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Accelerated Publications

Preferred Conformation, Orientation, and Accumulation of Dynorphin A-(1–13)-tridecapeptide on the Surface of Neutral Lipid Membranes†

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ABSTRACT: Infrared attenuated total reflection (IR-ATR) spectroscopy and capacitance minimization (CM) were used to study the secondary structure, orientation, and accumulation of dynorphin A-(1–13)-tridecapeptide (dynorphin$_{1-13}$) molecules on the surface of planar membranes prepared from 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine. The peptide assumed a helical structure oriented perpendicularly on the membrane surface. Binding from aqueous solutions containing 10 mM KCl saturated reversibly at about a bilayer area of 110 nm^2 per peptide molecule, an apparent dissociation constant of 11 μM, and rate constants of 2×10^2 s^{-1} (adsorption) and 2×10^{-3} s^{-1} (desorption). The results complement those obtained by vesicle-mediated hydrophobic labeling [Gysin, B., & Schwyzer, R. (1983) *Arch. Biochem. Biophys. 225*, 467–474]. They indicate that the behavior of this amphiphilic peptide in contact with neutral lipid membranes may be quite different from that in molecularly disperse or micellar solutions of detergents or lysolecithins and that, in the case of dynorphin$_{1-13}$, primary amphiphilicity overrules secondary amphiphilicity.

Dynorphin A-(1–13)-tridecapeptide (dynorphin$_{1-13}$)[1] has practically the same pharmacological profile as its parent, natural opioid peptide dynorphin A with 17 amino acid residues (Chavkin & Goldstein, 1981). In particular, it is a preferential agonist for κ-receptors whose structural requirements differ markedly from those of other opiate receptors (Schiller et al., 1982; Schiller & DiMaio, 1982). As shown by vesicle-mediated hydrophobic photolabeling (Gysin & Schwyzer, 1983), dynorphin$_{1-13}$, net charge 5+, interacts regioselectively with anionic liposomes: the N-terminal message segment, residues 1–4 (Chavkin & Goldstein, 1981), contacts the hydrophobic membrane layers and the address segment, residues 5–13 (Chavkin & Goldstein, 1981), remains in the aqueous phase. We have extended our studies to the interaction of dynorphin$_{1-13}$ with planar, neutral phosphatidylcholine membranes as revealed by infrared attenuated total reflection (IR-ATR) spectroscopy [see Fringeli (1977)] and capacitance minimization (CM) (Schoch et al., 1979a). The results indicated peptide accumulation on the membrane surface as well as conformation- and orientation-selective interactions that were quite similar to those of adrenocorticotropin-(1–24)-tetracosapeptide (ACTH$_{1-24}$; Gremlich et al., 1983, 1984; Gysin & Schwyzer, 1984). The main feature is a short α-helix oriented perpendicularly on the membrane surface. Binding saturated at an area of 110 nm^2, which corresponds to about 180 lipid molecules.

Experimental Procedures

Synthetic dynorphin$_{1-13}$ was an analytically and spectroscopically pure product obtained from Bachem, Bubendorf. IR-ATR spectra were obtained as described by Gremlich et al. (1983) with a Perkin-Elmer IR spectrophotometer, Model 983G. The membranes were prepared from 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (purchased from R. Berchtold, Berne) on a ZnSe internal reflection element. They were hydrated either with water vapor or by contact with liquid water. Deuterium exchange was measured after equilibration with D_2O vapor for 14 h. Before measurements, the samples were dried in a current of nitrogen at 20 °C. The spectra are the average of 32 runs with the absorbance of the pure lipid subtracted (the remaining ester carbonyl absorption on the

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[1] Abbreviations: dynorphin$_{1-13}$, dynorphin A-(1–13)-tridecapeptide; ACTH$_{n-m}$, synthetic adrenocorticotropin segments comprising residues n to m; CM, capacitance minimization; IR-ATR spectroscopy, infrared attenuated total reflection spectroscopy; SDS, sodium dodecyl sulfate.

