Reconstitution of Split Green Fluorescent Protein for Monitoring Conformationally Changed Protein

Jinyoung Jeong, Sangkyu Kim, Junhyoung Ahn, Kyung-Sook Park, Eunju Jeong, Moonil Kim†, and Bong Hyun Chung†

BioNanotechnology Research Center, Korea Research Institute of Bioscience and Biotechnology, P.O. Box 115, Yuseong, Daejeon 305-600, Republic of Korea

E-mail chungbh@kribb.re.kr; or kimm@kribb.re.kr; fax

TEL: +82-42-860-4445, FAX: +82-42-879-8594

We describe a new method for the detection of conformational change based on reconstitution of split green fluorescent protein (GFP). In this study, we used fluorescence complementation assay, which was designated (FCA), for monitoring conformationally changed protein. Particularly, we used maltose binding protein (MBP) as a model protein, as MBP undergoes its hinge-twist movement upon binding of the substrate. The common feature of this approaches that GFP as a reporter protein is split into two non-fluorescent fragments, which are genetically fused to N- and C-termini of MBP. Upon the binding of maltose, chromophores come closer together, leading to fluorescence production. This split GFP method involves the reconstitution of GFP, which is monitored by restored fluorescence intensity. As results, reconstituted GFP was observed to represent the fluorescence upon maltose binding *in vitro*, allowing the direct detection of the change of fluorescence intensity in response to maltose in a concentration- and time-dependent manner. Our results demonstrated that fluorescence complementation assay (FCA) can be used to monitor conformational alteration of target protein, providing a number of scientific and medical applications.

Reference

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