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Peptide Secondary Structure Induced by a Micellar Phospholipidic Interface: Proton NMR Conformational Study of a Lipopeptide

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ABSTRACT: The conformational change of the model peptide Ac-K-G-R-G-D-G-amide induced by a phospholipidic interface was investigated by proton nuclear magnetic resonance (^{1}H NMR). In aqueous solution, the free peptide is highly flexible and disordered, even in the presence of deuterated dodecylphosphocholine (DPC-d_{38}) micelles which mimic a membrane interface. The lipopeptide, obtained by grafting a lipid anchor [2,3-dipalmitoyl-D-(+)-glyceric acid] to the lysine side chain of the peptide, was studied by standard 2D ^{1}H NMR spectroscopy combined with distance geometry and simulated annealing calculations. When anchored to a micelle interface, the peptide acquires a definite turn (II/I′) conformation. We were also able to describe precisely the conformation of the diacylglyceric fragment of the lipopeptide in a lipid environment and to establish the average orientation of the peptide segment with respect to the micelle surface.

The ability of membranes to alter the conformation of peptides is currently being illustrated by an increasing number of examples: biologically active peptides such as hormones or neuropeptides, etc. (Wider et al., 1982; Zetta et al., 1983; Kaiser et al., 1984; Wakamatsu et al., 1987; Wooley & Deber, 1987; Olejniczak et al., 1988; Naider et al., 1989; Beschiaschvili & Seelig, 1991), toxins or antibiotics (Brown et al., 1982; Arseniev et al., 1985; Lee et al., 1986; Cavatorta et al., 1989; Inagaki et al., 1989; Bairaktari et al., 1990; Lakey et al. 1991), peptide signals (Giersasch, 1989; von Heijne, 1990; Karslake et al., 1990), and coat proteins (Schiksnis et al., 1987; O'Neil & Sykes, 1988; Shon et al., 1991). The interactions of a peptide ligand with a membrane interface and the re-

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sulting conformational changes can in fact be considered as possible prerequisites for the peptide–receptor binding process. Therefore, the study of these interactions may provide an insight into the molecular basis of peptide activities. The conformational change of a peptide induced by the membrane interface is only well documented for a few species, and no systematic study has been reported. Therefore, no general conclusion is available at the present time. Two main difficulties complicate the investigation of peptide–membrane interface interactions. First, a membrane interface is a heterogeneous medium, the physicochemical properties of which exhibit large gradients: hydration, ionic concentration, dielectric constant, "interfacial pH", and electrochemical potential. Secondly, interpretation of experimental data is generally obscured by the limited residence time of the peptide on the membrane surface, depending on its affinity for a membrane of a given composition.

As a step toward the understanding of lipid–peptide interactions at a molecular level, we propose a conformational study, based on NMR[1] experiments, that demonstrates the ability of a membrane interface to induce secondary structure in a model peptide. When combined with the use of deuterated micelles, proton NMR spectroscopy is a very suitable technique for studying the conformation and dynamics of peptides in a membrane-like environment (Brown et al., 1982; Wider et al., 1982; Arseniev et al., 1985; Schiksnis et al., 1987; Olejniczak et al., 1988; O'Neil & Sykes, 1988; Inagaki et al., 1989; Karslake et al., 1990). By coupling the peptide to a lipid fragment, we solved the problems resulting from the exchange between the free and bound forms of the peptide. The model hexapeptide *N*-acetyl-Lys-Gly-Arg-Gly-Asp-Gly-amide **4** was synthetized, and a dipalmitoylglyceric residue **2** was grafted to a fraction of this peptide at the NHζ group of the lysine side chain. Since we decided to start by investigating a zwitterionic peptide, the Arg-Gly-Asp (RGD) sequence was preferred to other possibilities, because this segment constitutes an important recognition sequence in proteins involved in cell-surface adhesion [see, for instance, the review article by Springer (1991)].

In this paper, we report the conformational study by proton NMR spectroscopy of (i) the free peptide in aqueous solution, (ii) the free peptide in aqueous solution in the presence of deuterated dodecylphosphocholine micelles, and (iii) the lipopeptide solubilized in dodecylphosphocholine micelles. We show that, whereas the free peptide exhibits a random coil form in the absence or presence of micelles in aqueous solution, the same amino acid sequence coupled to the lipid moiety is partly structured by the micelle interface. In addition, we were able to give what to our knowledge is the first detailed description of a lipopeptide structure in a lipidic environment.

