Self-Association of CaMKII-Delta in Low ATP/Low pH Conditions

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Calcium-Calmodulin Dependent Protein Kinase II (CaMKII), an enzyme critical for brain function and involved in learning and memory, becomes inactive and aggregates following ischemic insult such as seen with stroke or traumatic brain injury. The Hudmon Lab's working model is that loss of CaMKII signaling exacerbates glutamate excitotoxicity, in turn inducing astrocytes to release neurotoxic levels of ATP, causing secondary cell death. CaMKII can autophosphorylate resulting in autonomous activity, and research by Hudmon Lab reveals that CaMKII will inactivate and self-associate when activating under low pH and low ATP conditions. CaMKII is coded by four genes, and for this study we focus on the alpha and delta isoforms. Alpha is found primarily in neurons and readily aggregates under conditions mimicking ischemic stress. However, delta is primarily expressed in astrocytes and its response to ischemic stress is uncharted territory. We ask how CaMKII delta self-associates under autophosphorylation conditions mimicking ischemic stress (low pH, low ATP) and how it differs from CaMKII alpha self-association. We use real-time light scattering and sedimentation assay to elucidate the time-course of delta aggregation as well as the kinase's sensitivity to differing pH and ATP concentrations. Light scattering suggests that alpha and delta have a similar aggregation profile, but also that delta has reduced light scattering over time. Sedimentation analysis suggests delta truly does aggregate under these conditions and that it undergoes a molecular weight shift, indicative of autophosphorylation-induced inactivation. In future studies, we plan to investigate delta's kinase activity under aggregation conditions, perform the same experiments detailed here on the gamma isoform, and investigate delta and gamma aggregation directly in astrocytes. If we can understand how all the isoforms of CaMKII aggregate, it may prove to be a novel research topic for therapies aimed at neuroprotection in victims of ischemic insults.

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