Measurement of Proteosome Inhibition in Live Cells on the Analyst® GT, FLEXstation® and Gemini EM Microplate Readers Using the BD Biosciences Proteasome Sensor

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Introduction

The proteasome is a massive protein complex inside all eukaryotic cells (and some bacteria) that removes unnecessary proteins by breaking them down into short peptides. It consists of a tunnel-like core with a cap at each end (Figure 1). The caps recognize and bind to the targeted proteins and inject them into the central core that acts as a degradation chamber. In most cases, selected proteins become targeted for degradation when "labeled" with several copies of a small protein tag called ubiquitin.

The proteasome is crucial in cellular regulation because controlled proteolysis of key cellular factors regulates numerous biological processes such as cell cycle, differentiation, stress response, neuronal morphogenesis, cell surface receptor modulation, secretion, DNA repair, transcriptional regulation, long-term memory, circadian rhythms, immune response, and biogenesis of organelles[1]. Aberrations in proteasome activity are implicated in the pathogenesis of many diseases, including cancer.

To develop treatments for proteasome-related diseases, it is useful to identify compounds that modulate its activity. BD Biosciences Clontech has recently introduced the **BD Living Colors™ HEK 293 ZsGreen Proteasome Sensor Cell Line** that allows noninvasive monitoring of proteasome activity[2]. It was obtained by stably transfecting HEK 293 cells with the Proteasome Sensor Vector (pZsProSensor-1). The Protein Sensor Vector encodes a destabilized version of a green fluorescent protein, (ZsGreen ; zFP506.1) that is degraded by the proteasome and does not require ubiquitin modification[3]. To convert ZsGreen into a proteasomal substrate, its C-terminus was fused to a specific degradation motif that targets the fusion for removal by the proteasome. Under normal conditions, the fluorescence of the cells is very low because ZsProSensor-1 protein is rapidly degraded. However, under conditions where proteasome activity is inhibited, fluorescence increases as the ZsProSensor-1 protein accumulates. This live cell assay is highly sensitive and can be monitored by fluorescent microscopy, flow cytometry, and microplate fluorimetry. Below, we show that inhibition of proteasome activity by ALLN, a well-characterized proteasome inhibitor, can easily be measured Molecular Devices microplate fluorimeters.

Analyst® GT multimode reader delivers unsurpassed performance in a system that is designed for seamless integration into both workstation and robotic environments. The Analyst is designed to give top-quality data in fluorescence intensity, fluorescence polarization, time-resolved fluorescence (including HTRF®), absorbance, and luminescence in 96, 384, and 1536 well plate formats. Plates can be read from either the top or the bottom in fluorescence intensity and TRF modes and the user can choose to focus anywhere in the well with the software controllable z-height adjustment.

The FLEXstation® is a benchtop scanning microplate fluorimeter with integrated fluid transfer capability. The Gemini EM is a benchtop scanning microplate fluorimeter with bottom read capability.

Proteasome

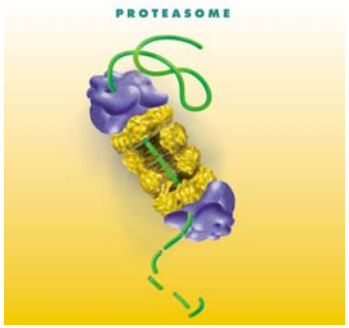

Figure 1. Image of the proteasome. US Department of Energy Genomics:GTL Program, http://www.ornl.gov/hgmis

Materials and Methods

Materials:

HEK 293 ZsGreen Proteasome Sensor Cell Line. BD Biosciences Clontech, Cat No. 631535.
Acetyl-leu-leu-norleu-al (ALLN) CalBiochem Cat. No. 208719
Black wall clear bottom 96-well microplate, Costar Cat No.3603

Analyst® GT multimode reader
FLEXstation Scanning fluorimeter & Integrated fluid transfer workstation
Gemini EM Scanning microplate fluorimeter

Methods:

The ALLN stock solution was prepared by dissolving in DMSO to a concentration of 10 mM. The HEK 293 ZsGreen Proteasome Sensor Cells were cultured in DME + 10% FBS + 1% Pen/Strep/L-glutamine + 200 ug/ml of G418. They were seeded overnight in 100 ul at a density of 30,000 cells/well in a Costar black wall /clear bottom 96-well plate. The cells were then treated with ALLN at dose of 0, 0.1, 0.3, 1, 3, 10 and 100 uM for 20 hours (N=12/group). The green fluorescence of ZsProsensor-1 in non-treated and treated cells was measured from the bottom in the three microplate readers.