Materials and Methods

*2,3-Dipalmitoyl-*D*-(+)-glyceric Acid* (**2**). D-(+)-Glyceric acid **1** (1.636 g, 15.43 mmol) obtained from the calcium salt with a Dowex H^+(AG 50W-X8) cation exchange resin, was dissolved in 20 mL of dioxane. To the cooled solution was added triethylamine (12.95 mL, 92.6 mmol) and drop by drop a solution of palmitoyl chloride (14.05 mL, 46.3 mmol) in 10 mL of dioxane, keeping the temperature under 35 °C. The solution was stirred for 2 h at room temperature and evaporated to dryness; the residue was dissolved in methanol and brought to pH 4 with 2 N hydrochloric acid. After evaporation to dryness, the compound was dissolved in dichloromethane and washed with a saturated solution of sodium hydrogen carbonate and then with water. The organic solution was dried with sodium sulfate, evaporated, and chromatographed with a column of silica gel eluted first with dichloromethane and then with ethyl acetate and 10% methanol to yield **2** (8.3%). R_f 0.65 (dichloromethane–methanol, 80:20). MS: (CI, NH_3), 600 (M + 18).

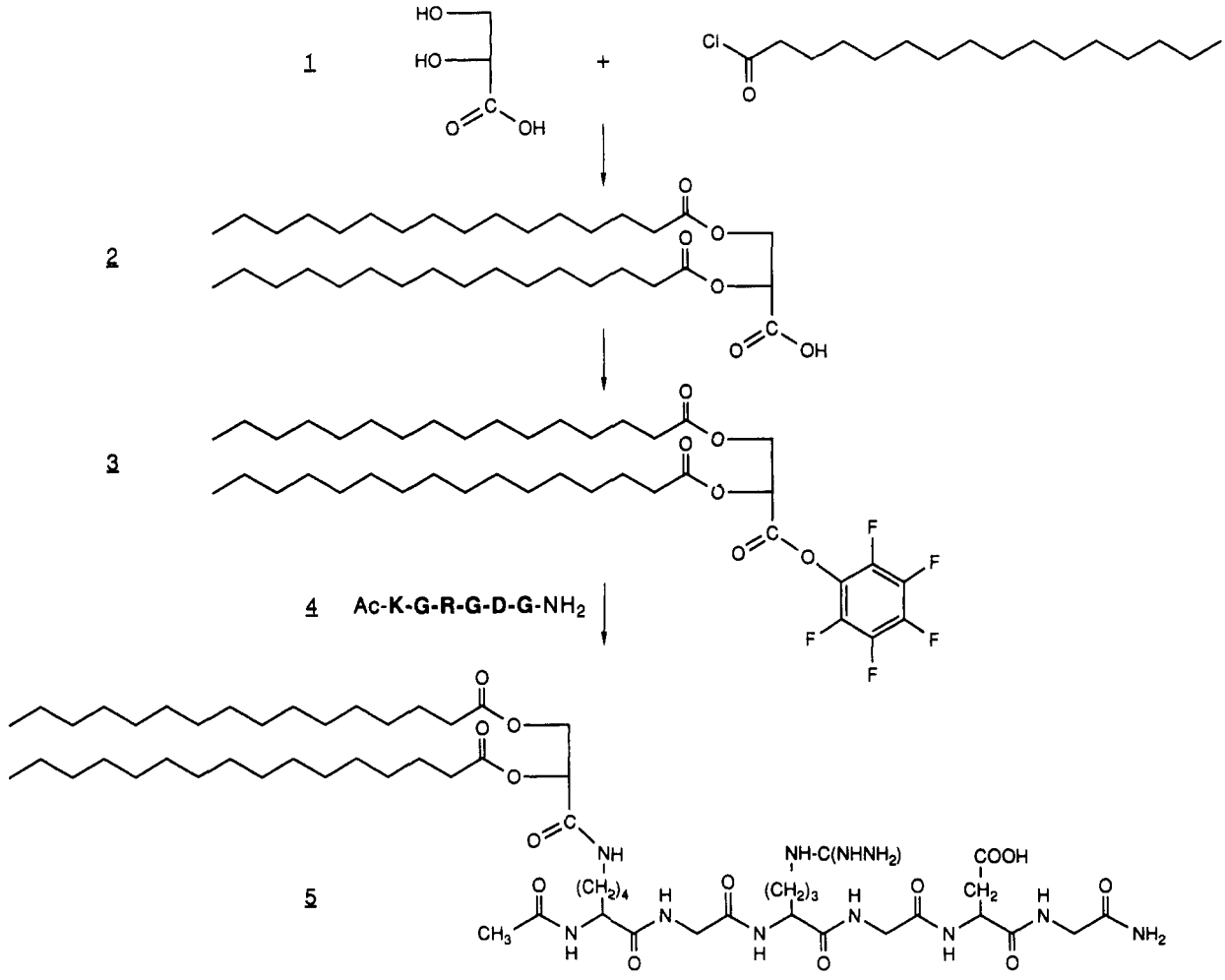
Ac-Lys-Gly-Arg-Gly-Asp-Gly-NH_2 (**4**). The peptide was synthesized by the Merrifield solid-phase method (Merrifield, 1963) on a Applied Biosystems 430A synthetizer. To obtain the peptide in the C-amide and N-acetylated form, the synthesis was performed on a 0.5 mmol *p*-methylbenzhydrylamine resin, and the acetylated peptide was formed on the peptide–resin at the end of the synthesis with acetic anhydride. The peptide was then cleaved from the resin by the standard low–high hydrofluoric acid protocol (Tam & Heath, 1983). After gel filtration on Bio-Gel P2, it was purified by MPLC on a 20-μm C18 100-Å column, using a 0–30% linear gradient of acetonitrile in 0.1% trifluoroacetic acid during 60 min at a 25 mL/min flow rate. The final purity of the peptide (99.5%) was checked by analytical RP-HPLC on a Nucleosil 5-μm C18 300-Å column using the above linear gradient during 20 min at a 1 mL/min flow rate. The retention time was 12.02 min, and the yield was 144 mg. Amino acid analysis gave the following peptide composition: Lys, 1.0 (1); Gly, 2.8 (3); Asp, 1.0 (1); Arg, 0.9 (1).

