Structure-Function relationship of Angiotensin-Converting Enzyme: Insights from mutagenesis and kinetic studies.

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Angiotensin-Converting Enzyme (ACE) is the principal regulator of blood pressure, electrolyte and fluid homeostasis. The somatic isoform of ACE (sACE) consists of two homologous domains which differ with respect to their substrate and inhibitor specificities. Structural elucidation of testis ACE (tACE), which is identical to the C domain of sACE, has enabled characterisation of the structure-function relationship of the enzyme via homology modelling of the N domain, kinetic and inhibitor studies. Two aspects of the ACE structure-function relationship were investigated: 1. The effect of specific regional substitutions of tACE with corresponding N domain sequence. 2. The nature of cooperativity between the N and C domains of full-length sACE. Chimeric constructs were generated, where the N and C domains were swopped-over or where specific regions of tACE were substituted with corresponding N domain sequence. The kinetic parameters of chimeric and mutant proteins were determined using synthetic peptide substrates. Structural alterations were assessed via homology modelling. Similar to wild-type sACE, CN-dom ACE, in which the C and N domains were interchanged, demonstrated evidence of negative cooperativity. When two identical domains are present as CC-dom ACE, positive cooperativity was evident. Thus, the N- and C domains of sACE may not necessarily function independently. Regional substitutions of tACE sequence with corresponding N domain sequence resulted in the acquisition of N domain-like catalytic properties by tACE with an observed shift in substrate-specificity from tACE to that of the N domain. The structural basis for these changes is discussed.