Significance of the S2' Binding Pocket in Angiotensin-Converting Enzyme C-Domain Specificity

Kröger, W.L., van Rensburg, H.G. and Sturrock, E.D.

Institute of Infectious Disease and Molecular Medicine, University of Cape Town, Observatory, 7925, South Africa

Angiotensin-converting enzyme (ACE) plays an important role in blood pressure regulation. Two isoforms, testis (tACE) and somatic (sACE) exist. sACE consists of 2 homologous domains, an N- and C-domain, each containing an active site with the Zn binding motif, HEMGH. Excluding the first 36 residues, tACE corresponds to the C-domain of sACE (1). Although similar, each domain of sACE has been shown to be more specific for certain substrates and more sensitive to certain inhibitors. For example, RXPA380, a phosphinic peptide inhibitor, is 1000-fold more selective for the C-domain, while RXP407, another phosphinic peptide inhibitor, specifically inhibits the N-domain. Ac-SDKP, a hematoregulatory peptide, has been shown to be an N-domain-specific substrate (2). Computer analysis of RXPA380 complexed to tACE, revealed that the pseudo-proline and tryptophan residues, the P1' and P2' groups respectively, play major roles in the specificity of the compound. Further analysis showed that the bent conformation of the proline residue allowed a more favourable interaction of the tryptophan with the S2' pocket. Examination of residues in this pocket revealed a number of differences between the C- and N-domains (3). To determine if particular residues in the S2' pocket of ACE confer C-domain specificity, these residues were converted to corresponding N-domain residues. A tACE gene fragment containing the residues of interest was sub-cloned into pGEM11Zf, and site-directed mutagenesis was performed. Mutants were confirmed by nucleotide sequencing, sub- cloned into pcDNA3.1+, and expressed in CHO cells. Mutant protein will be purified for future kinetic and comparative studies with wild type C- and N-domains.

[1] Acharya, K.R., Sturrock, E.D., Rirodan, J.F. and Ehlers, M.R. (2003) ACE revisited: A new target for structure-based drug design. Nature Reviews Drug Discovery. 2(11), 891-902

[2] Georgiadis, D., Beau, F., Czarny, B., Cotton, J., Yiotakis, A. and Dive, V. (2003) Roles of the two active sites of somatic angiotensin-converting enzyme in the cleavage of angiotensin I and bradykinin. Circulation Research. 93, 148-154

[3] Georgiadis, D., Cuniasse, P., Cotton, J., Yiotakis, A. and Dive, V. (2004) Structural determinants of RXPA380, a potent and highly selective inhibitor of the angiotensin-converting enzyme C-domain. Biochemistry. 43, 8048-8054