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Methods of Protein Misfolding Cyclic Amplification

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Abstract

Protein misfolding cyclic amplification (PMCA) amplifies infectious prions in vitro. Over the past decade, PMCA has become an essential tool in prion research. The current chapter describes in detail the PMCA format with beads (PMCAb) and several methods that rely on PMCAb for assessing strain-specific prion amplification rates, for selective amplification of subtypes of PrP^{Sc} from a mixture, and a PMCAb approach that can replace animal titration of scrapie material. Development of PMCAb-based methodology is important for addressing a number of research topics including prion strain evolution, selection and adaptation, strain-typing, prion detection, and biochemical requirements for prion replication.

Keywords

Prion protein; Prion diseases; Protein misfolding cyclic amplification; Sonication; Self-propagating protein states; Prion detection; Prion strains; Replication rate

1 Introduction

Protein misfolding cyclic amplification (PMCA) is the sole technique that can successfully amplify the disease-associated, infectious form of the prion protein (PrP^{Sc}) in vitro [1]. PMCA reactions consist of two alternating steps: sonication and incubation (Fig. 1). Sonication fragments PrP^{Sc} particles or fibrils into smaller pieces, a process that is believed to result in the multiplication of active centers of PrP^{Sc} growth. During the incubation step, small PrP^{Sc} particles grow by recruiting and converting the normal, cellular form of the prion protein (PrP^C) into PrP^{Sc}. Since its introduction in 2001 [2], PMCA has become an important tool in prion research. To date, amplification of PrP^{Sc} in PMCA can be achieved by using PrP^C in crude brain homogenate [1], PrP^C purified from normal mammalian brains [3], or recombinant PrP (rPrP) produced in *E. coli* [4, 5] as substrates. In recent years, PMCA was used for identifying cofactors that are involved in prion replication [4–6], assessing the impact of glycosylation on replication of prion strains [7, 8], assessing the ability of prion strains to replicate in heterologous substrate and to cross species barriers [9, 10], and elucidating prion interference and prion adaptation to new hosts or new replication environments [11–13]. Due to its capacity to amplify a single PrP^{Sc} particle [14, 15], PMCA provides one of the most sensitive approaches for detecting miniscule amounts of prion

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infectivity [14, 16, 17], including detection of prions in blood or peripheral tissues at preclinical stages of the disease [18, 19].

While PMCA-based methods offer tremendous opportunities for the development of new tools and assays in the field, a number of limitations have surfaced over the years. The efficiency of amplification depends to a large extent on the prion strain or type of Creutzfeldt-Jakob disease (if human prions are assayed). Amplification efficiency in PMCA could also vary significantly depending on minor variations in experimental parameters, including those that are difficult to control, such as the age of the sonicator's horn and individual patterns of horn corrosion. In addition, cross-contamination has been reported as one of the major issues that could undermine interpretation of the results [20].

Previously, our laboratory introduced a new PMCA format with beads (PMCAb) [21], an important modification that improved PMCA efficiency, sensitivity, and robustness. The current chapter describes in detail PMCAb and several methods that rely on PMCAb for assessing strain-specific PrP^{Sc} amplification rates, for selective amplification of PrP^{Sc} from a mixture, and a PMCAb approach that can replace animal titration of scrapie material. Further development of PMCA methodology is important for addressing broader scientific topics including prion evolution, behavior of prion strains, strain-typing, and prion amplification requirements.

2 Materials

All solutions are prepared with deionized water purified using a Barnstead MicroPure UV water system (Thermo Scientific), unless noted otherwise.

