Detection of PARP-induced ADP-ribosylation using a BMG LABTECH microplate reader

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- **PARP enzyme activity determined using a chemiluminescence assay**
- **The luminescence readout is reproducible and linear over a wide enzyme concentration range**
- **The kinetic parameter K_M and the IC_{50} value for an enzyme inhibitor were calculated**

Introduction

PARP (Poly(ADP-ribose) polymerase) family enzymes are involved in the regulation of transcription, DNA repair, and chromatin remodeling. These enzymes use nicotinamide adenine dinucleotide (NAD) as a substrate to build poly(ADP-ribose). Due to various links to diseases, PARP enzymes are targets for pharmaceutical drug development.
In this application note we describe the use of a chemiluminescent assay to determine PARP activity on the CLARIOstar multimode microplate reader. The assay allows kinetic analysis of PARP enzymes and evaluation of inhibitor potency.

Assay Principle

PARP activity is followed *in vitro* by detecting the incorporation of biotinylated ADP-ribose as a consequence of either enzyme target protein modification or auto-modification. The reaction principle is shown in Fig. 1

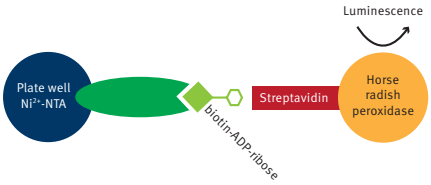

Fig. 1: PARP chemiluminescent assay principle.

Hexahistidine-tagged PARP enzyme or protein substrate is immobilized on Ni^{2+}-chelating microplates. The reaction is started by adding biotinylated NAD^+. The PARP enzyme uses the NAD^+ to synthesize biotinylated poly(ADP-ribose). This polymer is either added to the PARP enzyme itself or transferred to a protein substrate on the microplate (histone). After a washing step streptavidin-conjugated horseradish peroxidase is added and will bind to the biotinylated poly(ADP-ribose). After adding a substrate to the horseradish peroxidase, chemiluminescence is released and can be measured.

Materials & Methods

- Ni-NTA-coated, opaque, white 96-well microplates (5-PRIME)
- Streptavidin-conjugated horseradish peroxidase (Jackson Immunoresearch)
- Biotinylated NAD^+ (Trevigen)
- SuperSignal West Pico chemiluminescent substrate (Thermo Fisher Scientific)
- Microplate reader from BMG LABTECH

All standard chemicals and disposables were obtained through normal distribution channels.

Enzymatic reactions

Hexahistidine-tagged PARP enzyme or protein substrate was immobilized on Ni^{2+}-chelating plates. ADP-ribosylation reactions were started by addition of NAD^+ (2 % biotinylated) at 20°C. Reactions were stopped by addition of 7 M guanidine hydrochloride. Plate wells were washed with reaction buffer, incubated for 30 minutes with TRIS-buffered saline containing 0.02 % Tween-20 (TBST) and 1 % (w/v) BSA, and washed with TBST. After incubation with streptavidin-conjugated horseradish peroxidase (0.5 µg/ml) another washing step was done. After adding SuperSignal West (50 + 50 µl, undiluted) as substrate for the peroxidase chemiluminescence was detected in the CLARIOstar microplate reader using the following instrument settings.

Instrument settings

All measurements (linear range check, K_M determination and inhibitor dose-response) were done in endpoint mode.

Optic:	top optic used
Measurement interval time [s]:	1.00
Presetname:	Enliten ATP
Emission:	full range (no filter)
Gain:	needs to be adjusted prior the measurement
Focal height:	needs to be adjusted prior the measurement

Results & Discussion

For validation of the ADP-ribosyltransferase assay the linear range of signals obtained by a dilution series of biotin-ADP-ribosylated enzyme was determined (Fig. 2). The results show that the signal is linear over a wide range of ADP-ribosyl concentrations.

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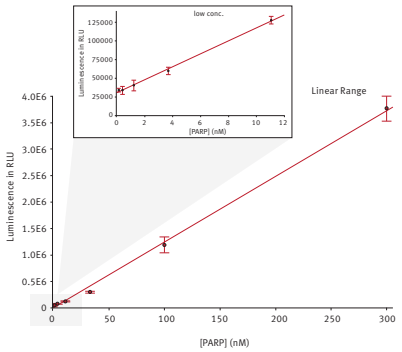

Fig. 2: A dilution series of a PARP-family enzyme under assay conditions, illustrating the linear range of the signal. The insert zooms into the low nM concentration range.

The kinetic parameters of a PARP enzyme family member were determined using initial reaction rates. Independent experiments showed that the biotin moiety linked to the co-substrate had no influence on the reaction kinetics (results not shown).

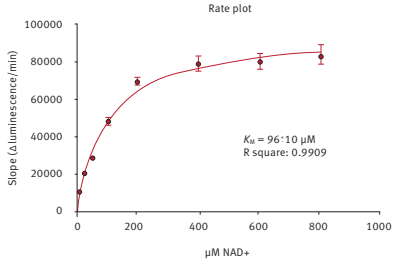

Fig. 3: Rates plot of the NAD$^+$-dependent ADP-ribosylation catalysed by a PARP-family enzyme.

Knowledge of K_M allowed the determination of inhibitor dose-response curves and experimental parameters (IC_{50}).

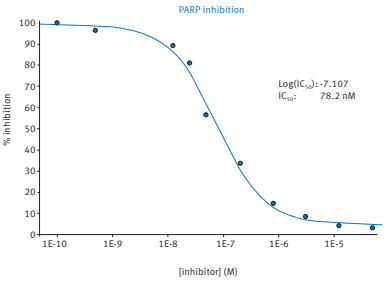

Fig. 4: Dose-response curve for inhibition of a PARP-family enzyme with a clinical PARP inhibitor (Olaparib).

Conclusion

An ADP-ribosylation assay of PARP enzymes carried out in a BMG LABTECH microplate reader shows signal linearity over a wide range of enzyme concentrations (0.015 to 300 nM). The assay allows enzyme characterization and calculation of different parameters that are important for the development of drug like enzyme inhibitors.