For the FLEXstation and Gemini EM, the settings were Ex/EM = 484/510 with a 495 nm emission cutoff filter and PMT set to Auto. For the Analyst GT, the band-pass filters were 485-20 excitation and 530-25 emission. A 50% transmission dichroic mirror was used. The sensed volume was focused at 1 mm above the well bottom.

Dose Response Curves

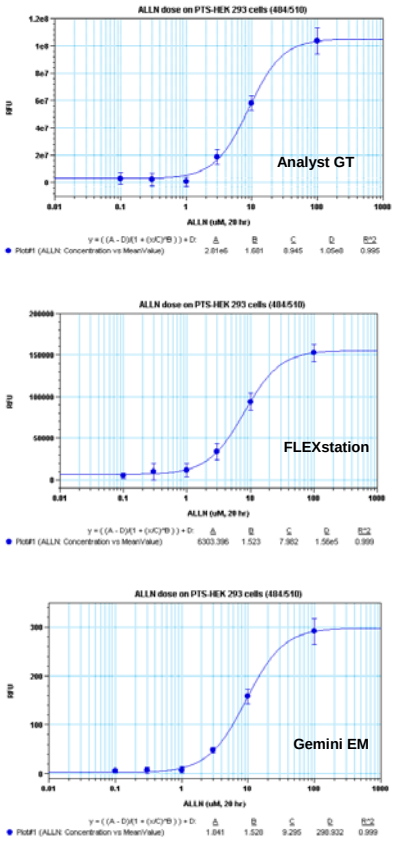

Figure 2. Proteasome inhibition in the HEK 293 ZsGreen Proteasome Sensor Cell Line measured in Analyst® GT (A), FLEXstation (B), and Gemini EM (C). The Z' factors ranged from 0.54 to 0.66 for the three instruments at the 10 uM dose level.

Abstract

Protein degradation by the proteasome is crucial to normal cell function. Disruption of proteasome activity is involved in many pathological conditions including cancer. Thus considerable attention is currently being given to identifying compounds that modulate proteasome activity. BD Biosciences Clontech has recently introduced the BD Living Colors™ HEK 293 ZsGreen Proteasome Sensor Cell Line that allows noninvasive monitoring of proteasome activity. The cell line is stably transfected with the Proteasome Sensor Vector which encodes a destabilized version of a green fluorescent protein. Under normal conditions, the protein is rapidly degraded and the fluorescent signal is very low. If proteasome activity is inhibited, the protein accumulates and fluorescence increases.

In this poster, we show that the dose-dependent inhibition of proteasome activity by ALLN (a known proteasome inhibitor) can easily be measured in the Analyst® GT, the FLEXstation® and the Gemini EM. At 10 uM (~the EC_{50}), the Z' factor was 0.54 – 0.66. Thus this assay system is well-suited for high-throughput screening of candidate modulators of proteasome activity in the Molecular Devices microplate fluorimeters.

Results and Conclusions

As expected, ALLN inhibited proteasome activity in a does-dependent manner (Figure 2). The EC_{50} was approximately 10 uM. At that concentration, the Z' factor was 0.54 – 0.66. Thus this assay system is well-suited for high-throughput screening of candidate modulators of proteasome activity.

References

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2. Clontechniques April 2004, 'http://www.bdbiosciences.com/clontech/archive/APR04UPD/Living_Colors.shtml')
3. Clontechniques April 2003, ('http://www.bdbiosciences.com/clontech/archive/APR03UPD/pdf/ProteasomeSensor.pdf')

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