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ORIGINAL ARTICLE

Preference toward a polylysine enantiomer in inhibiting prions

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Abstract Differential anti-prion activity of polylysine enantiomers was studied. Based on our recent discovery that poly-L-lysine (PLK) is a potent anti-prion agent, we investigated suppression of prions in cultured cells using poly-D-lysine (PDK). The results showed that PDK was more efficacious than PLK to inhibit prions. Protein misfolding cyclic amplification assay demonstrated improved efficacy of PDK in inhibiting plasminogen-mediated prion propagation, corresponding to the enantio-preference of PDK observed in cultured cells. Furthermore, our study demonstrated that polylysines formed a complex with plasminogen. These results propose to hypothesize a plausible mechanism that elicits prion inhibition by polylysine enantiomers.

Keywords Polylysine · Enantiomers · Prion · Inhibition · Plasminogen

K. S. Jackson and J. Yeom contributed equally to this work.

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Introduction

Prion diseases are fatal neurodegenerative disorders caused by infectious proteinaceous pathogens termed prions (Prusiner 1998; Ryou 2011). They are composed of scrapie prion protein (PrP^{Sc}), a misfolded isoform of cellular prion protein (PrP^{C}) abundantly expressed in neuronal cells (Prusiner 1998; Ryou and Mays 2010). Prion diseases include scrapie in sheep and goats, bovine spongiform encephalopathy (BSE) in cattle, chronic wasting disease in deer and elk, and Creutzfeldt–Jakob disease (CJD) in humans (Ryou 2007, 2010). While prion diseases pose a potential risk for human public health, either prevention or therapy is currently unavailable (Ryou 2011).

Polylysine is an artificial homo-polypeptide composed of several to hundreds of lysine residues which are linearly linked at the α-carbon groups by peptide bonds. Polylysine has been a useful model polypeptide to study formation and transition of the secondary structure of polypeptides (Greenfield and Fasman 1969). It was also frequently used as a biologically compatible medium to adhere cultured cells in vitro (Yavin and Yavin 1974) and as a non-viral vehicle to deliver nucleotide drugs (Luo and Saltzman 2000). Furthermore, biological activity of polylysine was reported to inhibit microbes (Shima et al. 1984), viruses (Langeland et al. 1988), and neoplastic cell growth (Arnold et al. 1979).

Recently, we demonstrated that plasminogen stimulates PrP^{Sc} propagation, playing a role as an auxiliary factor, while exclusion of plasminogen, destruction of plasminogen structure and interference of plasminogen–PrP interaction dampen stimulated PrP^{Sc} propagation (Mays and Ryou 2010). Our discovery on prion replication mechanism triggered development of anti-prion agents using polylysines that target the interaction of plasminogen with PrP. In our subsequent studies, polylysine as a form of poly-L-lysine (PLK) exhibited potent inhibitory effects against prion propagation in both cell-based and animal models of disease (Ryou et al. 2011). When ScN2a cells, a murine neuroblasotma cell line chronically infected with RML mouse-adapted scrapie prions (Butler et al. 1988), were incubated with PLK, the level of PrP^{Sc} was effectively decreased in a concentration responsive manner and the cells were completely cured with no reappearance of PrP^{Sc} even after the extended periods of cell culture. Most importantly, treatment with PLK significantly delayed the onset of disease in prion-infected mice and lowered the PrP^{Sc} level in their brains.

Polymerization of L-enantiomers of lysine leads to generation of PLK, whereas polymerization of D-enantiomers leads to generation of poly-D-lysine (PDK) (Fig. S1). PLK and PDK possess the same molecular formula and connectivity of the atoms, but only differ in spatial configuration of amino and carboxyl groups regarding the chiral α-carbon. Because the stereoisomers of compounds differ in their biological activities (David 1997), we hypothesized that the ability of polylysine stereoisomers is different to inhibit prion propagation.

In this study, we compared the anti-prion activity of the stereoisomers of polylysine by measuring the PrP^{Sc} levels in two independent cell lines chronically infected by different prion strains. We then investigated differential cytotoxicity of PLK and PDK to reveal whether the efficacious PLK and PDK concentrations to inhibit PrP^{Sc} propagation are below the cytotoxic concentrations. Furthermore, we ascertained the effect of polylysine with different physical states and investigated plausible scenarios by which polylysine stereoisomer-mediated anti-prion activity and enantio-preference were elicited.