2.1 Preparation of 10% Normal Brain Homogenate for Protein Misfolding Cyclic Amplification

1. Conversion buffer: to prepare 50 ml, use 5 ml of Ca²⁺-free and Mg²⁺-free 10 phosphate-buffered saline, pH 7.4 (10 PBS), 1.5 ml of 5 M sodium chloride, 0.1 ml of 0.5 M ethylenediaminetetraacetic acid, pH 8.0 (EDTA), 1 tablet of protease inhibitors (cOmplete, EDTA-free), 5 ml 10% (w/v) Triton X-100, and RNase-free water. Sterilize by filtration through 22 µm syringe-driven filter unit (SLGV033RB); keep on ice.
2. Brains from healthy weanling hamsters, perfused immediately after euthanasia with PBS, pH 7.4, supplemented with 5 mM EDTA (*see* Note 1). Three brains will produce about 30 ml of PMCA substrate (*see* Note 2).
3. 30 ml Potter-Elvehjem tissue grinders with PTFE pestle (358049, Wheaton Science Products, Millville, NJ) (*see* Note 3).

¹If brains are to be homogenized immediately after dissection, keep on ice in a 50 ml tube with PBS, pH 7.4 supplemented with 5 mM EDTA. Alternatively, brains can be flash frozen in liquid nitrogen and stored at −80 °C until needed.

²We observed that with time the quality of 10% normal brain homogenate prepared in conversion buffer decreases, even if stored at −80 °C without repeated freezing and thawing. To avoid this problem, we prefer to prepare a new batch of 10% normal brain homogenate every 3–6 months. Flash-frozen brains can be stored at −80 °C for at least 2 years.

³To keep the tissue grinder cold, keep the glass tube and PTFE pestle on ice. Only when both parts of the tissue grinder are cooled will the pestle fit properly inside the glass tube and move up and down well during homogenization.

4. Laboratory balance to weigh brains.
5. Refrigerated microcentrifuge.
6. Motor-driven homogenizer (Heidolph, Elk Grove Village, IL).
7. RNase-free tubes and RNase-free aerosol barrier pipette tips.

2.2 Protein Misfolding Cyclic Amplification with Beads (PMCAb)

1. RNase-free 0.2 ml thin-wall tubes with domed caps (14230205, Fisher Scientific).
2. Polytetrafluoroethylene (PTFE) spheres, 3/32'' diameter (B000FMUEXG, www.amazonsupply.com).
3. Brain homogenate prepared as in Subheading 3.1.
4. Seeds: the source of scrapie material depends on the experimental design. We usually use diluted 10% brain homogenate from terminally ill animals, which is prepared the same way as NBH (*see* Subheading 3.1, **steps 1–7**).
5. Sonicator with a microplate horn (Q700 with 431MPX, Qsonica, Newtown, CT) at 37 °C (*see* Note 4).
6. Floating rack (*see* Note 5).
7. Proteinase K (P8102S, New England BioLabs, Ipswich, MA).
8. 10% sodium dodecyl sulfate (SDS, Fisher Scientific).

2.3 Protein Misfolding Cyclic Amplification with Beads Using Partially Deglycosylated Substrate (dgPMCAb)

1. All materials as mentioned for PMCAb (*see* Subheading 2.2).
2. Peptide-N-Glycosidase F (PNGase F, glycerol-free, New England BioLabs).
3. Laboratory rotator.
4. Incubator.

2.4 Protein Misfolding Cyclic Amplification with Beads in RNA-Depleted Environment

1. All materials as mentioned for PMCAb (*see* Subheading 2.2).
2. RNase A (R4875, Sigma-Aldrich, St. Louis, MO): dissolve in water to prepare a 10 mg/ml solution; store at –20 °C in small aliquots.
3. Laboratory rotator.

⁴The temperature inside the horn can be maintained by coiled rubber tubing attached to a circulating water bath. Alternatively, the horn with the acoustic chamber can be placed in a 37 °C incubator.

⁵We make floating racks by punching small holes in a piece of dense foam available in craft stores (Fig. 4). When inserting the tubes into the foam rack, maintain a 1 mm space between the lid and the surface of the foam to ensure no contact between the lid and water in the horn. The tubes should be positioned about 2 cm away from the center and from the edges of the horn and about 3 mm from the bottom. Make sure the rack floats in the center of the horn.