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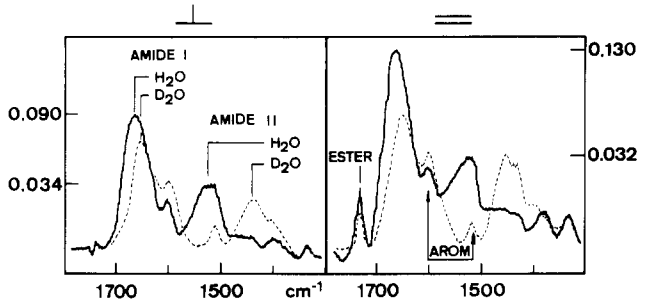

FIGURE 1: IR-ATR absorbance spectra of the amide I and amide II regions of dynorphin$_{1-13}$ in contact with neutral membranes. The molar ratio of peptide to lipid is 1:75 (similar spectra were obtained with 1:50 and 1:100 ratios). Parallel (right panel) and perpendicularly (left panel) polarized incident light corresponds to a plane of polarization perpendicular and parallel, respectively, to the plane of the membrane surface. The solid line was obtained with H_2O and the dotted line with D_2O.

right panel of Figure 1 is less than 1% of the total).

Changes in the capacitance minimization potential $V_{C\,min}$ following addition of dynorphin$_{1-13}$ to one side ("cis") of a planar lipid bilayer were measured according to Schoch et al. (1979a). The lipid was 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine and the aqueous phase contained 9 mM KCl and 2 mM 3-(*N*-morpholino)propanesulfonic acid buffer, pH 7.2. Both aqueous compartments were stirred thoroughly after the additions.

Results and Discussion

Representative IR-ATR spectra are shown in Figure 1. The amide I band culminated at 1665 cm^{-1}. Its position, shape, and shift from 1645 cm^{-1} (peptide on the ZnSe plate without lipid) indicated an induction by the membrane of a short α-helix comprising about six to nine amino acid residues (Nevskaya & Chirgadze, 1976). The amide II band, located at 1530–1540 cm^{-1}, while not possessing special diagnostic value, did not exclude a short helix (Nevskaya & Chirgadze, 1976). Comparison with model compounds derived from phenylalanine and tyrosine showed that the bands at 1608 and 1515 cm^{-1} were caused mainly by aromatic C=C stretching vibrations. As expected, treatment with D_2O shifted the amide I and II resonances to 1655 and about 1435 cm^{-1}, respectively, whereas the aromatic bands were unaffected. The amide I absorbance increased, and that of the amide II band decreased when the polarization of the IR beam was changed from parallel to perpendicular with respect to the membrane surface. The dichroic ratios, about 1.4 and 0.9 for amide I and II, respectively, were similar to those reported for ACTH$_{1-24}$ (Gremlich et al., 1983, 1984).

Adsorption of dynorphin$_{1-13}$ out of a 0.1 M aqueous solution onto preformed lipid multibilayer membranes yielded IR spectra exhibiting the same spectroscopic features as the membrane containing incorporated dynorphin$_{1-13}$ in Figure 1. Again, the behavior was similar to that of ACTH$_{1-24}$ (Gremlich et al., 1983, 1984).

Adsorption of dynorphin$_{1-13}$ from aqueous solutions onto neutral bilayer membranes was indicated also by CM studies (Figure 2). Dynorphin$_{1-13}$ dissolved in buffer solution was added to final concentrations of (a) 8.5, (b) 17, (c) 46, (d) 88, and (e) 130 μM. The polarity of $V_{C\,min}$ indicated adsorption of positive charges to the cis side of the bilayer. At (1) the bilayer broke and was quickly re-formed. $V_{C\,min}$, which reflects the asymmetry of the surface potentials on the two sides of the bilayer, returned to the base-line value, indicating that the two lipid layers had become mixed. The reestablishment of the prerupture value shows the reversibility of the

