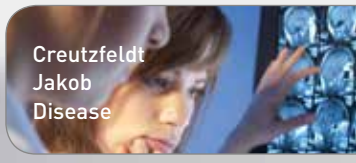
FLUOstar® Omega

Decrease time, increase throughput in prion and amyloid seeding assays



BMG LABTECH

The Microplate Reader Company

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Save time, run your seeding assays in days!

The FLUOstar® Omega microplate reader represents the best combination of performance and flexibility for all of your life science and R&D applications. In particular, it provides the perfect platform for the measurement of prion and amyloid seeding assays in the microplate format.

Thanks to its robustness and precision, the FLUOstar Omega was chosen by Rocky Mountain Labs, Montana, as the reference reader for the development of the RT-QuIC assay (Wilham et al., 2010), and for numerous peer-reviewed publications.

The FLUOstar Omega is a high-performance, automated microplate reader offering the following dedicated features for seeding assays:

- High quality German engineering and manufacturing for higher robustness and functionality
- Shaking and incubation over long periods of time (20-68 hours)
- Ability to withstand prolonged and continuous high-speed shaking
- Specifically designed microplate carrier for highest microplate stability even in thorough shaking conditions
- Linear, orbital, and double-orbital shaking
- Incubation up to 45°C or 65°C
- Top and bottom reading
- Data collection without interruption and output to BMG LABTECH's MARS data analysis software and/or Excel®
- Includes eight standard filters and dedicated multi-user Control and MARS data analysis software

Selected references

- Wilham JM et al., *PLoS Pathog.,* **2010.** Rapid end-point quantitation of prion seeding activity with sensitivity comparable to bioassays
- Kyle LM et al., *PLoS One,* **2013.** Introducing a Rigid Loop Structure from Deer into Mouse Prion Protein Increases Its Propensity for Misfolding *In Vitro*
- Haley NJ et al., *PLoS Pathog.,* **2013.** Prion-seeding activity in cerebrospinal fluid of deer with chronic wasting disease
- Mathiason CK et al., *J. Virol.,* **2013.** Susceptibility of domestic cats to chronic wasting disease
- John TR et al., *Prion,* **2013.** Early detection of chronic wasting disease prions in urine pf pre-symptomatic deer by real-time quaking-induced conversion assay
- Elder AM et al., *PLoS One,* **2013.** In vitro detection of prionemia in TSE-infected cervids and hamsters
- Peden et al., *Acta Neuropathol. Commun.,* **2014:** The prion protein protease sensitivity, stability and seeding activity in variably protease sensitive prionopathy brain tissue suggests molecular overlaps with sporadic Creutzfeldt-Jakob disease
- Orrú et. al., *N. Engl. J. Med.,* **2014:** A test for Creutzfeld-Jakob disease using nasal brushings
- Henderson DM et. al., *J. Gen. Virol.*, **2015**: Quantitative assessment of prion infectivity in tissues and body fluids by real-time quaking-induced conversion
- Orrú et. al., *J Clin Microbiol,* **2015:** Detection and discrimination of classical and atypical L-type bovine spongiform encephalopathy by real-time quaking-induced conversion

BMG LABTECH Microplate Reader used to Monitor Amyloid Formation

Sarah Shammas and Ann-Christin Brorsson, Department of Chemistry and Cavendish Laboratory, University of Cambridge

Introduction

Amyloid fibrils are implicated in a number of diseases, known as amyloidosis, including type II diabetes and Alzheimer's disease. Each disease is associated with the misfolding of a particular protein into linear aggregates (fibrils) that accumulate in the body's organs as plaques. Various different species may be formed during the aggregation process, including linear precursors known as protofilaments, which often appear in the early stages. Although only a handful of proteins are implicated in amyloidrelated diseases, there is evidence that amyloid formation could be a generic property of the polypeptide chain i.e. during appropriate (partially destabilising) conditions, "any" protein will form amyloid fibrils. Despite this property it is clear that the amino acid side chains contribute to the stability and the morphology of amyloid aggregates, as well as the kinetics of their formation. In this study various mutants of an amyloid forming protein V have been designed to investigate the effect of the mutation on the aggregation behavior.

