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Measurement of IP_3 by the Competitive Fluorescent Ligand Assay (CFLA) method

Azmeree JAHAN, Akihiro NEZU, and Akihiko TANIMURA

Division of Pharmacology, School of Dentistry, Health Sciences University of Hokkaido

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Inositol 1,4,5−trisphosphate (IP_3) is an important intracellular messenger in Ca^{2+} signaling and is involved in numerous responses to hormones, neurotransmitters, and growth factors. The IP_3 releases Ca^{2+} from intracellular stores through the IP_3 receptor (IP_3R), and this makes IP_3 measurement advantageous. There are several methods to measure IP_3, including (1) AlphaScreen technology (Taouji et al., 2009), and (2) fluorescence polarization for detection of the binding of IP_3 binding proteins and fluorescent analogues of IP_3 (Rossi & Taylor, 2011). These methods, however, required large amounts of IP_3 binding protein and accurate concentrations of functional binding sites. Presently, a conceptually new method for measuring IP_3, the Competitive Fluorescent Ligand Assay for IP_3 (CFLA−IP_3) has become available.

The CFLA－IP_3 uses Fluorescence Resonance Energy Transfer (FRET) which occurs between two different fluorescent molecules, CFP−labeled ligand binding domains of IP_3R (CFP−LBD), and fluorescent ligand (FL). The binding of FL to CFP−LBD causes FRET, and IP_3 competes with the FL in the binding to the LBD, and thereby decreases the FRET signal (Fig. 1).

Oura et al., developed a new high affinity fluorescent ligand for IP_3R, fluorescent adenophostin A by introducing the fluorescein unit at the 5'−position of ADA and its low affinity analogue (F−LL). It has been shown that the binding of these FL and CFP−LBD decreased the CFP signal and increased the FL signal due to FRET. In addition, F−LL−induced changes in the fluorescence ratio (CFP/FL) were reduced by the addition of IP_3 in a concentration−dependent manner. This method was further applied to measure the IP_3 concentration in cytosolic fractions of COS−7 cells with and without stimulation by ATP, and to examine the potency of IP_3R ligands.

These results show that the CFLA method is reliable, and the principles of this method are simply applicable to any receptor for which a fluorescent ligand is available.

References

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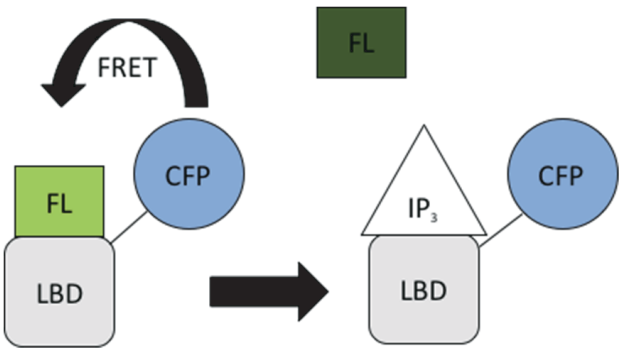

Figure 1. Competitive Fluorescent Ligand Assay for IP_3 (CFLA−IP_3). FL : Fluorescent ligand, CFP : Cyan fluorescent protein, LBD : Ligand binding domain of IP_3R.