*N-(Acetyl-Lys-Gly-Arg-Gly-Asp-Gly)-2,3-dipalmitoyl-*D*-(+)-glyceric Amide* (**5**). The pentafluorophenyl ester **3** was prepared from 100 mg of glyceric acid **2** (172 μmol) dissolved in 5 mL of dichloromethane, 38 mg (206 μmol) of pentafluorophenol, and 42.5 mg (206 μmol) of dicyclohexylcarbodiimide. This solution was stirred for 1 h at room temperature, filtered, evaporated to dryness and used without purification (R_f 0.8, dichloromethane–methanol, 80:20). Peptide **4** (55 mg, 86.85 μmol) was dissolved with 2.5 mL of water. To this solution were added 30 μL of diisopropylethylamine (173.7 μmol) and 1.5 equiv of activated ester **3** dissolved in 2.5 mL of dioxane. The solution was stirred for 20 h at room temperature, and the pH was maintained at 9.5 with diisopropylethylamine. After dioxane evaporation, the solution was lyophilized and the residue purified by HPLC with a reverse-phase 5-μm column (Nucleosil C8 SFCC) using acetonitrile–0.1% trifluoroacetic acid as eluent, to yield 15.5% of lipopeptide **5** [R_f (cellulose) 0.7, butanol–H_2O–acetic acid (66.7: 16.6: 16.7); retention time 17.60 min].

NMR Experiments. Two milligrams of free peptide **4** was dissolved in 0.4 mL of 10 mM phosphate buffer containing 0.1 mM EDTA and lyophilized in ether D_2O or H_2O–D_2O (90:10). The pH was adjusted to 6.0. For the experiments with micelles, 16 mg of deuterated dodecylphosphocholine (MSD Isotopes) was added to the solution; the solution was sonicated for 10 min with a Branson sonicator. The same procedure was applied to 2 mg of lipopeptide **5**, i.e., to a preparation with a lipopeptide to dodecylphosphocholine molar ratio of 1:27. Standard sets of 600-MHz two-dimensional phase-sensitive experiments (COSY and NOESY; Wüthrich, 1986; Markley, 1989) were performed on a Bruker AMX 600 spectrometer. 200-MHz spectra were recorded on a Bruker ACP 200 spectrometer. In general, 48 (COSY) or 80 (NOESY) transients were acquired with a recycling delay of 1 s. A total of 512 increments of 2K data points were collected

[1] Abbreviations: COSY, correlated spectroscopy; DG, distance geometry; DPC, dodecylphosphocholine; EDTA, ethylenediaminetetraacetic acid; MPLC, medium-pressure liquid chromatography; HPLC, high-performance liquid chromatography; NMR, nuclear magnetic resonance; NOE, nuclear Overhauser enhancement; NOESY, nuclear Overhauser enhancement spectroscopy; SA, simulated annealing.

Scheme I



for each 2D experiment, yielding a digital resolution of 6 or 8 Hz/point in both dimensions after zero filling. Shifted squared sine-bell functions were used for apodization. Three lipopeptide NOESY spectra were recorded with mixing times of 50, 80, and 150 ms; 150- and 300-ms mixing time values were used for the NOESY spectra of the free peptide.

Distance Geometry/Simulated Annealing (DG/SA) Calculations. Lipopeptide structures were generated from experimental NMR data using the DSPACE program (Hare Research, Inc., Woodinville, WA) running on a Sparcstation 2 (Sun Microsystems). This software combines distance geometry and simulated annealing (DG/SA) algorithms (Summers et al., 1990).

Results and Discussion

Synthesis of the Lipopeptide 5. The synthesis of the lipopeptide (see Scheme I) started from D-(+)-glyceric acid **1** which was esterified with palmitoyl chloride into 2,3-dipalmitoyl derivative **2**. The carboxylic function was activated via the pentafluorophenyl ester **3** which was not purified and was used immediately for the coupling with peptide **4**. The main difficulty encountered in the synthesis of the lipopeptide **5** is its low solubility in water, necessitating tedious repetitive injections for the preparative HPLC.