Materials and methods

PLK and PDK

PLK30–70 and PDK30–70 were purchased from Sigma-Aldrich (St. Louis, MO). The molecular weight of these polylysines ranges from 30 to 70 kDa with the average at ~50 kDa. Their average polymerization degrees (the number of lysine monomers per polymer molecule) were ~240 ranging 144–336. PLK300 and PDK300 were also purchased from Sigma-Aldrich and their average molecular weights are 300 kDa. PLK52 and PDK52 were purchased from Alamanda Polymers Inc. (Huntsville, AL). The molecular weight of these polylysines ranges from 47 to 57 kDa with the average at ~52 kDa. Their deduced average polymerization degrees were ~250 ranging 225–275. The properties of PLK and PDK are summarized in Table S1.

Anti-prion efficacy assay in cells

Anti-prion activity of PLK and PDK was assayed as previously described using two different cell lines with chronic prion infection (Ryou et al. 2003, 2011). ScN2a and SMB cells were initially seeded at 2 % confluency in culture dishes (60-mm in diameter, Corning, Lowell, MA) and cultured with Dulbecco's modified Eagle's medium (DMEM) containing 10 % fetal bovine serum, 1 % penicillin–streptomycin, and 1 % Glutamax under 5 % CO_2 and saturated humidity conditions. All cell culture reagents were purchased from Invitrogen (Carlsbad, CA). Treatment of cells with various concentrations of the PLK30–70/PDK30–70 and PLK52/PDK52 pairs began in 3 h as the cells anchored onto the plastic surface of culture containers by directly adding PLK and PDK to culture media. Incubation lasted for 6 days with a media replacement at the fourth day using fresh culture media containing PLK and PDK.

To measure anti-prion activity of immobilized PLK and PDK, culture dishes were coated with each polylysine stereoisomer using 0.1 mg/ml filter-sterilized stock solution prepared in water. After one half of a ml was aseptically transferred to a cell culture dish, it was gently rocked to evenly coat the surface for 5 min. Once the excess solution was removed, the surface of dish was thoroughly rinsed with a largely volume of sterile water and allowed to dry for several hours. The same numbers (5×10^5) of ScN2a cells were plated and cultured under the same condition described above for 5 days until cells became confluent.

Once the incubation period ended, cell lysate was prepared with Tris/NaCl buffer containing detergents (20 mM Tris, pH 8.0; 150 mM NaCl; 0.5 % Nonidet P-40; 0.5 % sodium deoxycholate). An aliquot (~30 μg) of cell lysate was analyzed for Western blotting for total PrP and β-actin. The rest (2 mg) of cell lysate was incubated with 20 μg/ml proteinase K (PK) for 1 h at 37 °C with vigorous shaking. PK-resistant PrP^{Sc} pelleted by centrifugation for 1 h at 16,000×g at 4 °C was detected by Western blotting. Detailed procedure for Western blotting is described elsewhere (Mays et al. 2008). Monoclonal anti-PrP antibodies, 6H4 (Prionics, Zurich, Switzerland) and 5C6 (gifted from

G. Telling, Colorado State University), and anti-actin antibody, pan Ab-5 (Lab Vision Corp., Fremont, CA), were used for Western blotting. Densitometry analysis of Western blots was carried out using Doc-It Image Analysis software (UVP, Upland, CA).

Cytotoxicity assay

MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide] assay was used to measure cytotoxicity of PLK and PDK. The assay was carried out as previously described (Ryou et al. 2003, 2011). ScN2a cells were plated in a 12-well culture plate and incubated with PLK and PDK by the same method described for anti-prion activity assay. Following 6-day treatment, the cells were incubated for 2 h with DMEM containing 0.5 mg/ml MTT. Purple MTT formazan products converted by dehydrogenases and reductases of live cells were extracted in 0.05 N HCl–isopropanol and quantified by colorimetric readouts at 570 nm with background subtraction at 650 nm using DTX-880 Multimode Detector (Beckman-Coulter, Brea, CA).

In vitro prion propagation assay

Protein misfolding cyclic amplification (PMCA) (Saborio et al. 2001) was used to assay prion propagation in vitro. Plasminogen-mediated prion propagation assay was carried out as described previously (Mays and Ryou 2010). Briefly, purified human plasminogen (0.5 μM, Haematologic Technologies, Essex Junction, VT) was included in the PMCA reactions composed of the mixture of diseased and normal mouse brain extract (1:2,500 v/v). The effect of PLK and PDK was estimated in the PMCA reactions with plasminogen in which various concentrations of individual polylysine enantiomers were added. In this analysis, PLK300 and PDK300 were used in replacement of PLK30–70/PDK30–70 or PLK52/PDK52 to demonstrate that PLK and PDK with greater molecular weights are also capable of displaying anti-prion activity shown by PLK30–70/PDK30–70 or PLK52/PDK52. After 96 cycles of PMCA finished, the samples were analyzed by Western blotting as described previously (Mays et al. 2009).

