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Characterisation of drug-like inhibitors of deadenylase enzymes by AMP detection and differential scanning fluorimetry

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Introduction:

In eukaryotes, the removal of the poly (A) tail of cytoplasmic mRNA (deadenylation) is a crucial step in post-transcriptional gene regulation. The main enzymes involved in regulated mRNA deadenylation are the Caf1 and Ccr4 ribonucleases whose activities are attributed to the DEDD (Asp-Glu-Asp-Asp) or the EEP (endonuclease-phosphatase) domain respectively. Two magnesium ions are required at the active site for activity[1]. These enzymes are possible drug targets in diseases such as metastatic cancer, osteoporosis, bone repair and obesity. To facilitate the discovery, development, and characterisation of small drug-like inhibitors of these enzymes, we recently developed a sensitive fluorescence-based assay for deadenylase activity based on detection using an oligonucleotide probe complementary to the RNA oligonucleotide substrate[2]. The aim of this study was to develop more biochemical assays useful for characterisation of molecular inhibitors.

Method:

First, we expressed human Caf1/CNOT7 enzyme in bacteria cells and purified to homogeneity using His-trap affinity chromatography. Next, we used a chemiluminescence-based detection assay of AMP, a reaction product of deadenylase enzymes to determine enzyme activity as a function of concentration in comparison with recently developed fluorescence-based assay. Inhibitory Concentrations (IC_{50}) of drug-like molecules of Caf1/CNOT7 was also determined. In addition, differential scanning fluorimetry (DSF) also known as thermal shift assay was evaluated as a means to characterise binding of compounds to Caf1/CNOT7 enzyme. IC_{50} and thermal shift data are given as mean ± SEM (n=3).

Results

The results indicate that a minimal amount of enzyme is required for assays using the AMP detection when compared to the fluorescence-based assay which requires a ten-fold amount of enzyme. The IC_{50} values determined comparing both methods were shown to be in a similar range. In addition, thermal shift assays showed enzyme inhibition by a shift in curve in the presence of the inhibitors.

Conclusion

The AMP detection is a highly sensitive assay with low background/noise ratio that can be used as an alternative to the previously developed fluorescence-based assay for screening of compound libraries and the determination of IC_{50} values. Also, the DSF is a useful assay to determine the binding mode of inhibitory compounds. These assays complement existing tools available for the characterisation of drug-like inhibitors of deadenylase enzymes.

References

1. Winkler and Balacco, 2013.

2 Maryati et al, 2014.

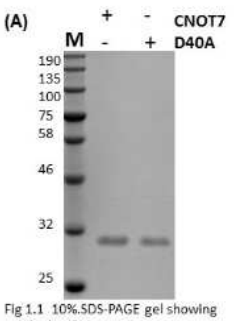

Fig 1.1 10%.SDS-PAGE gel showing Purified Caf1/CNOT7

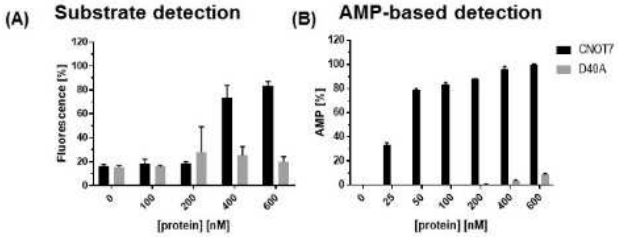

Figure 1.2 . Dose/response activity assay-fluorescence-based assay compared to AMP-based assay.

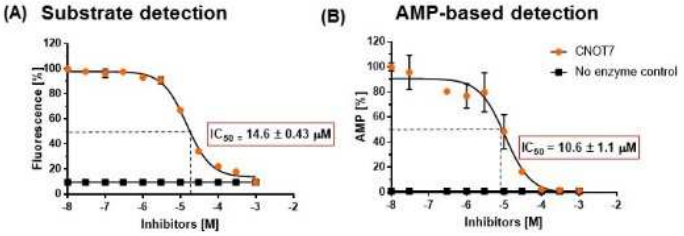

Figure 1.3 Determination of IC50 comparing fluorescence-based assay and AMP product-based assay

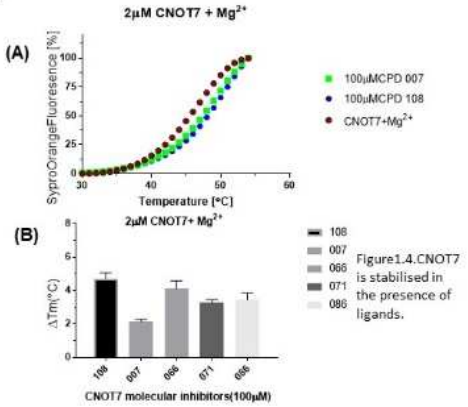

Figure1.4.CNOT7 is stabilised in the presence of ligands.