Interaction of Melanotropic Peptides with Lipid Membranes[a]

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Although the melanotropic peptides most certainly interact with protein receptors on the melanocyte/melanoma cell membrane, the lipid phase is supposed to be important as a catalyst for the peptide-receptor interaction.[1] The present work studies the interaction of the tridecapeptide α-melanocyte-stimulating hormone (α-MSH) and the biologically more active analogue [Nle^4,D-Phe^7]-α-MSH, with pure lipid bilayers, through the analysis of the electron paramagnetic resonance (EPR) signal of the 3-doxyl cholestane spin label (CSL) incorporated in the lipid phase. Oriented multibilayers were prepared with dimyristoylphosphatidyl choline (DMPC) and dimyristoylphosphatidyl glycerol (DMPG), both preparations containing 10% of cholesterol (in moles) necessary for organizing the oriented films.[2] Both peptides are positively charged at physiological pH, and display stronger interaction with the negatively charged bilayer (DMPG) than with the neutral one (DMPC). The effect of the peptides on the degree of organization and dynamics of the oriented bilayers was analyzed by calculating the order parameter S, related to the angular amplitude of motion of the CSL long molecular axis, and the correlation time τ, related to the rotation rate of the steroid about its long axis.[3] FIGURE 1 shows that while the natural hormone decreases both S and τ, the analogue causes an opposite effect on DMPG + 10% cholesterol membranes.

Similar results, though less evident, were obtained with the same lipids organized in isotropic liposomes. Considering the difficulty in separating order from mobility in the analysis of the EPR spectra of spin probes incorporated in liposomes, the effect of the peptides on the membrane fluidity was monitored by the ratio of the low field/center field EPR line heights (h_{+1}/h_0). An increase in h_{+1}/h_0 is proportional to both a decrease in order and/or an increase in mobility.[3] FIGURE 2a shows the h_{+1}/h_0 ratios obtained at different temperatures, above the lipid phase transition. In agreement with the results obtained for oriented bilayers (FIGURE 1), it is evident that while α-MSH decreases lipid fluidity the analogue increases the fluidity. However, when DMPG liposomes were prepared without cholesterol, both peptides were found to decrease membrane fluidity, as shown in FIGURE 2b, with [Nle^4,D-Phe^7]-α-MSH displaying a stronger effect.

In non-cholesterol-containing membranes, both peptides cause the ordering effect already observed for divalent cations,[4] or cationic molecules.[5] These latter

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substances are supposed to interact at the membrane surface, neutralizing part of the surface charge, allowing for a better packing of the lipids. A specific hydrophobic interaction between peptides and lipids should also contribute to the packing of the latter, as both peptides are equally charged and [Nle^4,D-Phe^7]-α-MSH is slightly more effective in altering membrane fluidity. Considering the well-known condensing effect of cholesterol on liquid-crystalline membranes,[2] the present work shows that the interaction of α-MSH with lipid membranes depends both on membrane charge and degree of packing, although a specific interaction between α-MSH and cholesterol, or cholesterol-rich domains, cannot be ruled out. Cholesterol is an important component in biological membranes and has been found to modulate the activity of several membrane proteins.[6] The different interactions of the two peptides with lipid membranes might be related to their different conformations in both aqueous and lipid phases, or at the aqueous/lipid interface, and could partially explain their different biological activities.

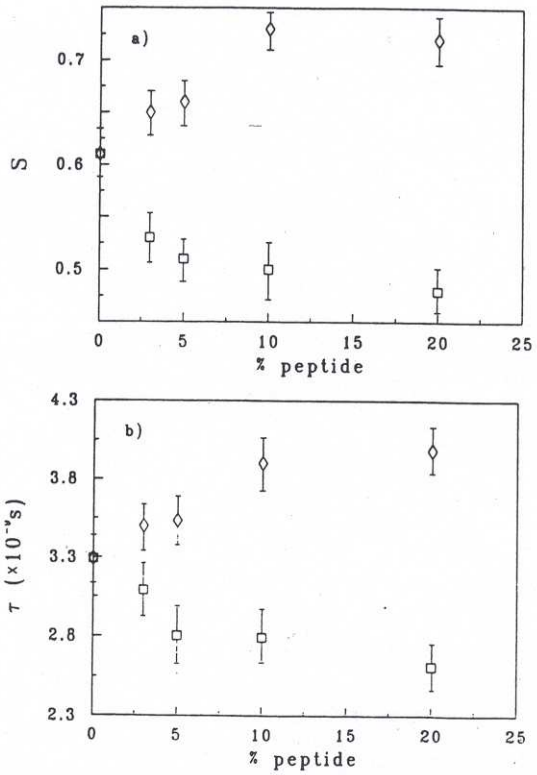

FIGURE 1. Effect of melanotropic peptides on DMPG + 10% cholesterol oriented films. Order parameter S (a) and rotational correlation time τ (b) of CSL incorporated in oriented multibilayers of DMPG + 10% cholesterol, in the presence of 10% α-MSH (squares) and [Nle^4,D-Phe^7]-α-MSH (diamonds), at 40°C. Cholesterol and peptide concentrations are reported as mole percent of phospholipid.

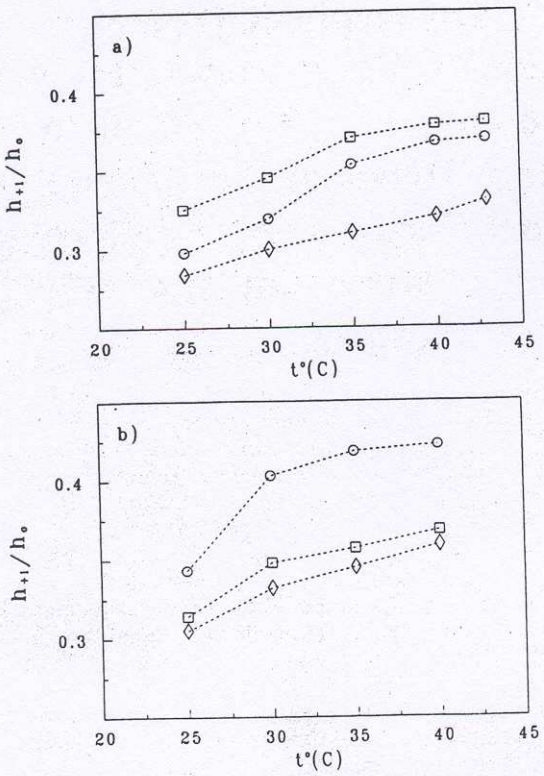

FIGURE 2. Temperature dependence of the effect of melanotropic peptides on DMPG liposomes. h_{+1}/h_0 values calculated from the EPR spectra of CSL incorporated in DMPG + 10% cholesterol (a) and DMPG vesicles (b), in the presence of 10% α-MSH (squares) and [Nle^4,D-Phe^7]-α-MSH (diamonds), and in the absence of peptide (circles).

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