The Use of Paramagnetic and Fluorescent Quenching Amino Acid TOAC for Evaluating Angiotensin I-Converting Activity

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Advantageously to other fluorescent quenching probes, the TOAC (2,2,6,6-tetramethylpiperidine-1-oxyl-4-amino-4-carboxylic acid), introduced earlier by us in chemistry [*J. Braz. Chem. Soc*. (1981) 14, 173; *J. Am. Chem. Soc*. (1993) 115, 11042] can be inserted at any position of an enzyme substrate. Following preliminarily study, this work examined the specificity of angiotensin I-converting enzyme (ACE) that cleaves the angiotensin I (AI, DRVYIHPFHL) to produce the angiotensin II and inactivates bradykinin (BK, RPPGFSPFR). TOAC-attaching AI analogues at positions 0, 1, 3, 5, 8, 9 and 10 indicated that the first four analogues are substrates for ACE with k_{cat}/k_m values of 11.9, 9.2, 3.2 and 2.0 respectively, in comparison with 15.4 $\mu M^{-1}.min^{-1}$ of the native AI. These results confirm that greater the proximity of the unnatural probe to the cleavage site (8-9), the smaller is the substrate specificity of analogues. Greater decrease in the substrate activity occurred with BK, where $TOAC^0$-BK and $TOAC^3$-BK presented k_{cat}/k_m of 20.9 and 38.9 respectively (against 202 $\mu M^{-1}.min^{-1}$ for BK). Other analogues were devoid of substrate activity. Interesting, EPR spectra indicated greater mobility for those analogues of AI and BK that were ACE substrates. These analogues clearly demonstrated the quenching property of TOAC affecting the Tyr^4 and $Phe^{5;\ 8}$ residues in AI and BK, respectively. The fluorescence intensity of labeled substrates decreased with increasing distance between both residues, thus suggesting extended structures for these active substrates. In addition, differences between EPR spectral lineshapes of TOAC-containing AI and BK substrates and their cleavage products allowed the monitoring of the ACE enzymatic reaction.