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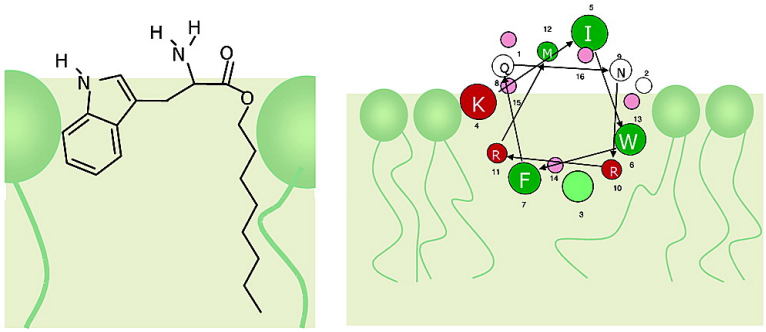

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Orientation and Conformation of Cell-Penetrating Peptide Penetratin in Phospholipid Vesicle Membranes Determined by Polarized-Light Spectroscopy

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There is an urgent interest how certain peptides interact with and translocate through biological membranes. The peptide "penetratin", corresponding to the 16-mer third helix of the homeodomain of the Antennapedia protein, has been reported to permeate eukaryotic cell membranes[1] and may be used as a carrier to transport oligonucleotides,[2] peptide nucleic acids,[3] and small proteins[4] into live cells. Although the translocation mechanism is not fully understood, recent findings suggest that penetratin enters live cells primarily through endocytotic pathways.[5] An important step toward understanding the internalization mechanism is to clarify the peptide−lipid interactions. Penetratin has been studied in membrane-mimetic environments both regarding affinity for the membrane[6] and preferred conformation. Great diversity is noted depending upon experimental conditions and model system; both β-sheet and α-helical structures have been reported.[7−12]

Here we assess the conformation and orientation of penetratin associated with phospholipid vesicle membranes using polarized-light spectroscopy. In addition to the peptide transitions absorbing in the far UV, we made use of the two tryptophan residues with isolated transitions at longer wavelengths. For comparison, tryptophan octyl ester (TOE) was used as a model chromophore (TOE is commonly used to model tryptophan fluorescence from proteins in membrane environments[13]).

Figure 1 shows LD spectra of TOE and of penetratin bound to POPG/POPC liposomes,[14] corrected for liposome background. In the LD spectrum of TOE (1:65 molar ratio) the L_b transition at 287 nm (Figure 1, inset) shows zero or slightly positive LD indicative of an orientation close to the "magic angle" (54.7°) to the membrane normal. The L_a and B_b transitions, at 270 and 220 nm, respectively, both give rise to a negative LD, suggesting a preferential orientation of these transition moments perpendicular to the membrane surface. Taken together these findings suggest that the TOE molecule is oriented in the membrane as schematically shown in Figure 2A, with the hydrophobic benzene ring of the indole pointing into the membrane and its hydrophilic groups oriented outward toward the water.

Figure 1 also shows the LD spectrum of penetratin, at a low peptide to lipid molar ratio (1:110) chosen to ensure that all peptide is bound to the membrane and to avoid aggregation.[6] At longer wavelengths where the LD spectrum is dominated by the indole chromophore of the tryptophan residues, one can clearly see contributions from the L_a (270 nm) and L_b (287 nm) transitions, both showing positive LD. The B_b transition is overlapped by the $n \rightarrow \pi^*$ and the $\pi \rightarrow \pi^*$ amide transitions and cannot be distinguished. Since both L_a and L_b have positive LD, indicative of an orientation parallel with the membrane surface, the B_b should also give a positive contribution, though overlapped by the negative amide LD. We conclude that the two tryptophan residues are on average oriented with their planes preferentially parallel with the surface of the membrane.

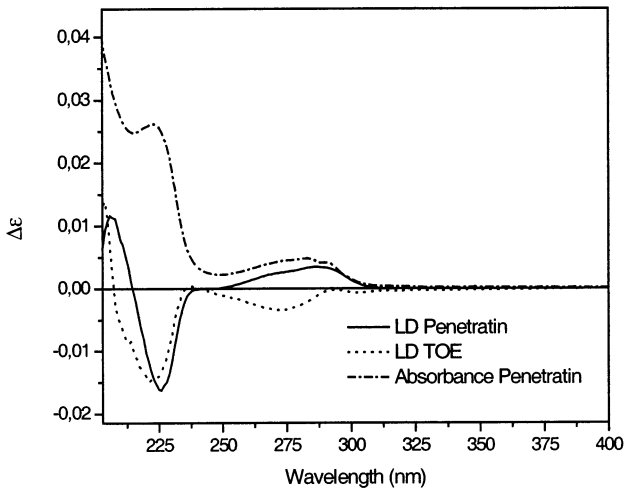

Figure 1. LD spectra of TOE (1:65 chromophore to lipid molar ratio, spectra multiplied by a factor 2 to match the Trp content of penetratin) and of penetratin (1:110 peptide to lipid molar ratio) bound to flow deformed POPG/POPC (20:80 molar ratio) liposomes. Liposomes were prepared by dissolving POPG/POPC in chloroform, which was removed by evaporation. The lipid film was dispersed in 5 mM phosphate buffer (pH 7) containing 50% w/w sucrose to a final concentration of 3.75 mg/mL lipids. Liposomes were sized by extrusion through 100 nm polycarbonate filters. Inset: transition moments of indole chromophore.[20,21]

Both Trp residues in penetratin have been inferred from fluorescence measurements to be in an environment of similar polarity and to be accessible to the solvent[15] and, hence, could be expected to orient in a comparable fashion although an average signal is obtained.