NMR Data for the Free Peptide 4. Figure 1a shows the 4.6–4.0 ppm region of the free peptide 600-MHz 1D proton spectrum recorded at 15 °C (D_2O solution). The corresponding NOESY spectrum (H_2O–D_2O, 90:10) exhibited few and weak cross-peaks. As regards the interresidue correlations, only CHα–NH (*i*,*i*+1) cross-peaks were detected. They enabled us to assign the signals of the three G residues, since the K, R, and D spin systems were readily identified. Neither the scarce NOE pattern nor the other NMR parameters

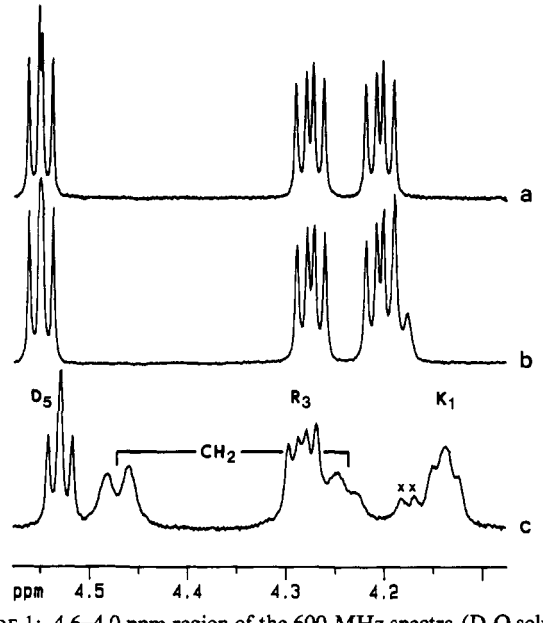


FIGURE 1: 4.6–4.0 ppm region of the 600-MHz spectra (D_2O solution at 15 °C) of the free peptide (A), of the free peptide in the presence of dodecylphosphocholine (DPC) micelles (B), and of the lipopeptide solubilized in DPC micelles (C). These spectra display the K1, R3, and D5 CHα resonances and the signals of the methylene group of the glyceric backbone. Asterisks indicate residual protonated DPC signals.

Table I: Proton Chemical Shifts of the Lipopeptide (in ppm) Measured at 5 °C

G6	NH 8.57; $CH_2\alpha$ 3.87, 3.80; NH_2 7.58, 7.21
D5	NH 8.36; $CH\alpha$ 4.53; $CH_2\beta$ 2.67, 2.61
G4	NH 8.68; $CH_2\alpha$ 3.91
R3	NH 8.30; $CH\alpha$ 4.28; $CH_2\beta$ 1.88, 1.73; $CH_{2\gamma}$ 1.59; $CH_2\delta$ 3.14; $NH\epsilon$ 7.50; NH_2 7.08, 6.69
G2	NH 8.80; $CH_2\alpha$ 3.88
K1	NH 8.60; $CH\alpha$ 4.15; $CH_2\beta$ 1.72; $CH_{2\gamma}$ 1.39, 1.32; $CH_2\delta$ 1.47; $CH_{2\epsilon}$ 3.17; $NH\zeta$ 8.40; CH_3CO 1.99
glyceric	CH 5.35; CH_2 4.47, 4.25
sn-1 chain	CH_2CO 2.22; CH_2CH_2CO 1.53; $CH_2CH_2CH_2CO$ 1.22; $(CH_2)_n$ 1.2; CH_3 0.75
sn-2 chain	CH_2CO 2.42; CH_2CH_2CO 1.58; $CH_2CH_2CH_2CO$ 1.25; $(CH_2)_n$ 1.2; CH_3 0.75

supported the existence of a preferential conformation, since, first, J(NH–CHα) values ranged between 6 and 7 Hz, and, secondly, no significant temperature dependence of the chemical shifts was observed between 5 and 45 °C. A second NOESY experiment performed at 200 MHz displayed no convincing cross-peak, thus enabling the NOE frequency dependence to be ruled out as a possible source of the poor result obtained at 600 MHz. In the presence of dodecylphosphocholine micelles, the peptide spectrum (Figure 1b) was identical to that previously obtained in pure aqueous solution (Figure 1a). No significant chemical shift or line width difference was observed between the two spectra (i.e., the chemical shift variation was less than or equal to 0.01 ppm). The same applied to the NOESY spectra. Therefore, our NMR experiments did not allow the detection of any interaction between the free peptide and the micelle surface.

