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Superparamagnetic Nanoparticle Capture of Prions for Amplification

Michael B. Miller  
*Dartmouth College*

Surachai Supattapone  
*Dartmouth College*

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Bovine spongiform encephalopathy, Creutzfeldt-Jakob disease, and other prion diseases are caused by an infectious agent that contains PrPSc, a misfolded conformer of the normal cellular prion protein (PrPC) (24). Low-abundance sources of prions, such as blood, may still transmit disease (17, 23). Prion diseases currently have no therapy, nor can prions be specifically removed from contaminated material.

Inoculation bioassay serves as the gold standard for specific detection of prion infectivity. For sensitive detection, protein misfolding cyclic amplification (PMCA) has emerged as a rapid alternative to bioassay. PMCA exploits prion multiplication mechanisms to amplify PrPSc in vitro using PrPC substrate (1, 5). Analogous to amplification of DNA sequence template by PCR, PrPSc template seeds the conversion of PrPC substrate in PMCA, resulting in propagation and amplification of the PrPSc conformation. Each serial PMCA round increases the detection sensitivity exponentially but requires ~72 h (7). The application of PMCA is also limited by prion propagation inhibitors present in blood and other biological solutions (6). An effective method to concentrate prions would improve subsequent PMCA sensitivity and utility.

Nanotechnology presents many opportunities for fine control of molecular events. Certain iron oxide crystals less than ~25 nm in diameter exhibit superparamagnetism, with a net magnetization only occurring in the presence of an external magnetic field (15). MagnaBind and Dynal superparamagnetic beads contain many iron oxide crystals, dispersed such that no permanent magnetic order can form. This enables the whole particles to be superparamagnetic, allowing them to be rapidly attracted to a magnet and to lose magnetic interactions upon removal of the magnet (28). In molecular biology, superparamagnetic beads are often conjugated to specifically bind a target molecule.

Using superparamagnetic nanoparticles, we have identified a novel binding interaction with PrPSc. Magnetic capture of PrPSc may be applied to prion detection and prion decontamination.

**MATERIALS AND METHODS**

**Preparation of scrapie-infected and uninfected brain homogenate.** CD-1 mouse (prion strains RML, Me7, and 301C) and Syrian hamster (prion strains Sc237 and 139H) scrapie-infected brains were homogenized (Coviden tissue grinder; Coviden, Mansfield, MA) to 10% in phosphate-buffered saline (PBS), pH 7.4 (Cellgro, Manassas, VA). Uninfected CD-1 mouse and Syrian hamster brains (Biochemed, Winchester, VA) were homogenized in the same manner. The homogenates were initially clarified by centrifugation at 200 × g for 30 s and stored at −70°C. Freshly clarified 5% homogenate for each experiment was prepared by adding an equal volume of Tris-buffered saline (TBS; 50 mM Tris, 200 mM NaCl, pH 7.5, vortexing for 15 s, sonicating (Misonix 4000 with microplate horn; Qsonica, Newtown, CT) for 1 min, and centrifuging at 500 × g for 15 min.

**Preparation of magnetic particles.** The superparamagnetic beads used in these studies were MagnaBind (Pierce, Rockford, IL) or Dynal (Invitrogen, Carlsbad, CA) bearing either protein A or streptavidin conjugates. All magnetic particles were separated from solution with a magnetic particle separator (PureBiotech, Middlesex, NJ).

Nonbead nanoparticles were prepared as follows: 10-nm iron(II,III) oxide (Fe3O4, magnetic) nanoparticles (Sigma, St. Louis, MO) in toluene were mixed with an equal volume of methanol and magnetically separated. ~50-nm iron(II,III) oxide (Fe3O4, magnetic) nanopowder was also obtained from Sigma. For silanization (21), nanoparticles or nanopowder was resuspended in methanol to 0.11 mg/ml, to which was added 1/10 volume 3-(trimethoxy-silyl)propyl methacrylate (Sigma). Each was sonicated for 1 min at 70% power and then incubated for 4.5 h at 25°C with 300 rpm shaking. Each was then rinsed in methanol and then ethanol.