Assay Principle

One group of fluorescent dye's which have been shown to bind to amyloid fibrils are the anilinonaphthalene sulphonic acid's (ANS). These dyes are not "amyloid-specific", they bind to proteins via exposed hydrophobic patches on the surface, and are traditionally used to demonstrate the presence of unfolded or partly folded proteins. In this study the dye bis-ANS has been used to follow the aggregation process of protein V and mutants to give information about the kinetics and the hydrophobic properties of the formed amyloid aggregates. Measurements were performed on a BMG LABTECH microplate reader set at 30°C under constant shaking.

Results and Discussion

Figure 1 shows the kinetic plot of the aggregation process for two mutants of protein V (X and W).
At time = 0 s protein X has a very low fluorescence signal, this increases until it reaches a fairly stable level at around 7000 s. The increase likely represents conversion of the monomer into mature fibrils with hydrophobic surfaces. The fluorescence with protein W increases over the same timescale as protein X, however the signal for protein W is consistently around 2500 a.u. higher than the signal for protein X.

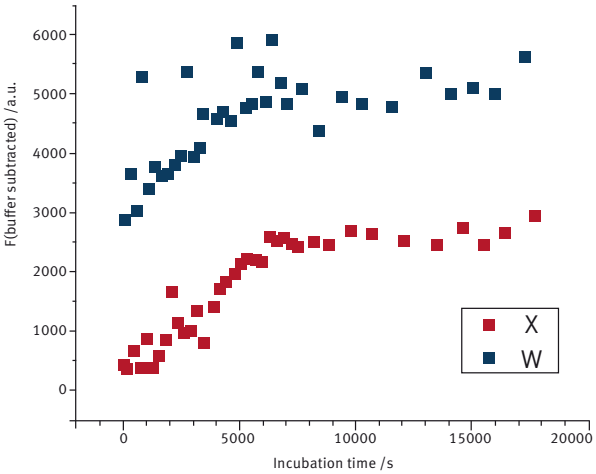

Fig. 1: Kinetics of aggregation of proteins X and W monitored by ex situ bis-ANS fluorescence

The non-zero initial fluorescence of protein W suggests the presence of aggregates, formed rapidly within the dead time of the experiment, that are able to bind bis-ANS. That these are not formed for protein X is interesting since it is generally thought that early oligomers represent the toxic species in amyloid diseases (rather than the fibrils).
The higher fluorescence signal found for Protein W after stabilization of the bis-ANS signal suggests that the mature aggregated species formed from this mutant are also more hydrophobic than species formed from protein X. Differences in the hydrophobic nature between aggregated species are also interesting to study since a hydrophobic species could have the potential to mediate toxicity by interacting with the cell membrane in vivo.

Conclusion

It was demonstrated that the BMG LABTECH microplate reader can monitor amyloid formation by performing fluorescence kinetics studies. The model protein V that has been used in this study is associated with amyloid disease, and the result enabled the author to gain information about the intrinsic determinants of protein aggregation which could be of significance in understanding the underlying biochemical mechanism of amyloid pathogenicity.

Following Abeta fibrillization/aggregation in real-time using a FLUOstar Omega

Frank Baumann, Hertie Institute for Clinical Brain Research, Tübingen, Germany

Introduction

Aggregation of the amyloid-β (Aβ) peptide is a fundamental hallmark for Alzheimer's disease. The formation of extra-cellular senile plaques will lead to synaptic and neuronal damages in clinical demented patients. The aggregation process of Aβ peptide is seen as seed driven. These seeds consist of small stable aggegrates of Aβ. It is thought that these aggregates are already present in early stages of Alzheimer's even before a patient experiences any symptoms. If this is true, determination of these early aggregates (aggregation seeds) would be an excellent diagnostic tool.
Here we present a cell-free assay that allows determination of the amount of aggregation seeds from brain tissue homogenates. The assay is run over 2-3 days using the FLUOstar Omega microplate reader from BMG LABTECH.