NMR Data for the Lipopeptide 5 in Micelles. Comparison of the amino acid resonances showed that the lipopeptide 1D spectrum (Figure 1c) clearly differs from that of the free peptide. Furthermore, unlike the free peptide spectra, the lipopeptide NOESY spectra displayed large and numerous cross-peaks. Figure 2 shows the region of the lipopeptide NOESY spectrum (recorded at 5 °C with a mixing time of 80 ms) containing the cross-peaks connected to the NH resonances (except for the NH–NH NOEs). The complete set of chemical shifts measured at 5 °C is listed in Table I. Signal assignment was achieved from COSY and NOESY experiments as follows.

Sequential assignment of peptide resonances was straightforward, since the lipopeptide NOESY spectra provided a complete set of NH–NH (i,i+1) and CHα–NH (i,i+1) correlations (the latter region is shown in Figure 2). J(NH–CHα) values of 5, 5.8, and 7.1 Hz were found for the K1, R3, and D5 residues, respectively, at 15 °C.

The glyceric C(1)H_2–C(2)H spin system[2] was easily identified because of the characteristic location of the C(2)H resonance (5.35 ppm). The two protons of the methylene group were markedly magnetically nonequivalent ($\Delta\delta$ = 0.22 ppm); a similar difference between the two protons of the C(1) glycerol methylene group was previously detected in the spectra of various sonicated phospholipid vesicles (Neumann et al., 1985). Furthermore, the two J(CH–CH_2) coupling constants were quite different (3.5 and 7.5 Hz at 15 °C). In the COSY network of the acyl chain protons, the discernible signals, on a 2D spectrum and with a decreasing chemical shift, were the α (CH_2CO), β (CH_2CH_2CO), and γ ($CH_2CH_2CH_2CO$) methylene protons and the terminal CH_3 group. The other methylene signals were not distinguishable. An interesting

[2] The standard nomenclature of the glycerol residue was used for the glyceric residue.

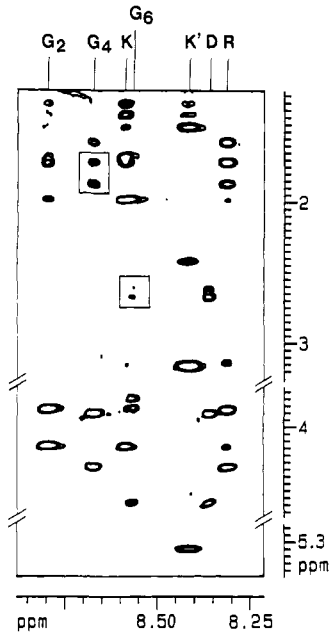


FIGURE 2: Part of the lipopeptide 600-MHz NOESY spectrum (H_2O/D_2O, 90:10 at 5°C; mixing time, 80 ms) containing the cross-peaks connected to the NH resonances (except for the NH–NH NOEs). The columns labeled K and K′ refer, respectively, to the backbone amide and $NH\zeta$ proton resonances of the K1 residue. Boxed cross-peaks in the G4 and G6 columns correspond to the NH(G4)–CHβ,–CHβ′(R3) and NH(G6)–CHβ,–CHβ′(D5) NOEs, respectively.

finding was that the *sn*-1 and *sn*-2 chains gave well-separated α-methylene resonances (Table I); only the upfield CH_2CO signal was dipolar coupled to the glyceric C(1)H_2 resonances and was thus assigned to the *sn*-1 chain. A small difference between the chemical shift of the *sn*-1 and *sn*-2 resonances was also observed for the β and γ protons (Table I). Chain discrimination in proton NMR spectra of phospholipids has been previously reported (De Bony & Dennis, 1981, and references therein); in all cases, the *sn*-1 α-methylene signal shifted upfield from the *sn*-2 signal. In summary, the lipidic resonances of the lipopeptide molecule were found to exhibit the same spectral features as the phospholipids. These features were related to the typical phospholipid conformation in which the *sn*-1 chain starts perpendicular to the layer surface, while the *sn*-2 chain starts parallel and then bends sharply at the α-carbon atom (Hitchcock et al., 1974; Seelig & Seelig, 1975; Haberkorn et al., 1977; Pearson & Pascher, 1979).

Effect of the Micelle Interface on the Peptide Conformation: Comparison of Free Peptide and Lipopeptide. The structure dependence of proton chemical shifts (δ) is now well established (Szilagyi & Jardetzky, 1989; Williamson, 1990), and the δ variations in peptide resonances can be used to detect conformational changes. We therefore compared the chemical shifts of the lipopeptide amino acid signals (δ_l) to the chemical shifts of the free peptide signals (δ_p). The differences (δ_l–δ_p) for the NH and CHα resonances of the six residues (at 5 °C) are presented in Figure 3. Only the N-terminal part of the peptide (K1–R3 segment) exhibited large significant chemical shift variations. Since the free peptide is in a random coil form, even in the presence of micelles, these variations indicated that the N-terminal peptide fragment of the lipopeptide acquires a preferential conformation in micelles. Moreover, when passing from the free peptide **4** spectrum to the lipopeptide **5** spectrum, a significant increase in the CHα line widths was observed for the residues of the K1–G4 fragment, whereas the D5 and G6 CHα line widths were not much affected. As

