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Development of Fluorescence Resonance Energy Transfer-Based Nanosensor for Measurement of Sialic Acid in vivo

Authors : Ruphi Naz, Altaf Ahmad, Mohammad Anis

Abstract : Sialic acid (5-Acetylneuraminic acid, Neu5Ac) is a common sugar found as a terminal residue on glycoconjugates in many animals. Humans brain and the central nervous system contain the highest concentration of sialic acid (as N-acetylneuraminic acid) where these acids play an important role in neural transmission and ganglioside structure in synaptogenesis. Due to its important biological function, sialic acid is attracting increasing attention. To understand metabolic networks, fluxes and regulation, it is essential to be able to determine the cellular and subcellular levels of metabolites. Genetically-encoded fluorescence resonance energy transfer (FRET) sensors represent a promising technology for measuring metabolite levels and corresponding rate changes in live cells. Taking this, we developed a genetically encoded FRET (fluorescence resonance energy transfer) based nanosensor to analyse the sialic acid level in living cells. Sialic acid periplasmic binding protein (sia P) from Haemophilus influenzae was taken and ligated between the FRET pair, the cyan fluorescent protein (eCFP) and Venus. The chimeric sensor protein was expressed in E. coli BL21 (DE3) and purified by affinity chromatography. Conformational changes in the binding protein clearly confirmed the changes in FRET efficiency. So any change in the concentration of sialic acid is associated with the change in FRET ratio. This sensor is very specific to sialic acid and found stable with the different range of pH. This nanosensor successfully reported the intracellular level of sialic acid in bacterial cell. The data suggest that the nanosensors may be a versatile tool for studying the in vivo dynamics of sialic acid level non-invasively in living cells

Keywords : nanosensor, FRET, Haemophilus influenzae, metabolic networks
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