Non-denaturing gel electrophoresis of plasminogen–polylysine complexes

Purified human plasminogen (0.5 μM, final concentration) was mixed with PLK or PDK (0–500 μg/ml, final concentration) in 20 μl of 10 mM Tris buffer (pH 7.5) containing 5 % glycerol (v/v). After incubation for 30 min at room temperature, the samples were subjected to 8 % non-denaturing polyacrylamide gel. The electrophoretic procedure was carried out using the modified method described elsewhere (Park and Raines 2004). The mobility of the plasminogen–polylysine complexes in the gel was visualized by staining with Silver Stain Kit (Pierce, Rockford, IL).

Measurement of particle size

The formation of particles composed of plasminogen and polylysine enantiomers was achieved by the similar manner described above. Briefly, purified human plasminogen (180 μg) and PLK/PDK (100 μg) were mixed in 10 mM Tris buffer (pH 7.5) (200 μl, final volume). The particle size of plasminogen–polylysine complexes was determined by dynamic light scattering (DLS) measurements using a Zetasizer Nano-ZS (Malvern, UK) (Bae et al. 2003). The instrument was equipped with a He–Ne laser (4 mW, 633 nm) and set up to collect 173° angle scattered light. Number distributions are presented as the mean particle size.

PrP^{Sc} destabilization assay

The ability of PLK and PDK to destabilize PrP^{Sc} was measured by the method described in our previous publication with modification (Lim et al. 2010). Brain homogenate (10 %, w/v) prepared in PBS from RML-infected CD-1 mice at the terminal stage was diluted 1:10 in 0.1 ml reaction buffer (50 mM sodium acetate buffer, 1 % NP-40) at acidic to neutral pH (pH 4, 5, 6, and 7). PLK52 and PDK52 were added to the reaction at a final concentration of 1 or 100 μg/ml. This PLK/PDK concentration was sufficient to exhibit anti-prion activity and enantio-preference in cell-based anti-prion efficacy assays. After incubation at 37 °C for 3 h with shaking at 300 rpm, 0.1 ml of PBS was added to the reaction. A 50 μl aliquot of samples were treated with PK under the same conditions described in the section above. The level of PrP^{Sc} was measured by Western blotting.

Results and discussion

To address the hypothesis that the stereoisomers of polylysine differ in anti-prion efficacy, we compared the level of PrP^{Sc} in ScN2a cells incubated with PLK and PDK. PLK30–70 gradually decreased the PrP^{Sc} level in a concentration responsive manner and removed PrP^{Sc} from cell culture below the limit of detection at 2 μg/ml (Fig. 1a). In contrast, PDK30–70 substantially decreased the PrP^{Sc} level at 0.25 μg/ml and completely removed PrP^{Sc} at the higher concentrations (Fig. 1a). Semi-quantitative analysis of the levels of PrP^{Sc} using densitometry demonstrated different profiles of anti-prion activity of PLK30–70 and PDK30–70

(Fig. 1b). These data suggested that the half-maximal concentration to inhibit PrP^{Sc} (IC_{50}) was 0.8 μg/ml (16 nM) for PLK30–70, which corresponds to that recently reported (Ryou et al. 2011). The IC_{50} of PDK30–70 was 0.15 μg/ml (3 nM), which is fivefold smaller than that of PLK30–70. These results demonstrate that the stereoisomers of polylysine retain different abilities to inhibit PrP^{Sc} propagation and that enantio-preference toward PDK elicits improved anti-prion efficacy.

The effect of PLK30–70 and PDK30–70 on expression of cellular proteins was negligible. The levels of β-actin and PrP^{C} detected as a part of total prion protein (PrP) contents remained the same in cells incubated with or without PLK30–70 and PDK30–70 (Fig. 1a). Our results suggest that the observed anti-prion activity of PLK and PDK was not due to modulation of the PrP^{C} abundance.

We then determined cytotoxicity of PLK and PDK. ScN2a cells showed no signs of cell death if they were incubated with up to 2 μg/ml of either PLK30–70 or PDK30–70 (Fig. 1c). PDK30–70 caused a drastic decrease of cell survival, leading to complete cell death at the higher concentrations, while PLK30–70 exhibited gradually increasing cytotoxicity at 4–10 μg/ml and caused complete cell death at >20 μg/ml. The dose responsiveness for PLK30–70 cytotoxicity in the current and previous (Ryou et al. 2011) studies was virtually identical. Taken together with the results of anti-prion efficacy assay in ScN2a cells, cytotoxicity study indicates that complete inhibition of PrP^{Sc} propagation by both PLK and PDK was achieved under non-toxic concentrations. Therefore, anti-prion activity of PLK and PDK appears to be involved in the processes of PrP^{Sc} propagation, but not to be facilitated by the mechanism that PLK and PDK inhibit survival of prion-infected cells.

