An Innovative EPR-Fluorescence Approach to Search for the Binding Site of a Peptide Receptor

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The present work proposes a pioneering approach aiming at investigating the binding site of a peptide in its membrane receptor. The vasoconstrictor angiotensin II (AII) and peptide fragments present in the putative region of its AT1 receptor were selected for the present study. The stable free radical TOAC (2,2,6,6-tetramethylpiperidine-1-oxyl-4-amino-4-carboxylic acid), introduced by us in the chemistry of peptides [*J. Braz. Chem. Soc.* (1981), 14, 173; *J. Am. Chem. Soc*. (1993) 115, 11042] was used to produce TOAC^{1}-AII, TOAC^{3}-AII and TOAC^{4}-AII analogues. Pharmacological experiments have indicated that only the first derivative maintained the AII activity. In respect to AT1 fragments, the sequences involving the (13-27 or P15) and/or the third extracellular loop (266-278 or P13) region were synthesized. In our view, the key element of the proposed strategy was the TOAC spin probe as it allowed: i) through EPR spectra, the detection of the interaction (decrease in the molecular motion) of the active TOAC^{1}-AII only in contact with P15-X-P13 fragments [X is a 6 or 22 $(CH)_2$ – spacer group], cyclized through a S–S bond linking P15 and P13 sequences; ii) through fluorescence spectra, the same finding, detected in this case, by the decrease in the fluorescence intensity as a consequence of the quenching effect induced by the paramagnetic TOAC close to the Tyr^{26} residue of P15-X-P13 during the agonist-receptor fragments interaction. Conversely, the two inactive AII analogues did not showed interactions with none of these receptor fragments. In conclusion, this combined and different spectroscopic strategy seems to be of value for this or other types of applications involving monitoring of macromolecular interactions.