peptide studies, we were able to show that both RP-HPLC mobile and stationary phases induce specific conformations in peptides that affect their elution behavior. However, the binding affinity to the hydrophobic stationary phase lipid surface was found to be the predominant driving force in the preliminary induction of conformation and, thus, retention behavior. The development of a system using CD spectroscopy to study the conformational state of lipid immobilized peptides highly supports the proposition that specific conformations are induced during the RP-HPLC elution process and that the nature, as well as the extent, of these induced conformations is closely related to the retention times of the model peptides.

The relationship found between the retention times of more complex peptides, such as the melittin analogs studied here, with their propensity to adopt an α-helical conformation when bound to the C_{18}-coated CD cells, indicates that not only can RP-HPLC be used to study the induced conformation of closely related peptides, but also that it can be used to evaluate the structural changes that result from modifying one or several residues of a given peptide sequence. These results, in conjunction with earlier work that illustrated the correlation between biological activity and both RP-HPLC retention times and CD mean residue ellipticities in the presence of lipid surfaces (Blondelle and Houghten, 1991, 1992; Blondelle et al., 1993; Pérez-Payá et al., 1994), suggest that RP-HPLC can be a useful tool for the design of analogs of a given peptide having improved biological activities.

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