FLUORESCENCE MICROSPECTROSCOPY FOR TESTING THE DIMERIZATION HYPOTHESIS OF BACE1 PROTEIN IN CULTURED HEK293 CELLS

SPENCER GARDEEN, JOSEPH L. JOHNSON, AHMED A HEIKAL, *Chemistry and Biochemistry, University of Minnesota Duluth, Duluth, MN, USA*.

Alzheimer's Disease (AD) is a neurodegenerative disorder that results from the formation of beta-amyloid plaques in the brain that trigger the known symptoms of memory loss in AD patients. The beta-amyloid plaques are formed by the proteolytic cleavage of the amyloid precursor protein (APP) by the proteases BACE1 and gamma-secretase. These enzyme-facilitated cleavages lead to the production of beta-amyloid fragments that aggregate to form plaques, which ultimately lead to neuronal cell death. Recent detergent protein extraction studies suggest that BACE1 protein forms a dimer that has significantly higher catalytic activity than its monomeric counterpart. In this contribution, we examine the dimerization hypothesis of BACE1 in cultured HEK293 cells using complementary fluorescence spectroscopy and microscopy methods. Cells were transfected with a BACE1-EGFP fusion protein construct and imaged using confocal, and differential interference contrast to monitor the localization and distribution of intracellular BACE1. Complementary fluorescence lifetime and anisotropy measurements enabled us to examine the conformational and environmental changes of BACE1 as a function of substrate binding. Using fluorescence correlation spectroscopy, we also quantified the diffusion coefficient of BACE1-EGFP on the plasma membrane as a means to test the dimerization hypothesis as a fucntion of substrate-analog inhibitition. Our results represent an important first towards examining the substrate-mediated dimerization hypothesis of BACE1 in live cells.