Assay Principle

The assay uses Thioflavin T to follow the amyloid formation (Fig. 1). Thioflavin T is a benzothiazole salt that is known to show increased fluorescence when bound to beta sheet-rich structures, such as in amyloid fibrils of Aβ.

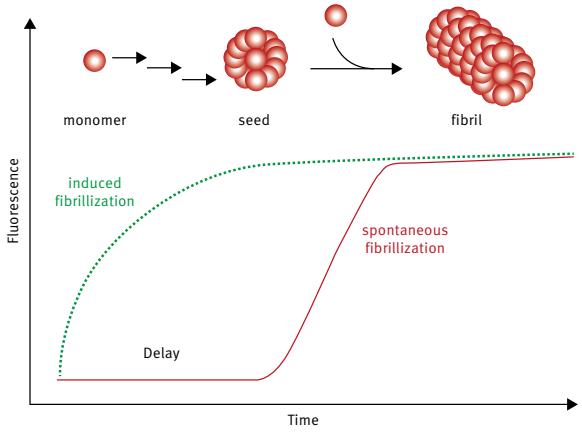

Fig. 1: Fibrillization process followed over time

Before aggregation or fibrillization can start a critical amount of initial aggregation seeds need to be present or spontaneously formed. This is a thermodynamically unfavoured process even in the presence of excess monomeric Aβ, kinetically slow and results in a delay in time before measurable aggregation starts. Once enough seeds have formed a massive and steep increase of fibrillization can be monitored by following the increase of ThT fluorescence due to incorporation into newly formed fibrils.

After some time a plateau is reached indicating the end of the reaction. The delay time is significant and can be shortened by exogenous addition of aggregation seeds. These seeds accelerate the increase in fluorescence in relation to their amount.

Results & Discussion

The time until the signal starts to increase is the lag time. The MARS data analysis software offers the possibility to create 4-parameter fits of the signal curves from which the lag times are calculated (lag times correspond to the EC20 value of the fit). Initial fibril seeds are formed until the lag time is reached. Considering this, the lag time can be used as a measure to compare different brain homogenates (Fig. 2).

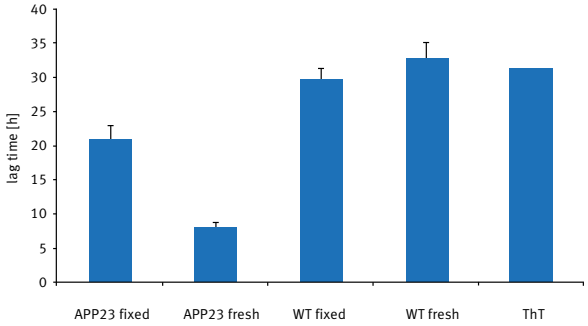

Fig. 2: Lag times of samples containing mice brain homogenates. Fixed and fresh frozen transgenic (APP23) and WT mice are compared. As a control thioflavin T only (ThT) was measured on the same microplate (n=1). Error bars refer to 3 biological replicates.

From figure 2 it can be followed that the lag times of the wild type are bigger compared to the lag times obtained for the tg mice. Further a difference can be seen between fixed and fresh-frozen APP23 samples. As expected the fresh samples induce Aβ deposition much faster. Nonetheless, the fixed APP23 samples show compared to the WT a significantly lower lag time indicating that fixation in formaldehyde is not sufficient to prevent Aβ aggregation.

Conclusion

With the help of the FLUOstar Omega microplate reader it is possible to prove that the in vitro assay is reliable to detect seeding activity in brain samples. In addition it allows quantitative comparison of seeding activity which with only little effort can be statistically validated.

