BMC Pharmacology

Poster presentation

Identification of novel target proteins of cGMP/cAMP signaling pathway using cGMP/cAMP capture compound mass spectrometry (CCMS)

Yan Luo*, Olivia Baessler, Mirko Glinski, Michael Sefkow, Mathias Dreger and Hubert Koester

Address: Caprotec bioanalytics GmbH, Volmerstr 5, Berlin, Germany

Email: Yan Luo* - yan.luo@caprotec.com

* Corresponding author

from 4th International Conference of cGMP Generators, Effectors and Therapeutic Implications
Regensburg, Germany. 19–21 June 2009

Published: 11 August 2009

BMC Pharmacology 2009, **9**(Suppl 1):P42 doi:10.1186/1471-2210-9-S1-P42

This abstract is available from: http://www.biomedcentral.com/1471-2210/9/S1/P42

Background

The cyclic nucleotide monophosphates cGMP and cAMP play an essential role in many signaling pathways. The identification and profiling of cGMP- and cAMP-binding proteins is an important step in order to elucidate the molecular basis of these pathways. In order to develop a tool to specifically target cGMP- and cAMP-binding proteins, we synthesized cGMP- and cAMP Capture Compounds™ (CCs). CCs are trifunctional small molecule probes that carry a small molecule as the selectivity function attached to a scaffold that in addition contains a photo-activatable reactivity function and biotin as a sorting function. We here explored the capability of the cAMP- and cGMP Capture Compound Mass Spectrometry (CCMS) approach to display the proteome subset of cGMP-and cAMP-binding proteins.

Methods

In the CCMS workflow, the selectivity group (in this case, derivatives of cGMP or cAMP) first provides equilibrium binding of the CC to the target proteins. Second, upon irradiation, the reactivity function freezes this interaction through covalent cross-link to the target proteins. Third, using streptavidin magnetic beads, the CC-protein conjugates can be easily isolated from the complex protein mixture via the sorting function of the CC. The captured proteins are then directly subjected to proteolytic on-bead cleavage and automated LC-MS/MS based protein identification. We applied this workflow to protein mixtures from human derived HepG2 cells, and solubilized proteins from rat synaptosomes.

Results

From HepG2 cell lysate (500 µg protein input), regulatory subunits of PKA as well as cGMP-dependent protein kinases were captured and identified. From solubilized rat synaptosomes (60–80 µg protein input), in addition to the kinase subunits, several phosphodiesterases, Rap guanine nucleotide exchange factors, as well as HCN ion channels were captured and identified, with partially overlapping, but also partially distinct selectivity profiles observed with different CCs. In addition, several potential and *bona fide* interaction partners of cyclic nucleotide-binding proteins, such as IP3-receptors and A-kinase anchoring proteins were identified as well.

Conclusion

We here demonstrate that CCs with derivatives of cGMP or cAMP as selectivity group are unique tools to specifically target proteins in the cGMP/cAMP-dependent signaling pathways. Notably, cGMP-/cAMP-CCMS can successfully address even low-abundant transmembrane ion channels at an unprecedented sensitivity from minute amounts of complex protein mixtures.