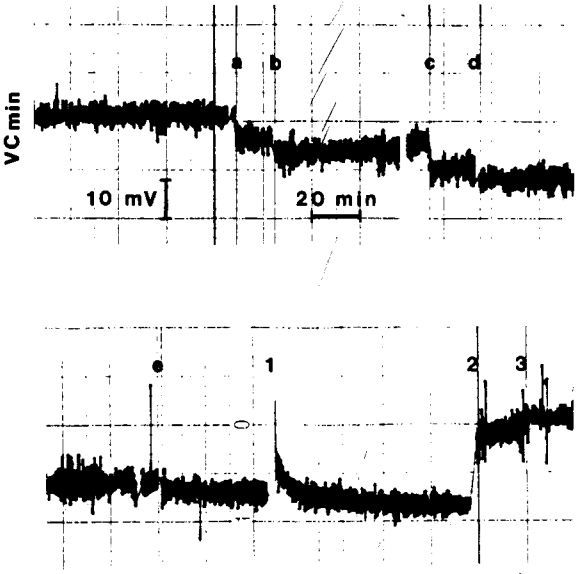

FIGURE 2: Changes in the capacitance minimization potential, $V_{C\,min}$, following addition of dynorphin$_{1-13}$ to one side (cis) of a planar lipid bilayer (see Experimental Procedures).

dynorphin$_{1-13}$ binding: the peptide initially adsorbed to the trans side of the bilayer desorbs with a time constant of about 500 s. At (2) the ionic strength on the cis side was raised to 210 mM with KCl. Taking account of the change in the liquid junction potential of the salt bridge (−4 mV), the total change in $V_{C\,min}$ is 22 ± 2 mV; i.e., the shielding appears to be complete. This is inconsistent with the Gouy–Chapman treatment of fixed charge surface potential and can best be explained by assuming that some of the charges are situated in the aqueous phase at a distance from the bilayer surface comparable with the Debye–Hückel length (about 0.5 nm at 210 mM KCl). At (3) the ionic strength on the trans side of the bilayer was also raised to 210 mM, and only the change in the liquid junction potential (+4 mV) is seen. The lack of significant changes in the surface potential itself confirms that dynorphin$_{1-13}$ is not present on the trans side, nor does it influence the trans surface potential, in contrast to ACTH$_{1-24}$ (Schoch et al., 1979b). Membrane conductance was not affected by dynorphin$_{1-13}$.

In summary, our CM studies showed adsorption localized on the side of the lipid bilayer to which the dynorphin$_{1-13}$ had been added. The binding was reversible and a preliminary analysis yielded K_d = 11 μM and a maximum surface density of 1 molecule/110 nm^2 in the presence of 10 mM KCl in the aqueous phase. These values were probably influenced somewhat by the mutual electrostatic repulsion of the bound peptide molecules. In particular, the indicated surface density may well be too low. Analysis of the reestablishment of the equilibrium surface potential following rupture of the bilayer yielded rate constants for adsorption and desorption of about 2×10^2 s^{-1} and 2×10^{-3} s^{-1}, respectively. Shielding studies at higher ionic strengths suggested that a charged part of the molecule extended into the aqueous phase.

The model derived from our data is shown in Figure 3. We assumed a helical structure extending from Tyr-1 to Pro-10. The helix is oriented perpendicularly to the membrane surface. The extended conformation of the C-terminal -Lys-Leu-Lys-OH segment with peptide bonds (H—N—C=O dipoles) perpendicular to the membrane surface was chosen in analogy to the C-terminal address segment, ACTH$_{11-24}$ (Gremlich et al., 1983, 1984). In our model, the message segment, dynorphin$_{1-4}$, contacts hydrophobic membrane layers and the address segment, dynorphin$_{5-13}$, the aqueous phase. This

