A Reagentless Sensor Based on Fatty Acid Amide Hydrolase

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Fatty acid amide hydrolase (FAAH) is an integral membrane protein that participates in termination of cannabinoid signalling mechanisms by catalysing the hydrolysis and thus inactivation of the endogenous cannabinoid anandamide and other natural lipids involved in signalling. Inhibition of FAAH activity, which increases the anandamide levels, is implicated in the treatment of pain, inflammation and many other disorders. Therefore, compounds that inhibit this enzyme are of medical and therapeutic significance.

The aim of the current work is to create an optical biosensor in order to test substrate/inhibitor effects of FAAH. Mutants of the rat FAAH protein (rFAAH) were engineered with unique surface accessible cysteine residues for site-specific labelling with fluorescent probes. The cysteine mutations (C269S and C299S) were introduced in regions of the FAAH protein where structural changes occur upon ligand/inhibitor binding. Both mutants and the wild type protein were purified from the membrane fractions after expression in *E. coli* BL21. Yields of about 1mg of pure protein per litre of culture were obtained for wild type and both mutants. The activity of the purified proteins was measured using aminomethyl coumarin arachidonoyl amide (AMC-AA) as the substrate. This substrate upon hydrolysis releases the fluorescent AMC whose emission was measured at 445nm. A selected group of Cysteine-specific fluorescence probes were tested for labeling of the mutants. Labeling experiments were carried out with 10x molar excess of the probe at 4C for 1-3 hrs. Excess label was removed by gel filtration and the labeling ratio measured spectrophotometrically. Samples with labeling ratios of around 1 were then tested for inhibition of the FAAH activity. Data will be presented on the fluorescence intensity changes upon titration with the strong FAAH inhibitor, MAFP (methyl arachidonyl fluorophosphonate). These preliminary data provide the basis for a reagentless sensor of FAAH.