In the amide absorption region of penetratin the LD of the $n \rightarrow \pi^*$ transition at 227 nm (polarized perpendicular to the amide plane) is negative, indicative of an orientation of this transition moment parallel to the lipid chains. Circular dichroism suggests 65% α-helix for penetratin in this sample (data not shown). The positive LD signal at 207 nm corresponds to an exciton $\pi \rightarrow \pi^*$ transition polarized parallel to the helix axis.[16] Taken together, our LD data indicate that the α-helical part of the peptide lies parallel to the surface of the membrane. If the entire peptide had been α-helical and all penetratin molecules were perfectly aligned parallel to the membrane surface, the extreme value of LD^r ($LD/A_{isotropic}$) theoretically expected for the $n \rightarrow \pi^*$ transition would be $(-3/8)S$, compared to $(-3/2)S$ if all the peptide bonds were perfectly coplanar with the membrane surface, with S being the orientation factor of the membrane ($0 \geq S \leq 1$).[16] From the observation of an LD^r value of $+0.075$ for the L_b transition, for which the maximum value is $(+3/4)S$, we can estimate a minimum value of S to be about 0.1.

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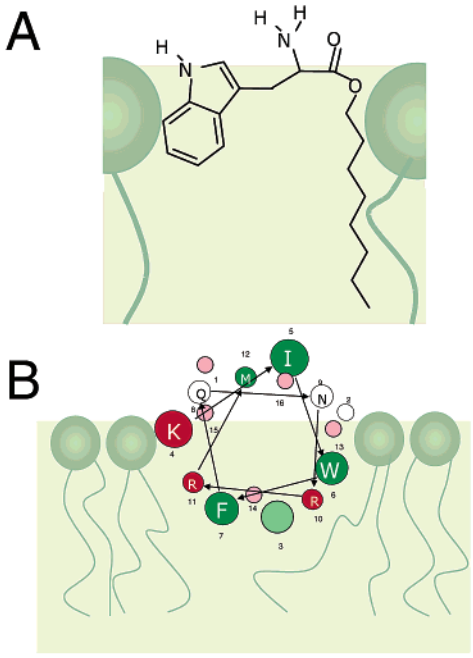

Figure 2. Schematic orientation of TOE (A) and of penetratin (B) at membrane interface. Penetratin represented by helical plot, with red denoting electropositive, white uncharged, and green hydrophobic residues. Only residues 4−12 (bright colors) are suggested to participate in the α-helix,[17] while the ends of the peptide, as suggested by n → π^* LD, adopt a more planar structure, which we believe indicates a strong, specific interaction with the membrane.

Comparing with LD^r for the n → π^* transition, this is found to be markedly more negative that expected for a 100% α-helical conformation. This deviation can be explained if the ends of the peptide adopt a conformation in which the planes of the amide bonds are parallel to the membrane surface. The orientation we observe for penetratin is unusually high for a solute molecule in our membrane system, which we take as an indication of a strong, specific interaction with the membrane.

In an NMR study on penetratin in triflouroethanol, it was suggested that residues 4−12 form a bent irregular helix.[17] Assuming that this region of penetratin adopts an α-helix in the POPG/POPC lipid system, a helical plot[18] shows that a hydrophobic region is expected to be flanked on both sides by positively charged amino acids (Figure 2B). With seven positive charges, it is highly likely that the penetratin molecule is located at the polar region of the membrane surface rather than in the hydrophobic interior of the lipid bilayer, in agreement with the orientation data. Thus, penetratin is probably positioned with the hydrophobic region of the α-helix facing the hydrophobic lipid chains and with the positive regions toward the polar headgroups of the lipids (Figure 2B). As a result of its amphiphilic character, with most of the polar and charged amino acids located on one side of the helix, it is not surprising if the helix adopts a flat orientation relative to the membrane interface. One could argue that the Trp residues in penetratin would be expected to orient similarly to TOE in the lipid bilayer, like coins dropped into a hairbrush, in contrast with the observed preferential orientation parallel with the surface. The latter orientation may be a result of the indole groups of penetratin being at the membrane interface where dispersive forces may favor an orientation parallel to the surface, whereas with TOE the indole is partly inserted between the lipid chains. An analogous orientation behavior parallel to a surface has been reported for benzene, when located at the surface of cylindrical micelles or a lamellar phase of cetyltrimethylammonium bromide/1-hexanol/water.[19]

In conclusion, LD spectra of penetratin indicate that a central α-helical part of the peptide, the ends of the peptide adopting a planar structure, and the planes of the two tryptophan residues all have in common that they adopt orientations parallel with the membrane surface. These findings could provide a clue about the mechanism of cell-uptake for penetratin. The peptide orientation parallel to the membrane surface is in agreement with the observation that the penetratin peptide does not form membrane pores;[6] thus it is unlikely that the peptide participates in any flip-over motion across the membrane. This is further supported by the observation that penetratin is almost entirely taken up by endocytosis.[5]

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Supporting Information Available: Linear dichroism theory, further details of the amide and indole chromophore, and additional data for *S*. This material is available free of charge via the Internet at http://pubs.acs.org.

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