

Method for Folding of Recombinant Prion Protein to Soluble β -Sheet Secondary Structure

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Abstract

A key event in the pathogenesis of prion diseases is the change in structure of the normal cellular form of the prion protein from a predominantly α -helix form to the β -sheet-rich prion protein found in disease-associated tissue. To allow more detailed structural research into PrP misfolding, it is necessary to have techniques which enable enrichment of the β -sheet content in recombinant PrP.

This method describes the procedure for inducing β -folding of recombinant PrP to resemble a disease-associated structure and ultimately produce soluble β -folded recombinant PrP.

Key words α -helix, β -sheet, β -folding, Misfolded, Protein conformation, Recombinant protein, Secondary structure, Conversion, Buffer exchange, Low pH refolding, Protein structure, Soluble

1 Introduction

From experimental data, it has been inferred that during prion disease the normal cellular prion protein (PrP^C) misfolds into a protease, detergent-resistant isoform [1–6] which has been termed PrP^{Sc}. Structurally PrP^C mainly consists of α -helices, while disease-associated PrP^{Sc} misfolds in such a way that the β -sheet content of the protein increases and the protein aggregates [7–10].

The following method describes the refolding of PrP into the β -sheet isoform based on the protocols published by Jackson et al. [11] and Coleman et al. [12]. Jackson [11] established the conditions required to switch native, α -helical recombinant PrP to a soluble, monomeric, β -sheet-rich form. In this method, following denaturation, the protein is slowly refolded in an acidic, reducing environment, which promotes β -sheet formation. The product formed exhibits partial resistance to PK digestion and can be a precursor to fibrils; both properties are indicative of disease-associated PrP^{Sc}.

The ability to refold recombinant PrP has enabled the investigation of folding pathways [13], prion seeding [14], and the

thermodynamic stability of disease-associated PrP [15]. Manipulation of the structure of PrP will enable the understanding of how the protein is induced to misfold during disease as well as how the β -sheet structure influences the behavior of PrP during pathogenesis.

2 Materials

1. High-quality recombinant protein (*see Note 1*).
2. Serological pipettes and pipette boy.
3. Dialysis tubing and clips.
4. $2\times$ magnetic stirrer and large fleas (*see Note 2*).
5. 4×5 L beakers.
6. Dithiothreitol (DTT).
7. Guanidine HCl (Gnd-HCl).
8. Tris-acetate.
9. Sodium acetate.
10. Acetic acid (*see Note 3*).
11. Ultrapure water (Milli-Q).
12. Unfolding/denaturing buffer: 6 M Gnd-HCl, 100 mM DTT, 10 mM Tris-acetate, 10 mM sodium acetate, pH 8.
13. Folding buffer: 10 mM Tris-acetate (pH 4), 10 mM sodium acetate.
14. pH meter.
15. Vivaspin 20 columns with a 10 kDa molecular weight cut-off (MWCO) (Sartorius).
16. Centrifuge.

3 Methods

1. Dissolve lyophilized recombinant protein to a final concentration of 0.04 mg/mL in unfolding/denaturation buffer. Incubate overnight at room temperature.
2. The following day, take dialysis tubing with the correct MW cutoff, and cut to size (*see Note 4*).
3. Fold over one end three times and clip shut.
4. Take a serological pipette, and transfer approximately 25 mL of the protein solution into the dialysis tubing tube (*see Note 5*).

5. Fold over the top end several times and clip. You want a small air bubble to remain in the tube which will cause the tube to float in the buffer.
6. Check for leaks before you place the tubing into the dialysis buffer.
7. Dialyze at 4 °C against folding buffer supplemented with 1 mM DTT, twice for 2 h each. 5 L of dialysis buffer is used each time, and the buffer is continually mixed by magnetic stirrer to speed up the diffusion.
8. Continue dialysis in folding buffer (no DTT) at 4 °C for 2 days with a total of four buffer changes, with 5 L of fresh buffer each time.
9. After the dialysis is complete, very carefully take out the tubes, and undo the clips on one end. Insert a serological pipette and carefully recover the sample (*see Note 6*).
10. Concentrate the protein using a centrifugal concentrator with the appropriate MWCO if required (*see Note 7*).
11. Confirm β -sheet content increase using circular dichroism spectroscopy (CD). It is also worth testing for increases in PK resistance compared to the original native recombinant protein.
12. Store β -folded PrP in folding buffer at 4 °C. Avoid using other buffers or water for storage as the protein will potentially slowly lose its β -sheet content.

4 Notes

1. Full-length and C2 fragments of human and mouse recombinant PrP have been successfully refolded using this procedure.
2. Preferably set up two magnetic stirrers, one at room temperature for the denaturing step and one set up in a cold room (4 °C) for the refolding steps.
3. Acetic acid is used for lowering the pH of the refolding buffers.
4. For example, Thermo Fisher Scientific SnakeSkin Dialysis Tubing with a 10 K MWCO with a 22 mm internal diameter. The tubing will usually come with instructions for converting volume of liquid into length of tubing. Always cut extra to fold over to close the ends.
5. Transfer sample to dialysis tubing very carefully. It is very easy to drop the slippery tubing and spill your sample.
6. Pipette the sample up, and wash the sides of the tubing to help dislodge any PrP remaining bound to the tubing.

7. For example, Vivaspin 20 columns with a 10 kDa MWCO for full-length recombinant protein (23–230).

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