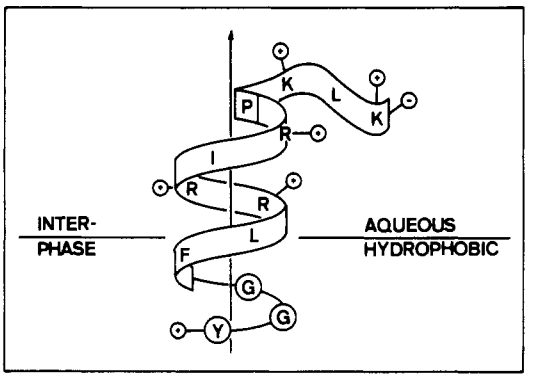

FIGURE 3: Model of dynorphin$_{1-13}$ conformation and orientation on neutral, hydrated lipid membranes. The code used is F = Phe, G = Gly, I = Ile, K = Lys, L = Leu, P = Pro, and Y = Tyr. Circles indicate peptide residues at the end of the helix that cause destabilization in hydrophobic surroundings (see text). Hydrophobic designates regions with reduced water activity and concentration (e.g., hydrogen belt), not necessarily the hydrocarbon layer.

arrangement follows from the results of our vesicle-mediated hydrophobic labeling studies (Gysin & Schwyzer, 1984) and from the shielding observed on raising the ionic strength on the cis side in the CM experiments (Figure 2). However, hydrophobic interactions with side-chain elements of Leu-5, Arg-6,7, and Ile-8 are easily possible according to space-filling models and may have remained undetected in the labeling experiments (low rate of carbene insertion into aliphatic C–H bonds). Projection of the N-terminus of a helix into a hydrophobic phase amounts to about 10 kcal/mol. This destabilization can be accounted for by the free energy of transfer of the hydrophobic side chains of the dynorphin helix from aqueous into micellar surroundings (Jencks, 1975), which is in the range of −13 to −20 kcal/mol. Thus, the observed binding, K_d = 11 μM ($\Delta G°$ = −6.6 kcal/mol), is reasonably accounted for by our model (detailed report in preparation).

With circular dichroism, dynorphin$_{1-13}$ is shown to have very little order in water (Maroun & Mattice, 1981). This agrees with our measurements on dynorphin$_{1-13}$ films on the internal reflection element without lipid. Dynorphin$_{1-13}$ increases its helix content to 19% in 3.6 mM sodium dodecyl sulfate (SDS) but not in 80 μM lysolecithin, and it was concluded that "helix formation is seen only when the polar head group is anionic" (Maroun & Mattice, 1981). Our results with IR-ATR spectroscopy, however, indicated a strong increase of helicity on contact with neutral lecithin membranes. Moreover, circular dichroism spectra (to be published elsewhere) showed an increase of dynorphin$_{1-13}$ helicity in 2,2,2-trifluoroethanol/water mixtures similar to that observed in SDS solutions (Maroun & Mattice, 1981). Thus, as suggested earlier (Pitner & Urry, 1972), this solvent appears to mimic influences of membranes on peptide conformation very well. The behavior of amphiphilic peptides in contact with lipid membranes may be quite different from that in molecularly disperse or even micellar solutions of detergents or lysolecithins.

Dynorphin$_{1-13}$ thus interacts with 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine membranes in a manner similar to that of ACTH$_{1-24}$ (Gremlich et al., 1983, 1984; Gysin & Schwyzer, 1984). In the absence of significant Coulombic interactions, the amphiphilic primary structure of dynorphin$_{1-13}$ (more hydrophobic message segment followed by a more hydrophilic, charged address segment; Gysin & Schwyzer, 1984) and hydrophobic forces may be the principal reasons for strong binding and orientation at the membrane–water interphase, as with ACTH$_{1-24}$. The reduced activity of water in the interphase is expected to favor internal hydrogen bonding and formation of peptide helices. However, the dynorphin$_{1-13}$ helix (Figure 3) also displays a considerable amount of "secondary amphiphilicity" (hydrophobic amino acid side chains on one side of the helix axis, hydrophilic side chains on the other); this should orient the helix axis parallel to the membrane surface and not perpendicular as observed (Figure 1). In our case, membrane binding caused by the amphiphilic primary structure apparently overruled that arising from the secondary amphiphilic helix.

Registry No. Dynorphin$_{1-13}$, 72957-38-1; 1-palmitoyl-2-oleoyl-*sn*-3-phosphocholine, 26853-31-6.

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