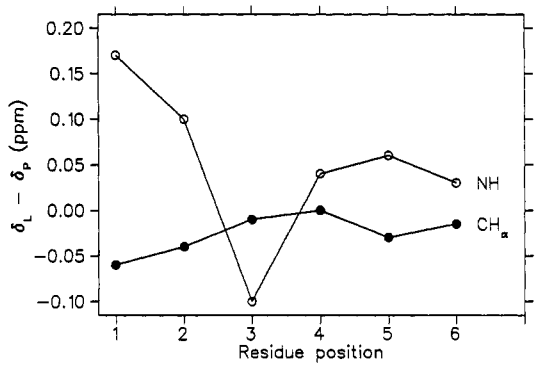


FIGURE 3: Difference (in ppm) between the chemical shifts of the lipopeptide and the free peptide (δ_l–δ_p) measured at 5 °C for the NH and CHα resonances of the six amino acid residues of the model peptide.

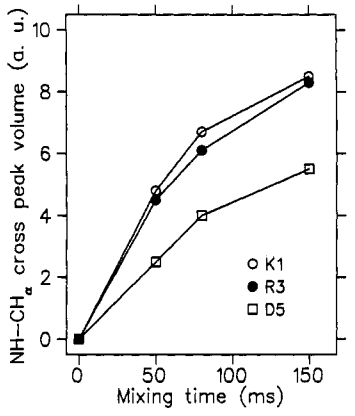


FIGURE 4: Buildup curves of the intraresidue NH–CHα NOE relative to the K1, R3, and D5 residues.

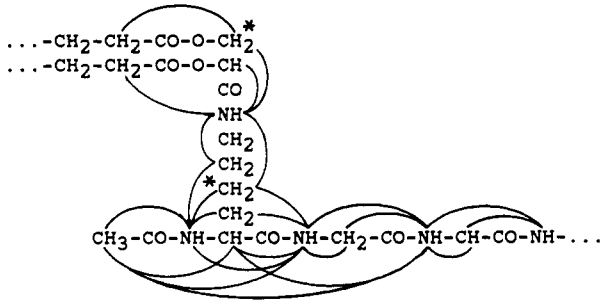


FIGURE 5: Schematic representation of the most interesting NOEs obtained at 5 °C with a mixing time of 80 ms.

shown in Figure 1c, the K1 and R3 CHα signals were much broader than the D5 signal. At 5 °C, the former signals are no longer resolved, and their line width was similar to that of the glyceric CH signal. In contrast, the J multiplicity was still observed on the D5 resonance. Furthermore, as regards comparable types of NOE, the cross-peaks corresponding to D5 and G6 protons were always less intense than those of other amino acids; for example, the NH(G6)–CHβ,–CHβ'(D5) correlations were considerably weaker than the NH(G4)–CHβ, –CHβ(R3) NOEs (Figure 2). Another example of this is shown in Figure 4, where the NH(D5)–CHα(D5) NOE buildup curve is compared to the corresponding curves for K1 and R3. From this set of observations, we can conclude that the D5–G6 terminal segment is highly flexible and is mainly located outside the interface. The K1–G4 fragment, on the contrary, is embedded in the micelle interface, and its dynamics closely resemble that of the glyceric moiety. In the following sections, the conformation of the lipopeptide is analyzed using the NOE data set and distance geometry calculations.

Lipopeptide NOE Constraints and Distance Geometry/Simulated Annealing (DG/SA) Calculations. Figure 5 shows a schematic representation of the most interesting NOEs, obtained with a mixing time of 80 ms at 5 °C. They strongly suggest that, to some extent, the lipopeptide acquires a predominant conformation in the micelle. As mentioned above, the NOEs between G4 and D5 and between D5 and G6 were scarce and were associated with a correlation time obviously different from that of the remaining fragment. Consequently, the data concerning these residues were discarded from the analysis described below. The distance constraints used in the DG/SA calculations were collected from 30 proton–proton NOEs obtained at 5 °C with a mixing time of 80 ms. Additional restraints were derived from the J(CH–CH_2) coupling constants of the glyceric backbone and from the J(NH–CHα) coupling constants of residues K1 and R3, measured at 15 °C. DG/SA structures are displayed in Figures 6 and 7.

Conformation of the Peptide Fragment. Figure 6 shows nine different superimposed DG/SA structures of the peptide backbone. Clearly, the first half of the peptide fragment acquires a highly preferential turn conformation. One can easily demonstrate that two characteristic NOE contacts, αN(K1,G2) (strong) and αN(K1,R3) (weak), are the key data of this structure. The turn conformation belongs to the II,I′ subfamily. Of course, no predominant conformation was obtained for the second half of the peptide. These results confirm that chemical shift variations such as those illustrated in Figure 3 contain relevant information about peptide conformational changes.