4. Incubator.

2.5 PMCA-Based Titration

1. All materials as mentioned for PMCAb (*see* Subheading 2.2).

2.6 Estimation of Amplification Rate in PMCA

1. All materials as mentioned for PMCAb (*see* Subheading 2.2).

3 Methods

Low efficiency of protein misfolding cyclic amplification can be caused by several factors:

1. Presence of blood in NBH, when using nonperfused brains to prepare the substrate.
2. Degradation of PrP^C in NBH due to prolonged storage and multiple freeze/thaw cycles.
3. Inefficient sonication due to a very old or damaged horn (*see* Note 6).
4. Inadequate sonication protocol (*see* Note 7).

Once sonication protocol is established, prions can be amplified very efficiently. All PMCA reactions should be set up and performed in a way that minimizes cross-contamination of the samples (*see* Note 8), since even a miniscule amount of prions can generate false-positives [20].

3.1 Preparation of 10% Normal Brain Homogenate for Protein Misfolding Cyclic Amplification

1. Keep tissue grinders, conversion buffer, and fresh brains on ice (*see* Notes 1 and 3). Place on ice a 50 ml tube for collection of the homogenate.
2. Tare a Petri dish on a balance, transfer brains into the Petri dish, record the weight quickly, and place the Petri dish with brains on ice to keep brains cold.
3. Calculate the amount of conversion buffer needed to prepare a 10% homogenate. Each gram of brains requires 9 ml of conversion buffer. If the calculated volume of conversion buffer exceeds 15 ml, work in batches in order not to overfill the grinder.

⁶The surface of the horn weathers over time developing cracks and cavities. When positioning the tubes, avoid severely damaged areas. If PMCAb results show significant inconsistency, or overall efficiency of the sonication becomes low, replace the horn. Resurfacing of the horn by polishing can also prolong its life (Qsonica offers resurfacing services).

⁷Different prion strains may require particular modifications to ensure maximum amplification efficiency. If a strain is difficult to amplify, lowering the starting dilution of seeds, lowering dilution between rounds, or shortening the sonication pulses with more cycles within one round of amplification may be useful [11, 23].

⁸All reusable materials, e.g., dissection instruments and homogenizers, should be decontaminated thoroughly between uses. We decontaminate them by soaking them in sodium hydroxide (2 N NaOH) or undiluted household bleach for 1 h, then rinsing with water, and autoclaving at 134 °C for 1 h. If practical, designate separate sets of reusable materials for tissues infected with different strains and for noninfected tissues. Although the risk of contaminating PMCA samples through the water in the microplate horn is low, the horn should also be decontaminated before critical PMCA experiments by wiping with a generous amount of 2 N NaOH. Prolonged incubation with NaOH degrades the metal surface of the horn and should be avoided. Unless different strains need to be compared, avoid amplification of several strains with one microplate horn at the same time.

4. Attach the pestle of the tissue grinder to the motor-driven homogenizer.
5. Transfer the brains into the glass tube of the grinder, and add the needed volume of the ice-cold conversion buffer.
6. Turn on the homogenizer and operate on a speed that is easy to control. Run the rotating pestle up and down through the tube, and process the tissue until homogeneous. Make sure the grinder remains cold. Cool on ice, if needed.
7. Pour the prepared 10% brain homogenate into a precooled 50 ml tube. Keep on ice. If working in batches, combine all homogenate to produce a homogenous pool, and then aliquot for storage as desired (*see Note 2*).
8. Before assembling a protein misfolding cyclic amplification reaction, thaw the 10% normal brain homogenate, aliquot into RNase-free 1.5 ml microcentrifuge tubes, and centrifuge 2 min at $500 \times g$. Carefully collect the supernatant and use it as a substrate for PMCA (10% NBH). Leftovers of the precleared NBH can be stored at -80°C for 3–6 months.

3.2 Protein Misfolding Cyclic Amplification with Beads (PMCAb)

A single PMCAb round consists of multiple cycles, each of which consists of brief sonication and incubation at 37°C (Fig. 1). In a serial PMCA, the end products of a single PMCA round are diluted to fresh NBH to seed the next PMCAb round (Fig. 1).

