

P002 β -secretase activity in human platelets
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β -site Amyloid Precursor Protein (APP) Cleaving Enzyme (BACE) catalyses β -secretase type cleavage of APP to generate the A β peptide. With time, the A β peptide aggregates and results in the formation of the amyloid plaques that characterise Alzheimer's disease (AD) neuropathology. Human platelets are known to contain full-length and processed APP. We examined the presence of a β -secretase-like proteolytic activity in human platelet samples. Platelets were isolated from blood from 42 AD and 29 control individuals, lysed (25 mM HEPES, pH 7.2) and fractionated into particulate and soluble fractions by centrifugation at 100,000 g for 1 h. The particulate fraction pellet was re-suspended in lysis buffer containing 2% CHAPS. Samples, corrected for protein concentration, were assayed for β -secretase activity in triplicate using the internally-quenched fluorogenic substrate (MCA)EVKMDAEFK(DNP)-NH₂ (Calbiochem) in 50 mM sodium acetate, pH 4.5 at 37°C. Activity was assayed continuously over 30 min by excitation at 325 nm and emission at 393 nm using a fluorescence spectrophotometer. Our study indicated that robust β -secretase-like activity was detectable in platelets, in AD samples and control samples.