Real-Time Quaking Induced Conversion Assay for Prion Seeding

Maggie Nakamura, BMG LABTECH

Introduction

Prions are transmittable pathogens that cause an abnormal folding of a brain protein in both humans and animals. Infection results in brain damage and is fatal. Some examples of these neurodegenerative diseases are Scrapie, Bovine Spongiform Encephalopathy, and Creutzfeldt-Jakob Disease. Previously, prions were studied using lengthy bioassays where infected animals were studied over long periods of time (1-6 months). This was both time consuming and costly to maintain the infected animal. Researchers at Rocky Mountain Laboratories in Hamilton, Montana have developed a prion seeding assay called Real-Time Quaking Induced Conversion Assay (RT-QuIC) that gives end point quantitation for measuring the levels of prions in infected samples. This assay is faster and yields higher throughput compared to previous methods. The assay can be completed in as few as 20 hours and is as sensitive, if not more so, as whole animal models.

Assay Principle

Combining parts of the original Quaking Induced Conversion (QuIC) assay and the amyloid seeding assay (ASA), the RT-QuIC assay is used to estimate the relative amount of prion seeding. The assay measures serial dilutions of samples, statistically estimating the seeding dose (SD). Very small amounts of infectious prions are added to normal prion protein to seed or cause the misfolding of the prion proteins as seen in the disease. The assay is quantitated by measuring serial dilutions of the samples and determining the loss of seeding activity, which is the end point dilution.

The fluorescent dye thioflavin T (ThT) is used as a prion seeding marker. When ThT is added to recombinant prion proteins, it becomes incorporated when polymerization occurs causing an increase in fluorescence over time.

BMG LABTECH's Omega series of readers have the ability to shake and incubate microplates over long periods of time. A POLARstar Omega was used to measure RT-QuIC samples every 15 minutes for 20-68 hours while alternately shaking and resting for a minute.

Results and Discussion

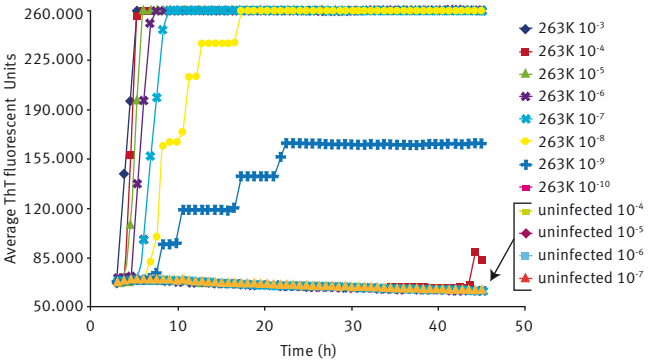

Fig. 1: RT-QuIC sensitivity: analysis of dilutions of a scrapie hamster brain homogenate stock 263K 80 - 85 dags post infection (DPI).

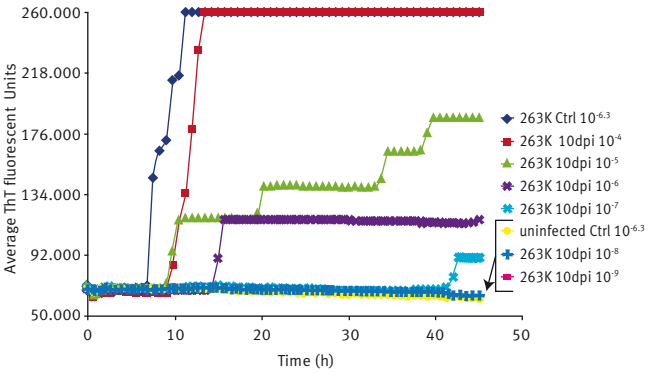

Fig. 2: RT-QuIC end-point dilution analysis of three 263K-inoculated preclinical 10 days post injection hamster BHs.

The SD50/gram of tissue for the 85 DPI samples (10E12) was higher than the 10 DPI (10E8.2) because it had a longer time for onset.

