ABSTRACT

Biochemical and Structural Studies of Nitric Oxide Synthases and CaM Kinase I in Rat Heart

Aldrin Verno Gomes

This thesis describes the isolation and partial characterization of the constitutive and inducible forms of nitric oxide synthase (NOS) from rat heart, the interaction of these proteins and calmodulin-dependent protein kinase I (CaM kinase I) with calmodulin (CaM), and the proteolytic degradation of two of these proteins by calpain.

A 135kDa heart protein was identified as the endothelium derived (eNOS) isoform of NOS on the basis of its ability to produce [^{3}H]-L-citrulline from [^{3}H]-L-arginine, its requirement for Ca^{2+}, CaM and other co-factors needed for nitric oxide (NO) production by NOS, its predominance in the particulate fraction (100,000 xg pellet) of the heart homogenate, and its recognition by anti-eNOS antibody. Another 130kDa heart protein that was induced by lipopolysaccharide was shown to be the inducible (iNOS) isoform of NOS on the basis of its ability to produce [^{3}H]-L-citrulline from [^{3}H]-L-arginine, its lack of requirement for exogenous Ca^{2+} and CaM, its predominance in the cytosolic fraction (100,000 xg supernatant) of the heart homogenate, and its recognition by an anti-iNOS antibody. Studies with cardiomyocytes showed that eNOS was constitutive while iNOS was inducible.

Gel electrophoretic studies showed that a peptide derived from CaM kinase I (CaMKIp) was capable of binding CaM in a 1:1 ratio. The binding of iNOS peptide to CaM was, however, much more complex. CaMKIp was found to bind CaM with a very high affinity (K_d <1nM). Fluorescence studies using mutants of CaM with single and several methionine residues replaced by leucine showed that the C-terminal half of CaMKIp bound the N-terminal domain of CaM while its N-terminal half bound the C-terminal domain of CaM. CaM was capable of interacting strongly with iNOS peptide both in the presence and absence of calcium, unlike the other CaM-binding peptides investigated (CaMKIp and eNOS peptide). Far-UV circular dichroism (CD) studies showed that CaMKIp adopted an α-helical conformation on interacting with CaM. Near-UV CD studies showed that the Trp residue in CaMKIp is involved in interaction with CaM.

NMR spectroscopic studies showed that both domains of CaM are involved in binding CaMKIp and that a continuous α-helix is formed from residue Trp 303 to Gln 320 of CaMKIp. These NMR and CD data suggest that the CaM-binding region of CaM kinase I significantly increases in α-helical content on binding CaM, when compared to the autoinhibited structure. These results provide the foundation for experiments addressing the potential role of partial α-helices in the middle of CaM-binding regions and their recognition by CaM.

Experiments on crude homogenates suggest that nNOS may serve as a substrate for calpain *in vivo*, accounting partially for its rapid degradation. eNOS and CaM kinase I were, however, not hydrolyzed by calpain. The presence of PEST regions in most CaM-binding proteins reflects their susceptibility to proteolytic degradation and suggest that these proteins are degraded via similar mechanism(s).