Combining in-situ proteolysis and microseed matrix screening to promote crystallization of PrP-nanobody complexes

Romany N. N. Abshkharo1, Sameh Soror1,*, Els Pardon1, Hassan El Hassan1, Giuseppe Legname2, Jan Steyaert1 and Alexandre Wohlkonig1

1- VIB Department Structural Biology, Vrije Universiteit Brussel, Pleinlaan 2, 1050 Brussel, Belgium
2- Scuola Internazionale Superiore di Studi Avanzati - International School for Advanced Studies (SISSA-ISAS), Trieste, Italy, Institute for Neurodegenerative Diseases

*Contact author soror@vub.ac.be

ABSTRACT

Prion proteins (PrP) are difficult to crystallize, probably due to their inherent flexibility. Several PrP structures have been solved by nuclear magnetic resonance (NMR) technique; however, only three structures were solved by X-ray crystallography. Here we combined in-situ proteolysis with automated microseed matrix screening (MMS) to crystallize two different PrP/Camelidae nanobody (Nb) complexes. Nanobodies are single-domain antibodies derived from heavy-chain-only antibodies of camels. Initial crystallization screening of mouse prion (23-230) in complex with a nanobody (Nb_PrP_01) did not produce any crystals. However, in-situ proteolysis gave poor diffraction thin needle-crystals. We used these microcrystals as nucleants for automated MMS. Good-quality crystals were obtained from mouse PrP(89-230)/Nb_PrP_01 which diffracted to 2.1 Å resolution using synchrotron radiation. Human PrP(90-231)/Nb_PrP_01 crystals diffracted to 1.5 Å resolution. This combined strategy benefits from the power of the MMS technique without suffering from the drawbacks of the in-situ proteolysis. It proved to be a successful strategy to crystallize PrP/nanobodies complexes.

INTRODUCTION

Conversion of the cellular monomeric prion protein PrPc into the pathogenic isoform PrPres The transition mechanism is still unknown, however it has been shown that PrPc exhibits an α-helical fold as the infectious isoform (PrPres), is formed of β-sheets. Camelidae antibodies are a class of immunoglobulin that lacks the light chains. It is named heavy-chain antibody and also referred to as nanobodies (Nb). They are composed of a single domain and possess all features of conventional antibodies making them one of the smallest functional immunoglobulin. Due to the absence of light chains, they are referred to as variable domain of heavy chain antibody or VH-HH. Recently, nanobodies have been used to help in crystallizing difficult proteins due to their promising tool to crystallize prion proteins.

RESULTS

1. Our results highlight how we combined different crystallization techniques: in-situ limited proteolysis and MMS, to produce well-ordered crystals that diffract to high resolution.

2. Our protocol details how using limited proteolysis of a complex between two proteins can produce poor-quality crystals, which can be used as nucleants for homologous and heterogenous seeding in a high-throughput microseed matrix screening to obtain crystals of better diffraction quality.

3. Nb_PrP_01 aided in producing good quality crystals (2.1 Å) of mouse PrP(89-230), which has never been crystallized before and high resolution crystal structure of human PrP(90-231) (1.5 Å)1).

CONCLUSIONS

REFERENCES