Conclusion

Prion seeding can be measured faster and in a higher throughput using the RT-QuIC assay and a microplate reader. Some of the transmissible spongiform encephalopathies that have been shown to work using RT-QuIC include hamster and sheep scrapie, deer chronic wasting disease, Creutzfeldt-Jakob Disease (CJD), and Bovine Spongiform Encephalopathy (BSE). The Omega series of plate readers from BMG LABTECH are both functional and robust to withstand the many days of shaking at high speeds required for this assay.

FLUOstar® Omega - Technical Specifications

Due to the modularity of BMG LABTECH's instruments, all or combinations of the features below can be installed at purchase or upgraded at any time. Please contact your local representative for more details or a quote.

Detection Modes	Fluorescence Intensity - including FRET UV/Vis Absorbance Spectra Luminescence (flash and glow) - including BRET Time-Resolved Fluorescence - including TR-FRET Alpha Technology	
Measurement Modes	Top and bottom reading Endpoint and Kinetic measurements Sequential Multi-Excitation measurements Sequential Multi-Emission measurements Ratiometric measurements Well Scanning	
Microplate Formats	Up to 384-well plates, 1536-well plates in absorbance, user-definable	
Light Source	High energy xenon flash lamp	
Detectors	Side window photomultiplier tube	
Optical Filters	Excitation and emission filter wheels for 8 filters each	
Spectral Range	240 - 740 nm or 240 - 900 nm Absorbance Spectrometer: 220 - 1000 nm	
Sensitivity	FI	< 0.2 fmol/well Fluorescein
	TRF	< 30 amol/well Europium
	High-End TRF for Omega	< 3 amol/well Europium
	LUM	20 amol/well ATP DLReady™ certified
	Alpha Technology	< 100 amol/well* (384)
	ABS with Spectrometer	Spectral range: 220 - 1000 nm Full spectrum captured in < 1 s/well Selectable spectral resolution: 1 - 10 nm OD range: 0 to 4 OD Accuracy: < 1% at 2 OD Precision: < 0.5% at 1 OD and < 0.8% at 2 OD
Read Times	Flying mode: 9 s (96), 16 s (384)	
Reagent Injection	Up to 2 built-in reagent injectors Injection at measurement position (6 to 384-well) Individual injection volumes for each well (3 to 500 µL) Variable injection speed up to 420 µL/s Up to four injection events per well Reagent back flushing	
Shaking	Linear, orbital, and double-orbital with user-definable time and speed	
Gas Vent	System to inject an atmosphere or to pull a vacuum into the reader	
Incubation	+5°C above ambient up to 45°C or 65°C	
Software	License-free software package including Reader Control and MARS Data Analysis Software	
Dimensions	Width: 44 cm, depth: 48 cm, height: 30 cm; weight: 28 kg	
Accessories		
Atmospheric Control Unit (ACU)	Oxygen Regulation: 1 - 19% using nitrogen to purge. Carbon Dioxide Regulation: 0 - 20% using 100% moisture free carbon dioxide to purge. Dimensions (ACU only): Width: 44 cm, height: 17 cm and depth: 19 cm; Weight: 5 kg	
Stacker	Plate handler for up to 50 microplates - continuous loading feature	
THERMOstar	Microplate Incubator and Shaker	
LVis Plate	Microplate designed to measure 16 low volume (2 µL) samples and standard cuvettes. Incorporating NIST filter and holmium oxide standards for instrument performance test. Sensitivity: 2 ng/µL dsDNA	
Filters	Optimized for dyes, fluorophores and specific assays Filters for all applications from UV to NIR Customized filters available upon request	
Upgrades	Upgrades to include options such as additional detection modes, reagent injectors, extended temperature control, etc. are available. Please contact your local representative for more information.	

* Limit of detection < 100 amol of biotinylated and phosphorylated polypeptide (P-Tyr-100 assay kit, PerkinElmer, #6760620C), measured in white 384 small volume microplates (17 µL/well)

Limit of detection was calculated according to the IUPAC standard: $3x(SD_{blank})/slope$

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