Binding of N-terminally TOAC-labeled Angiotensin II to Large Unilamellar Vesicles

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The bioactive peptide hormone angiotensin II (AII, DRVYIHPF) is the main effector of the renin-angiotensin-aldosterone system. The paramagnetic amino acid 2,2,6,6-tetramethylpiperidine-1-oxyl-4-amino-4-carboxylic acid (TOAC) is used as a probe to investigate the conformation of peptides. We have investigated the conformational properties of angiotensin II labeled at the N-terminus (TOAC[1]-AII) in the presence of phospholipid large unilamellar vesicles (LUV) prepared from zwitterionic 1-palmitoyl-2-oleoyl phosphatidylcholine (POPC) and negatively charged 1-palmitoyl-2-oleoyl phosphatidylglycerol (POPG). Experiments were conducted at pH 4.0, 7.0, and 10.0 to determine the effect of peptide charge on peptide-membrane interaction. Electron paramagnetic resonance (EPR) spectra showed that the peptide interacts with POPC:POPG LUV (1:1, mol:mol) at pH 4.0, indicating the presence of two components corresponding to two peptide populations, one bound to vesicles and the other in the aqueous phase. Binding occurred to a much lesser extent at pH 7.0, and, at pH 10.0, no interaction was observed. In the case of POPC:POPG, the interaction was also evidenced by circular dichroism (CD), but binding-promoted spectral changes were only observed at pH 4.0. No interaction between the peptide and POPC LUV, was observed at the three pHs, both by EPR and by CD, in contrast with studies with zwitterionic micelles, where an interaction with these aggregates was still observed. The results suggest that the interaction between TOAC[1]-AII and bilayers is strongly modulated by electrostatic forces. Since the membrane milieu is believed to mediate the access of peptides to their receptors, the current study provides contributions to the understanding of the mechanism of signal transduction at a molecular level.

Keywords: Angiotensin II, circular dichroism, EPR, large unilamellar vesicles, TOAC,

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