Conformation and Orientation of the Lipid Fragment. Using the complete NMR data set (NOEs, coupling constants, and proton signal nonequivalence), DG/SA calculations gave a single conformation for the glyceric backbone. This is de-

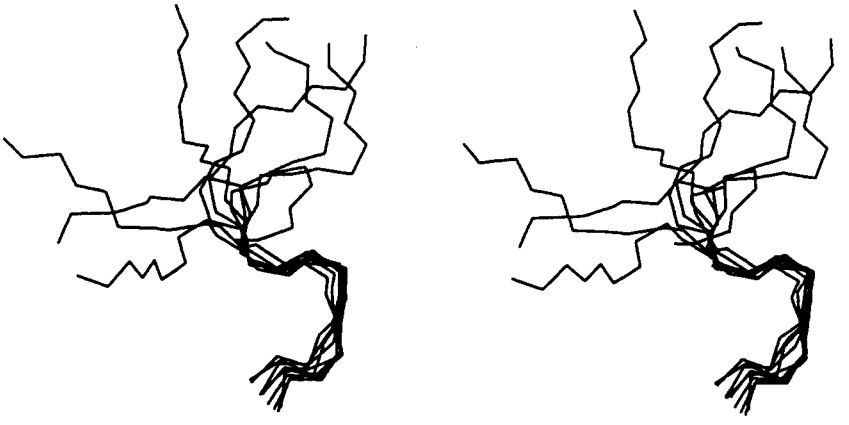


FIGURE 6: Nine superimposed DG/SA structures showing only the peptide backbone of the lipopeptide.

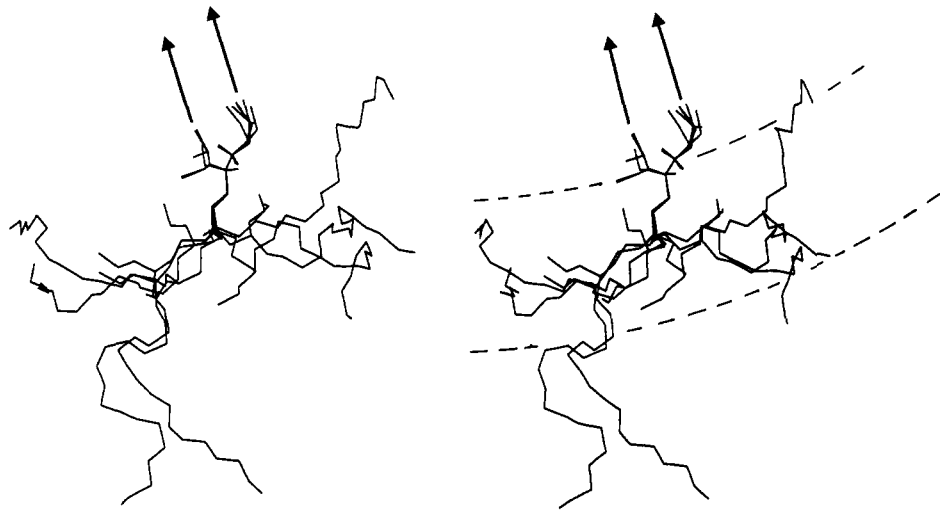


FIGURE 7: Seven superimposed DG/SA structures of the lipopeptide. Only the backbone atoms extending from the C-terminal amino acid residue to the first methylene carbons of the acyl chains are shown. Arrows roughly indicate both the starting direction of the chains and the direction of the normal to the micelle surface. Doted lines simulate the micelle interface.

picted in Figure 7, showing seven superimposed structures. The conformation of the glyceric backbone together with the average starting direction of the acyl chains are close to those typically observed in membrane glycerophospholipids and described above. It is important to note the conformational rigidity of the glyceric segment shown by the very small dispersion of the calculated structures. Therefore, one can reasonably assume that the local normal to the micelle surface is roughly parallel to the glyceric backbone. The direction of this normal is also the average orientation of the acyl chains schematically indicated by arrows in Figure 7.

Orientation of the Peptide Fragment with Respect to the Micelle Surface. The existence of a strong NOE network extending from the glyceric backbone to the peptide backbone through the Lys side chain leads to distance constraints allowing a complete description of the average conformation of the structured part of the lipopeptide. As a result of the fairly rigid conformation of the glyceric backbone, the Nζ–Cϵ bond of the K1 side chain is maintained in the direction previously defined as the normal to the micelle surface. Then the K1 side chain bends at the δ carbon and lies parallel to the micelle surface. Lastly, the peptide turn is restrained to explore a spatial domain identified as the micelle interfacial region, and the plane defined by the turn structure is perpendicular rather than parallel to the micelle surface (see Figure 7). Nevertheless, no hindrance should a priori prevent the free rotation of the peptide around the Nζ–Cϵ bond.