**Binding assays.** Unless otherwise noted, 25 μl of beads (5 mg/ml) or 2 mg of magnetite (10-nm nanoparticles or ~50-nm nanopowder, as described above) was rinsed twice in 50 μl PBS plus 0.5% Triton X-100 and then incubated in 150 μl of assay buffer (TBS, 1% Triton X-100, 1% Tween 20) with 5 μl of clarified 5% brain homogenate overnight at room temperature with 10-rpm end-over-end rotation. IgG 89-112 anti-PrPSc antibody (18) was added to designated samples at 7.5 μg/ml. Particles were separated from the solution and rinsed twice in 500 μl of wash buffer (TBS, 0.05% Tween 20) before analysis of bound molecules. PrPSc/PrPC comparison reactions were carried out in TBS with 3% NP-40 and 3% Tween 20 for 2 h, followed by four 1-ml washes in TBS with 2% Sarkosyl.

**PMCA.** Following binding, samples were resuspended in 10% CD1 mouse or Syrian hamster brain homogenate, which was prepared in conversion buffer (PBS, 1% Triton X-100, Roche Complete mini protease inhibitor) (6) with an additional 4 mM EDTA. One round of PMCA consisted of 30 s of microplate horn sonication pulses every 30 min for 24 h at 90% power.

**Prion protein detection.** Bound PrPSc was detected by subjecting beads to limited proteolysis in 50 μl (25 μg/ml for mouse, 50 μg/ml for hamster) of limited protease K (Roche, Indianapolis, IN) in PBS, 1% Triton X-100. Proteolysis proceeded for 30 min (mouse) or 60 min (hamster) at 37°C and 750-rpm shaking and was terminated by the addition of 17 μl of 4X sample buffer (217 mM Tris, 8 M urea, 2% Sarkosyl, 0.1% SDS, 50 mM Tris, pH 6.8). The homogenates were initially clarified by centrifugation at 200 × g for 30 s and stored at −70°C. Freshly clarified 5% homogenate for each experiment was prepared by adding an equal volume of Tris-buffered saline (TBS; 50 mM Tris, 200 mM NaCl, pH 7.5, vortexing for 15 s, sonicating (Misonix 4000 with microplate horn; Qsonica, Newtown, CT) for 1 min, and centrifuging at 500 × g for 15 min.

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PrPSc, while streptavidin-conjugated Dynabeads did not. MagnaBind beads conjugated to streptavidin also bound PrPSc. Neith
er protease treatment nor boiling abrogated MagnaBind-protein A interaction with PrPSc. Neither pro-
tease treatment nor boiling abrogated MagnaBind’s ability to bind PrPSc molecules (Fig. 1B), further suggesting that MagnaBind beads alone bind PrPSc.

MagnaBind beads are composed of silanized superparamagnetic iron oxide. Dynabeads also contain superparamagnetic iron oxide but are completely enveloped by a polystyrene coat of uniform thickness, which presumably prevents interaction with PrPSc. Superparamagnetism is exhibited only by small nanoparticles. To further dissect the MagnaBind-PrPSc interaction and to identify simple reagents for prion capture, we tested defined particles with a composition similar to the MagnaBind silane-coated iron oxide. Silanized magnetite (Fe3O4) nanoparticles captured PrPSc in a dose-dependent manner (Fig. 2). Interestingly, magnetite nanoparticles alone (nonsilanized) also captured PrPSc, with 10-nm nanoparticles and ≤50-nm nanopowder performing similarly. This suggests that the prion-capturing activity of MagnaBind beads can be recapitulated by magnetite nanoparticles. Moreover, PrPSc appears to interact directly with the Fe3O4 metal surface.

Distinct strains of prions infect different brain regions and display various biochemical properties, despite possessing identical PrP amino acid sequences (4). We assessed whether multiple prion strains can be magnetically captured and found that MagnaBind beads bound all strains examined, including mouse strains RML, 301C, and Me7 and hamster 139H (Fig. 3). This result suggests that magnetic nanoparticles could be used as a general prion capture reagent, including for strain 301C, which are mouse-adapted bovine spongiform encephalopathy (BSE) prions (3). Magnetic particle capture of prions appears to target a general feature of PrPSc that is conserved between strains and shared among prions from different animal species, which bear different prion protein sequences.

We next examined the selectivity of magnetic capture for...
**PrPSc.** Magnetic particles did not capture the normal prion protein conformer PrP\(_C\) from uninfected mouse brain tissue (Fig. 4A), suggesting specificity for the disease-associated conformer, PrP\(_Sc\). Furthermore, silver stain analysis of total protein indicated that magnetic particles bound minimal protein from both uninfected and scrapie-infected brain (Fig. 4B). Other metals, minerals, and resins have been found to interact with normal and disease-associated prion protein (2, 12, 16, 25). Our findings indicate that superparamagnetic iron oxide particles capture PrP\(_Sc\) selectively and efficiently.