1. Fill the microplate horn of the sonicator with 350 ml of water and make sure it reaches 37°C before starting PMCA (*see Note 4*).
2. Thaw a sufficient amount of prepared brain homogenate (*see Subheading 3.1*). To distinguish between amplified material and the residual amount of seeds, include round 0 in the experiment. Round 0 should contain the same amount of seeds as the round 1, but not subjected to sonication and incubation cycles.
3. Using clean forceps, distribute 1–3 PTFE spheres (beads) into each PMCA tube [21].
4. Aliquot precleared NBH: 90 μl per tube, if using tenfold dilution between rounds (*see Note 7*).
5. If several PMCAb rounds are planned, keep rounds 0 and 1 on ice, and freeze the rest at -80°C .
6. If using 10% scrapie brain homogenate (BH) as a source of seeds, prepare corresponding dilution: sonicate 80–100 μl of 10% BH in a 0.2 ml PMCA tube for 30 s using Qsonica microplate horn at 150–200 Wt sonication power. Centrifuge the tube briefly to bring all liquid down before opening the tube. Dilute sonicated 10% brain homogenate into conversion buffer to achieve the desired dilution. Usually, the final dilution of scrapie brain 10^{-4} (for 263 K) provides sufficient amount of seeds for amplification in the first round, leaving the residual amount of the original seeds undetectable by standard immunoblotting.

7. Add 10 μ l of the diluted seeds to 90 μ l of NBH in rounds 0 and 1. Close the tubes of round 0 and freeze them at -20°C until ready to analyze.
8. Close the tubes of round 1 and place in a floating rack (*see* Note 5). Make sure that the tubes do not touch the bottom of the horn. To achieve minimal variations in sonication efficiency, position the tubes equidistantly around the center of the horn and horn surface. Cover the horn with foil to reduce evaporation.
9. Program the sonicator: as a rule, we use 30 s sonication pulses at 150–200 Wt delivered every 30 min during a 24-hour period. This gives 48 cycles within one round of PMCA (*see* Note 7). Start sonication.
10. The next day, after one round of PMCAb is completed, centrifuge the tubes briefly before opening. Thaw the aliquots for the next round, and add 10 μ l from completed round to the 90 μ l of the fresh substrate of the next round tubes (*see* Note 7).
11. Sonicate the tubes by the same protocol as during the previous round.
12. Store completed PMCAb rounds frozen at -20°C until ready to analyze. PrP^{Sc} produced in PMCAb and stored at -20°C retains its seeding abilities for at least 6 months.
13. To visualize amplification of PrP^{Sc} after PMCAb, we use immunodetection after proteinase K (PK) digestion: combine 10 μ l of the PMCAb reaction with 5 μ l of 1% sodium dodecyl sulfate (SDS) and 5 μ l of diluted PK, to achieve 20–50 $\mu\text{g/ml}$ final concentration of PK in the reaction, and incubate at 37°C for 1 h (*see* Note 9). Stop the reaction by adding sample buffer of your choice and denaturing the samples in a boiling water bath for 10 min. If round 0 without PK treatment is used as a weight marker in a gel and as a control for PK digestion, subject the tube with round 0 to a single 30 s sonication pulse to reduce sample viscosity, then add sample buffer, and boil with the rest of the samples. Proceed with the electrophoresis and Western blot as described elsewhere.

3.3 Protein Misfolding Cyclic Amplification with Beads Using Partially Deglycosylated Substrate (dgPMCAb)

Treatment of NBH with PNGase partially deglycosylates PrP^C and shifts the ratio of the three PrP^C glycoforms making mono- and unglycosylated forms more abundant. A shift in glycoform ratio changes the selectivity of amplification and can be used for selective amplification of classical or atypical PrP^{Sc} or for strain typing [8] (Fig. 2a).

1. Thaw sufficient amount of prepared brain homogenate (*see* Subheading 3.1).
2. Preclear brain homogenate as in Subheading 3.1, **step 8**, if it is not already done.
3. Add 1500 U/ml PNGase F to an aliquot in a RNase-free sterile tube and rotate at 37°C for 5 h.