At this point, it is of interest to focus on the average direction of the Arg side chain. DG/SA calculations indicated that all the Arg side chains (not shown in Figure 7) are either parallel to the micelle surface or point toward the lipidic phase but never toward the aqueous phase. This would be expected for guanido groups interacting with lipid phosphate groups. However, we have no direct evidence of such interactions.

As regards the localization of the disordered C terminal part of the peptide, the only information we obtained here concerns the pK variation of the D5 carboxylate group. Ionization of this group was followed by the pH dependence of the chemical shifts for both free peptide and lipopeptide. The carboxylate apparent pK increased from 3.8 to 4.2 when passing from the free peptide to the lipopeptide. Such an increase may be ascribed to the dielectric constant decrease of the D5 environment; in other words, the D5 residue is slightly "sensitive" to the presence of the uncharged (zwitterionic) micelle interface.

Conclusion

Our aim was to study the ability of a phospholipid interface to induce a definite structure in peptides which are inherently flexible molecules in aqueous solution. For this purpose, we performed NMR experiments on a model hexapeptide. The results show that (i) the free peptide in aqueous solution does not exhibit any preferential structure on the NMR time scale and (ii) when this peptide is grafted to a lipid fragment solubilized in deuterated dodecylphosphocholine micelles, it is strongly anchored within a membrane-like phospholipid interface, and, under these conditions, the N-terminal part of the peptide acquires a predominant turn conformation (turn II or I'). It is important to note that no conformational change of the free peptide was detected in the presence of micelles, and our work therefore demonstrates the general interest of lipid anchors for conformational studies of peptide interacting with a membrane surface.

Furthermore, we were able to describe precisely the conformation of the diacylglyceric fragment of the lipopeptide in a lipid environment. To our knowledge, this is the first time that the detailed conformation of a lipopeptide has been elucidated. The average orientation of the peptide segment with respect to the micelle surface was also established.

Nevertheless, it is still not clear why the peptide acquires a specific conformation in the lipid interface. Reduction in the dielectric constant and water molecule activities in the interfacial region is obviously part of the driving forces. The importance of the interactions of lipid phosphate groups with the positively charged Arg side chain is suggested by the average orientation of both the peptide backbone and the Arg side chain within the interface. Various experiments designed to clarify the roles of the dielectric constant, ions, and chemical composition of the lipid interface are now under way in our laboratory.

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Identification of the Active Site Residues in Dipeptidyl Peptidase IV by Affinity Labeling and Site-Directed Mutagenesis†

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ABSTRACT: The active site of dipeptidyl peptidase IV (DPPIV) was examined by chemical modification and site-directed mutagenesis. Purified DPPIV was covalently modified with [^{3}H]diisopropyl fluorophosphate (DFP). The radiolabeled DPPIV was digested with lysyl endopeptidase, and the peptides were separated by high-performance liquid chromatography. A single ^{3}H-containing peptide was obtained and analyzed for amino acid sequence and radioactivity distribution. A comparison of the determined sequence with the predicted primary structure of DPPIV [Ogata, S., Misumi, Y., & Ikehara, Y. (1989) *J. Biol. Chem. 264*, 3596-3601] revealed that [^{3}H]DFP was bound to Ser^{631} within the sequence Gly^{629}-Trp-Ser-Tyr-Gly^{633}, which corresponds to the consensus sequence Gly-X-Ser-X-Gly proposed for serine proteases. To further identify the essential residues in the active-site sequence, we modified the DPPIV cDNA by site-directed mutagenesis to encode its variants. Expression of the mutagenized cDNAs in COS-1 cells demonstrated that any single substitution of Gly^{629}, Ser^{631}, or Gly^{633} with other residues resulted in the complete loss of the enzyme activity and DFP binding. Although substitution of Trp^{630} → Glu or Tyr^{632} → Phe caused no effect on the enzyme activity, that of Tyr^{632} → Leu or Gly abolished the activity. These results indicate that the sequence Gly-X-Ser-(Tyr)-Gly is essential for the expression of the DPPIV activity.

Dipeptidase peptidase IV (DPPIV)[1] is a unique enzyme among dipeptidyl aminopeptidases. DPPIV is an ectoenzyme, in contrast to lysosomal and cytosolic localization of other DPPs, and it cleaves X-proline dipeptides from the NH_2 termini of peptides (Hopsu-Havu & Glenner, 1966; McDonald & Schwabe, 1977; Macnair & Kenny, 1979). Despite ex-

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[1] Abbreviations: DPPIV, dipeptidyl peptidase IV; DFP, diisopropyl fluorophosphate; HPLC, high-performance liquid chromatograpy; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; SDS, sodium dodecyl sulfate; *N*-glycanase, peptide *N*-glycosidase F.

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