Fast kinetics enzyme assay High-throughput screening of prolyl isomerase inhibitors

Abstract

The peptidyl-prolyl cis-trans isomerases (PPIases), or rotamases, are a family of ubiquitous enzymes that catalyze the cis-trans isomerization of the peptide bond on the N-terminal side of proline residues in proteins. PPIases are divided into three classes, cyclophilins (Cyps), FK-506 binding proteins (FKBPs) and the Pin1/parvulin class.
Cyclophilin(Cyp) is one of the key enzymes and plays critical roles during many biological processes and the unique enzyme Pin1 plays a key role in the pathogenesis of Alzheimer's disease and many human cancers. Pin1 also regulates the cell cycle and is a necessary enzyme for cell division. For these reasons, cyclophilins have emerged as potential drug targets for several diseases. Therefore, it is extremely important to screen for novel small molecule cyclophilin inhibitors.
Recently, a real time fluorescence monitoring system for Cyclophilin A was reported on a high-throughput screening format. PPIase activity has been assessed by assays based on protease-coupled isomer specific proteolysis using tetrapeptide derivatives, Ala-Xaa-Pro-Yaa-(pNA/MCA). Cyclosporin A inhibits the PPIase activity, and it has been used as tool compound during the setup.

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Suc-Ala-Ala-Pro(cis)-Phe-MCA
⬇ CypA
Suc-Ala-Ala-Pro(trans)-Phe-MCA
⬇ chymotrypsin
Suc-Ala-Ala-Pro-Phe-COOH + AMC

Screening using FDSS7000/6000/3000/uCell
AMC (λex=340 nm to 380 nm; λem=460 nm)

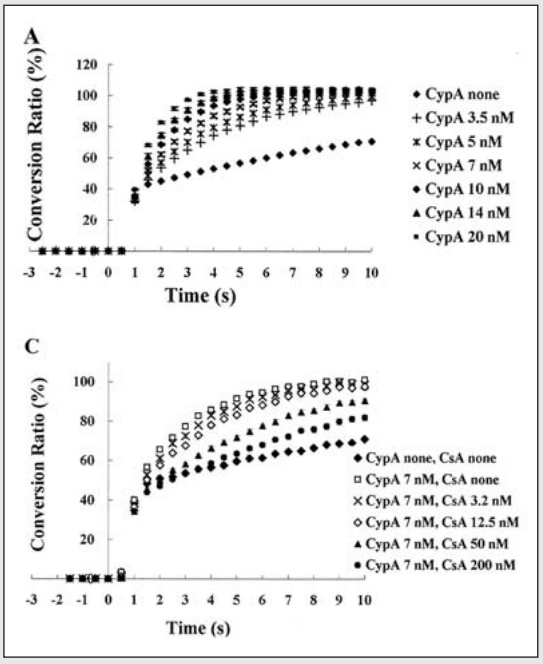

Fig. 1: Cyclophilin A (CypA) peptidyl-prolyl isomerase (PPIase) assay.

The protease-couple CypA PPIase assay carried out by measuring the increase of fluorescence intensity from liberated 7-amino-4-metylcoumarin(AMC). Then 15 μM fluorogenic peptide substrate that had been proline isomerized was hydrolyzed by 50 μM chymotrypsin, an isomer-specific protease.
(A) Time course of CypA activity. The reaction was performed for 60s, and at the beginning of 10 sec, the reaction was shown because the CypA-catalyzed cis-trans isomerization rate is extremely fast.
The fluorescence of the liberated AMC in each well was detected simultaneously by the FDSS6000 real time fluorescence detector. Activity was measured at the indicated concentrations of enzyme.
(C) Concentration dependence of CsA inhibition of CypA activity. Concentration of CypA and CsA are expressed as follows.

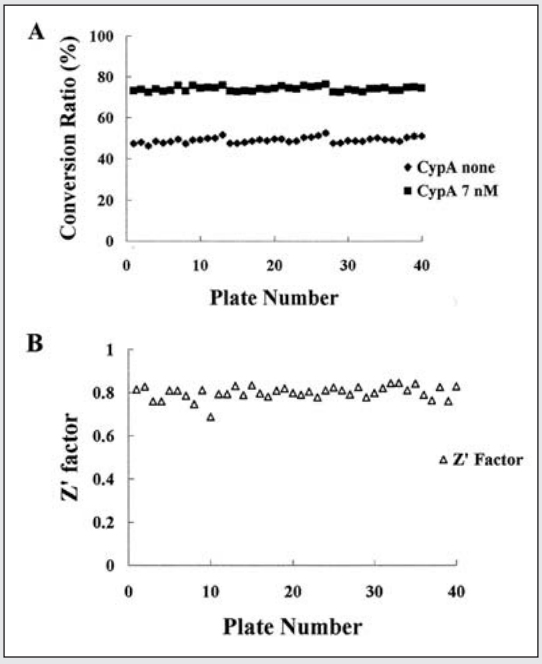

Fig. 2:

Evaluation of cyclophilin A(CypA) peptidyl-prolyl cis-trans isomerase (PPIases) assay performance. Forty 384-well plates that contain 16 control (CypA 7nM) and blank (CypA nne) wells, respectively, were used to determine the suitability of the assay for high-throughput screening. (A) The conversion ratio of substrate for each well was recorded, and average values corresponding to each plate were plotted. (B) The Z' factores were calculated for the each plate.

Summary

Dr. Mori and Prof. Uchida (Tohoku university) have established the High-Throughput Screening method for Prolyl Isomerase Inhibitors using a Real-Time Fluorescence Monitoring System. To use this method, Their group have finished an HTS successfully.

References

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