⁹Twofold dilution of the PMCAb reaction before immunodetection permits better PK digestion and helps to avoid smearing of the bands in the gel. Optimal final concentration of SDS and PK, as well as the time and temperature of the PK digestion reaction, can be strain dependent.

4. Proceed using the same method as for the PMCAb (*see* Sub-heading 3.2).

3.4 Protein Misfolding Cyclic Amplification with Beads in RNA-Depleted Environment

Prion strains from different species or certain PrP^{Sc} subtypes differ with respect to RNA dependency of amplification [8, 24, 25]. PMCAb amplification in an RNA-depleted environment can be used to assess their RNA dependency. If amplification of a strain or PrP^{Sc} subtype is found to be RNA independent, removing all RNA from NBH by treatment with RNases improves selectivity of amplification for such strain or PrP^{Sc} subtype (Fig. 2b).

1. Thaw a sufficient amount of prepared brain homogenate (*see* Subheading 3.1). Partially deglycosylated brain homogenate as in Subheading 3.3 can also be used for RNA depleting.
2. Preclear brain homogenate as in Subheading 3.1, **step 8**, if it is not already done.
3. Add 100 µg/ml RNase A to an aliquot in a sterile tube and rotate at 37 °C for 1 h.
4. Proceed using the same method as for the PMCAb (*see* Sub-heading 3.2).

3.5 PMCAb-Based Titration of Scrapie Material

To determine the titer of PMCAb-active particles in infectious material, serial tenfold dilutions are prepared and used to seed multiple rounds of PMCAb. As a result, infectious material diluted up to a single PMCAb-active particle can be amplified and detected by immunoblot. This method offers a fast and accurate way of quantitating prion titer and can replace prion titration by animal bioassay [15]. Perform titration in several independent repetitions to allow statistical analysis of the data (*see* Note 10).

1. Label PMCA tubes for all repetitions and rounds in the titration experiment, place PTFE spheres in all the tubes, and dispense NBH (*see* Subheading 3.2).
2. Prepare tubes for dilutions by dispensing 90 µl of conversion buffer into each tube (*see* Note 11). Keep the tubes open under the hood on the rack to eliminate handling, which might increase the risk of cross-contamination.
3. Sonicate 10% homogenate of scrapie material as in Subheading 3.2, **step 6**. Centrifuge the tube briefly to bring all liquid down before opening the tube.
4. Open the tube with sonicated 10% homogenate of scrapie material, mix the contents by careful pipetting up and down, and dispose of the tip.
5. With a new tip, transfer 10 µl of sonicated 10% scrapie homogenate into the next tube, and mix carefully but thoroughly by pipetting. This makes a 10⁻² dilution of scrapie material.
6. Continue diluting tenfold, always changing pipette tips between dilutions.

¹⁰We recommend sonicating no more than 30 tubes simultaneously, since overloading of the horn can change the efficiency of sonication.

¹¹If infectious material shows inconsistent amplification within one serial dilution, dilute scrapie material into 1% normal brain homogenate instead of conversion buffer to block possible sticking of PrP^{Sc} particles to the plastic tubes and tips. 1% normal brain homogenate is prepared by dilution of 10% pre-cleared NBH into conversion buffer. It can be sonicated in 100 µl of aliquots to further increase homogeneity. 1% NBH can be prepared in advance and stored at -80 °C.

