Single-well simultaneous measurement of MAP2K MEK1 activity and interaction with the MAP kinase ERK2 using AlphaScreen and AlphalISA® platforms

Mathieu Arcand, Philippe Roby, Roger Bossé, Sophie Dahan, Nathalie Rouleau, Martina Bielefeld-Sénéguy

Abstract

Protein kinases play crucial regulatory roles in important biological processes and represent attractive pharmacological targets. Most of today's kinase inhibitors block target phosphorylation by competing with ATP for binding to the catalytic cleft of the kinase. There is increasing demand for less promiscuous compounds including allosteric and protein-protein interaction modulators representing technological challenges for several screening approaches. Here we describe how AlphaScreen® and AlphalISA® platforms were used for the simultaneous detection of biomolecular interaction and phosphorylation. These techniques have proven to be versatile and powerful tools for monitoring protein-protein interactions as well as phosphorylation events. MEK1 and its substrate ERK2 were used as model. In their un-phosphorylated state, they form a complex that dissociates upon activation by phosphorylation. Data will be shown where MEK1 activity on ERK2 and binding are measured simultaneously. For a compound screen, such a twin detection set-up could provide immediate discrimination between ATP competitors, interaction inhibitors, and allosteric modulators.

Introduction

Activated by numerous stimuli, the ERK MAP kinase cascade is a key regulator of proliferation and cell death. Understanding how MEK1 and ERK2 interact is important for understanding many of the activities of this cascade and ERK2/2 and their upstream activators MEK1/2. We have used MEK1 and ERK2 as an experimental model to simultaneously monitor phosphorylation and interaction events.

Although, many reports on MEK1 and ERK2 regulation exist, phosphorylation and interaction events have always been observed separately. Here, we propose a method to monitor simultaneously both types of biomolecular events using AmpliLight Luminescence Prestained Homogeneous Assay (Alpha) technology, with MEK1 and ERK2 as an experimental model.

MEK1–ERK2 interactions and activities. In the unphosphorylated state, MEK1 and ERK2 are highly soluble. Activated by phosphorylation, MEK1-phosphorylated and unphosphorylated ERK2, moving in ERK2 activation and complex formation. Alpha technology relies on the binding of Donor and Acceptor beads. Excitation of Donor beads at 680 nm generates the release of singlet oxygen that can diffuse for 200 µm before losing its capacity to trigger light emission from Acceptor beads. This makes the technology suitable for observing many biochemical pathways such as phosphorylation reactions and protein-protein interactions.

Both AlphaScreen and AlphalISA Coposite antibodies are activated by singlet oxygen to produce light. In contrast, the fluorophores used in each Acceptor bead differ, thus conferring different emission properties to AlphaScreen and AlphalISA Acceptor beads. We took advantage these properties to simultaneously monitor phosphorylation and interaction events.

4 Methods

All assays were performed in 384 well plates (PerkinElmer) at 23°C in kinase buffer containing 20 mM Tris pH 7.4, 150 mM NaCl, 1 mM MgCl₂, 1 mM DTT, 0.1% Nonidet P-40 and 100 µM ATP. The MEK1 and ERK2 protein kinase reactions were incubated for 2h before addition of detection reagents. Donor and Acceptor beads were used at 20 µg/ml for each type and batches were run according to the AlphaScreen protocol. For phosphorylation-interaction assays, Donor beads were used at 40 µg/ml and AlphalISA® and AlphalISA Acceptor at 20 µg/ml. Assays were read twice with unmodified Envision readers, first with a stopgap 912 nm narrow bandwidth filter, then with a sumgap 915 nm narrow bandwidth filter.

5 MEK1-ERK2 Interaction

5.1 MEK1-ERK2 interactions can be detected with an antibody-free AlphalISA set up, and activation state of either protein greatly influences binding.

6 ERK2 Phosphorylation by MEK1

6.1 MEK1 kinase assay with ERK2. Kinase reactions were incubated at 30°C for 30min with 50 mU MEK1, 1 µg ERK2 and 100 µM ATP. MEK1 is highly soluble and ERK2 is phosphorylated by the presence of 0.5 µM ATP.

7 Phosphorylation-Interaction Assay

We have provided the first direct evidence that ERK2 phosphorylation triggers its dissociation from active MEK1. Both biomolecular events are intrinsically linked with interaction ERK2, matching ERK2 phosphorylation ERK2.

8 Competitor Titration Assays

Although, the same inhibitors affect MEK1 and ERK2 binding, their active MEK1 phosphorylation mechanism differs. The poorly selective ATP competitor causes competition with the phosphorylation, while partially rescuing its interaction with MEK1. OUI12, a reported Raf and MEK1/2 inhibitor displays allosteric effects on MEK1. In contrast, the MEK1/2 inhibitor SL327 affected MEK1 catalytic activity, but not its interaction with ERK2.

9 Pharmacological MEK1 inhibition

10 Summary

- MEK1-ERK2 binding was measured with AlphalISA beads
- The phosphorylation/activation states of both proteins affects their physical interaction
- ERK2 phosphorylation was detected with AlphalISA beads
- Both biomolecular events were simultaneously monitored by combining AlphalISA and AlphalISA beads
- These biomolecular events have also been monitored with the kinase pairs Raf1-MEK1 and Abl1-GSK3 (data not shown)
- Upon ERK2 phosphorylation, there is a marked decrease of its interaction with MEK1
- MEK1 phosphorylation and binding can be disrupted with a specific peptide or, more potently, by untagged ERK2
- Simultaneous monitoring of phosphorylation and interaction events allowed us to delineate distinct mechanisms of action for three MEK1 inhibitors: Staurosporine, OUI12 and SL327