Detection of prions by PMCA may be made more efficient, rapid, and sensitive if samples are concentrated for PrP\(_Sc\) prior to amplification. This depends on the concentration procedure leaving PrP\(_Sc\) with the autocatalytic ability to seed the conversion of PrP\(_C\). To determine whether magnetic particle-captured PrP\(_Sc\) retained seeding ability, we used bound PrP\(_Sc\) to seed PMCA reaction mixtures containing normal brain homogenate substrate. RML prion-infected mouse PrP\(_Sc\) successfully seeded the conversion of PrP\(_C\) to PrP\(_Sc\), causing amplification of PrP\(_Sc\) (Fig. 5A). We found that captured hamster Sc237 PrP\(_Sc\) also seeded PMCA, even after the bound PrP\(_Sc\) was washed stringently with the ionic detergent sarkosyl (Fig. 5B). Thus, magnetically concentrated PrP\(_Sc\) is competent for amplification by PMCA.

Magnetic capture of PrP\(_Sc\) could be useful to remove prions from potentially contaminated biological solutions. We treated a prion-contaminated solution with magnetic nanoparticles and detected no PrP\(_Sc\) in the supernatant (Fig. 6A). Both MagnaBind beads and magnetic nanoparticles captured all input PrP\(_Sc\), leaving none in the remaining fluid. To test for small amounts of residual PrP\(_Sc\) not detected by immunoblot, we used nanoparticle-treated supernatants to seed PMCA reaction mixtures with normal brain homogenate as substrate (Fig. 6B). Input PrP\(_Sc\) was amplified upon performing PMCA (Fig. 6B, lane 3); in contrast, supernatants treated with MagnaBind and ~10-nm nanoparticles showed no detectable PrP\(_Sc\), even after amplification by PMCA (Fig. 6B, lanes 5 and 7). This suggests that nanoparticle treatments are highly effective in removing PrP\(_Sc\) from contaminated samples.

**DISCUSSION**

Various materials have been reported to bind prion protein. Prion infectivity adheres to stainless steel (29), promoted by nickel and molybdenum, which in isolation also bind to PrP\(_C\) and PrP\(_Sc\) (16). Stainless steel has been proposed for prion...
concentration, for use in a coupled concentration-cell culture detection scheme (9). Prions also adsorb to various minerals found in soil (14, 20, 27). Phosphotungstic acid has been used to precipitate PrPSc in the laboratory (26) and may also be used to detect PrP protein was detected by anti-PrP (6D11) immunoblot.

Capture of prions by magnetic nanoparticles holds great potential to improve current methods of prion detection. Though the PMCA technique is very sensitive, particularly when serial amplifications are performed, each round requires 24 to 72 h (7). Nanoparticle-bound PrPSc is competent to seed PMCA reactions, facilitating the coupling of magnetic nanoparticle concentration with PMCA detection. Another technique, immunoprecipitation, may also be able to concentrate prions (18, 22), but antibodies directed against PrP may inhibit prion propagation (19), precluding such a detection scheme. Coupling of magnetic concentration with PMCA would improve current methods to detect low concentrations of prions, valuable in safeguarding biological materials for consumption and in medicine.

Prions may be transmitted to humans by transfusion of infected blood (23). Magnetic nanoparticle capture presents an opportunity to decontaminate biological products derived from potentially contaminated sources. Other methods, such as sodium hydroxide, sodium hypochlorite, and phosphotungstic acid treatments, destroy or remove prions (11, 26) but also damage the material of interest. In contrast, magnetic nanoparticles capture PrPSc with specificity. Innovative methods, such as filtration, have been proposed to remove prions from blood (13). Magnetic capture could potentially reduce the prion load in contaminated samples while at the same time facilitating detection. Following treatment with magnetic nanoparticles, we detected no remaining PrPSc, even after amplification, indicating that nanoparticle capture is effective for prion removal. The safety of treating biologically derived pharmaceutical products with iron oxide nanoparticles is further supported by their nontoxicity, demonstrated in clinical studies. The small size of nanoparticles enables passage through capillary beds, and iron oxide nanoparticles have been approved by the American Food and Drug Administration for use as a magnetic resonance imaging contrast agent (8). Thus, superparamagnetic nanoparticles may be used to simultaneously detect and decontaminate prion-contaminated materials.

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