7. After all dilutions of scrapie material are prepared, change gloves and open the tubes with the NBH substrate for the 1st PMCA round. Keep PMCA tubes with the substrate for the subsequent rounds at -80°C .
8. To seed NBH with prepared dilutions of scrapie material, mix $10\ \mu\text{l}$ of each dilution with $90\ \mu\text{l}$ of the NBH substrate in the corresponding PMCA tubes. Start with the highest dilution and move toward the more infectious ones. Close PMCA tubes immediately after adding the seeds. Change tips between dilutions.
9. Space PMCA tubes of the 1st round in a foam rack and place into a microplate horn of the sonicator (see Notes 5 and 10). Sonicate according to the protocol optimized for efficient amplification of the strain. For example, for 263 K scrapie material sonication for 20 s every 20 min within 24 h routinely allowed us to amplify 10^{12} -fold diluted brain material in only two to three rounds of PMCA (see Note 7).
10. After one PMCA round is completed, spin the tubes briefly and use to seed a subsequent round. Change gloves before opening the tubes with NBH substrate. In the case of tenfold dilutions between rounds (see Note 7), mix $10\ \mu\text{l}$ from the completed reaction with $90\ \mu\text{l}$ of the NBH substrate in the corresponding PMCA tubes for the following round. Start from the highest dilution and move toward the more infectious ones.
11. Repeat PMCA rounds as long as needed. Analyze by immunoblotting (see Subheading 3.2, **step 12**). Usually, with every round of PMCA, the amount of dilutions positive for PMCA-active particles gradually increases. When after several subsequent PMCA rounds no new positives are detected, the necessary number of rounds has been reached (see Note 12).
12. Calculate the titer of PMCA-active particles or PMCA₅₀ values. PMCA₅₀ values are determined by regression analysis in Sigma Plot (other software can be employed, too) using nonlinear least squares fitting of data to the sigmoidal equation:

$$F = (100 * \exp(-(A+B * x))) / (1 + \exp(-(A+B * x)))$$

where F is percent of positive PMCA reactions, x is logarithm of the scrapie material dilution fold, A and B are two fitting parameters that define the position of a limiting dilution transition on the x axis and the slope of the transition, respectively. After A and B are determined, PMCA₅₀ is calculated according to the equation:

$$\text{PMCA}_{50} = A/B$$

¹²One should be aware that spontaneous misfolding of PrP^C into PK-resistant conformations can occasionally happen upon harsh and prolonged sonication. Sometimes spontaneously formed PK-resistant PrP states also propagate from round to round increasing the probability of false-positive signals. The sonication conditions should be optimized for each strain, so that the strain is amplified efficiently within the lowest possible number of rounds. Processing several non-seeded controls for the same number of rounds helps to control for false-positives due to spontaneous misfolding and cross-contamination.

Because the titer of scrapie material is calculated per gram of tissue, whereas the assay volumes used for PMCAb are typically 100 μ l, the PMCAb₅₀ value has to be recalculated per gram of scrapie tissue (if PMCAb is conducted in 100 μ l, the PMCAb₅₀ value should be multiplied by 10).

3.6 Estimation of Amplification Rate in PMCAb

The amplification rate differs for each prion strain or PrP^{Sc} subtype and can be used as an individual characteristic of a strain [23, 26, 27]. Evaluating the amplification rate can also be useful for optimization of the sonication protocol. The amplification rate is defined operationally as the highest dilution between serial PMCAb rounds at which amplification is still capable of compensating the effect of dilution [27]. To evaluate a strain-specific amplification rate, a set of serial PMCAb reactions are conducted, where the dilution factors between serial rounds could range from 3-fold to 10⁶-fold (Figs. 2c and 3). However, in each serial PMCAb reaction, the dilution factor between rounds is fixed. The amplification rate is equal to the highest dilution factor at which PrP^{Sc} replication can be maintained steadily in serial PMCAb (Figs. 2c and 3).

1. Label PMCA tubes for all rounds of the experiment; place PTFE spheres in all the tubes (*see* Subheading 3.2, **steps 1–5**). To estimate amplification rate of one strain, four rounds are usually sufficient. Rounds 0 and 1 can be represented by only one tube each and contain 90 μ l of NBH substrate. The number of tubes for rounds 2–4 and the volume of NBH in these tubes, however, depend upon the number of dilutions and on the dilution factors to be tested.
2. Seed 90 μ l of NBH for rounds 0 and 1 with 10 μ l of scrapie material (*see* Subheading 3.2, **step 6**), and subject to the desired sonication protocol.
3. Transfer a portion of the completed round to the tubes with fresh substrate for a subsequent round. For example, to test if amplification sustains a threefold dilution, have 60 μ l of NBH in the tubes for rounds 2–4 and supplement them with 30 μ l of completed reaction from the previous round. In a typical assay, we increase the dilution factors with ~3-fold increments: $\times 3$ -fold, $\times 10$ -fold, $\times 30$ -fold, $\times 100$ -fold, etc. (Figs. 2c and 3). If the dilution factors call for a volume below 10 μ l, prepare an intermediate dilution of the completed round, and use 10 μ l of it to seed 90 μ l of the substrate. For example, to check if the amplification can be sustained at a 1000-fold dilution between rounds, mix 10 μ l of the completed round with 90 μ l of conversion buffer, then use 10 μ l of this dilution to mix with new 90 μ l of conversion buffer, and then use 10 μ l of the resulting dilution to seed 90 μ l of NBH in a subsequent round. Repeat the same dilution process in each round.
4. After four rounds of amplification are completed, analyze all rounds together by immunodetection after PK digestion (*see* Subheading 3.2, **step 12**).

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Fig. 1.

Schematic diagram that illustrates the principles of PMCA. Prion amplification is initiated by mixing diluted scrapie material with normal brain homogenate (NBH) used as a source of PrP^C. A single PMCA round consists of multiple repetitive cycles of sonication and incubation at 37 °C. A serial PMCA consists of multiple rounds, where each subsequent round is initiated by diluting PMCA products from the previous round into fresh NBH

Fig. 2.

Representative results from the experiments on PMCAb, dgPMCAb, and PMCAb in an RNA-depleted environment and an estimation of amplification rate. Each reaction was done in the presence of three PTFE spheres. The sonication protocol consisted of 30 s sonication pulses at 50% power performed every 30 min during 24 h. (a) PMCAb and dgPMCAb reactions were seeded with 10^{-2} dilution of brain material from the first passage of a synthetic strain LOTSS [22]. Ten microliters of reaction products from a completed round were used to seed 90 μ l of the subsequent round. In the first passage, brain-derived LOTSS represents a mixture of authentic PrP^{Sc} and atypical PrPres, which are selectively amplified in PMCAb (*upper panel*) and dgPMCAb (*lower panel*), respectively. Amplification of PrP^{Sc} was visualized with 3F4 antibody, while atypical PrPres was detected with SAF-84. (b) PMCAb in an RNA-depleted environment illustrates RNA dependency of amplification of 263 K scrapie material. The reactions were seeded with 10^{-3} diluted brain material from terminally ill hamsters. Ten microliters of reaction products from a completed round were used to seed 90 μ l of the subsequent round. Western blot was stained with SAF-84. (c). Estimation of an amplification rate for LOTSS. PMCAb reactions were seeded with 10^{-4} diluted brain material from the second passage of LOTSS. For the dilution factor $\times 30$, 10 μ l of a completed reaction were mixed with 20 μ l of conversion buffer, and 10 μ l of this dilution was used to seed subsequent PMCAb rounds. For the dilution factor $\times 100$, 10 μ l of a completed reaction were mixed with 90 μ l of conversion buffer, and 10 μ l of this dilution was used to seed subsequent PMCAb rounds. For the dilution factors $\times 300$ and $\times 1000$, 10 μ l

of a completed reaction was diluted threefold and tenfold, respectively, into conversion buffer and then diluted further by mixing 10 μ l with 90 μ l of conversion buffer each, and 10 μ l out of these dilutions were used to seed subsequent PMCAb rounds. An amplification rate of LOTTS was estimated to be 300-fold per PMCAb round. An increase in intensity of the signal from round to round illustrates the ability of synthetic strains to quickly adapt to an PMCAb environment and increase its amplification efficiency as previously described [23].

Western blot was stained with SAF-84

Fig. 3.

Schematic representation of a PMCAb experiment for estimating a strain-specific amplification rate. Several serial PMCAb reactions with incrementing dilution factors between rounds are initiated using the same scrapie material. In each individual serial PMCAb reaction, the dilution factor between rounds is kept constant. The highest dilution factor at which PrP^{Sc} amplification fully compensates the effect of dilution determines the amplification rate

Fig. 4.

A floating rack for holding PMCAb tubes in the microplate horn is made from a 6 mm extra firm foam sheet available in craft stores