Although PLK30–70 and PDK30–70 used in the studies are the polymers with the same molecular weight ranges, it was unclear whether specific batches of PLK30–70 and PDK30–70 described above represented the polylysine stereoisomers with the similar degree of polymerization. PLK and PDK with different degrees of polymerization, although within the given range, may mislead the interpretation of the outcome of the study. Thus, we performed independent anti-prion efficacy tests using PLK and PDK with a relatively narrow molecular weight range (47–57 kDa). Due to the difficulty in controlling the degree of polymerization during chemical synthesis, we were not able to use PLK and PDK with the exactly identical molecular weight. Anti-prion activity of PLK52 closely resembled the results obtained with PLK30–70, and PDK52 and PDK30–70 likewise were similar (Fig. 2). These results verify the conclusion from our initial results shown in Fig. 1a.

Next, we examined whether PLK and PDK are efficacious to inhibit another prion strain. In SMB cells (Clarke and Haig 1970), a murine mesenchymal brain cell line chronically infected with 139A scrapie prions, incubated with PLK30–70 and PDK30–70, the level of PrP^{Sc} was decreased in a PLK/PDK concentration-dependent manner (Fig. 3). As in ScN2a cells, PDK30–70 was more potent than PLK30–70 to inhibit prions in SMB cells (Fig. 3). These results show that anti-prion activity of PLK and PDK is not limited to a certain prion strain and that inhibition of prions in a stereoisomer-preferred manner is independent of prion strains and host cell types. It remains to be tested whether PLK and PDK are effective to suppress BSE and CJD prions to ensure utility of these compounds for future clinical application.

To study dependence of anti-prion activity upon the physical state of polylysines, we measured the effect of

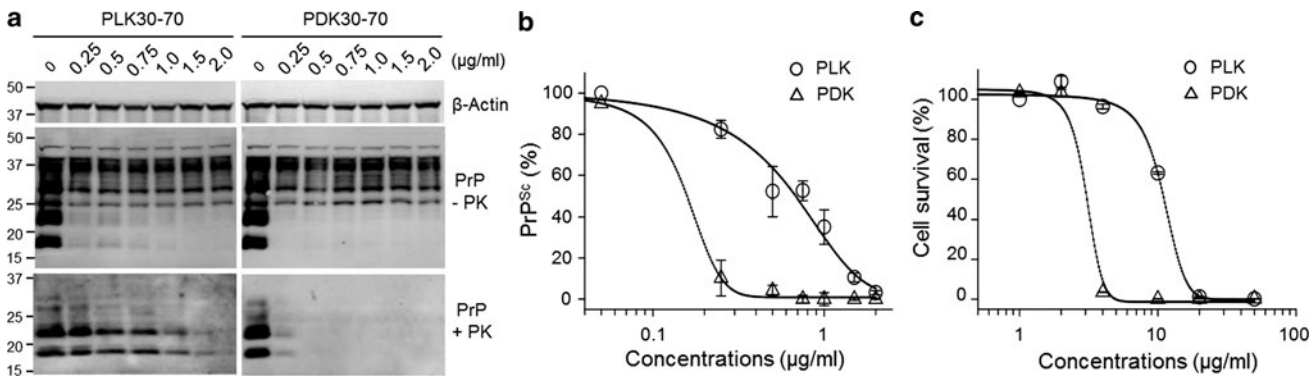

Fig. 1 Efficacy of PrP^{Sc} inhibition by PLK and PDK. **a** The levels of PrP^{Sc}, total PrP, and β-actin from ScN2a cells incubated with PLK30–70 or PDK30–70 (0–2.0 μg/ml). **b** Dose-responsive PrP^{Sc} inhibition curve based on densitometry of the PrP^{Sc} levels detected in **a**. **c** Cytotoxicity curve of ScN2a cells incubated with PLK30–70 or PDK30–70. Data represent the mean of three independent assays. PrP, prion protein; −/+ PK, digested with or without proteinase K. The −PK blot shows di-, mono- and un-glycosylated PrP^{C} bands and internally proteolyzed PrP fragments in each sample. The +PK (PK-resistant PrP^{Sc}) blot shows di-, mono- and un-glycosylated PrP^{Sc} bands in each sample

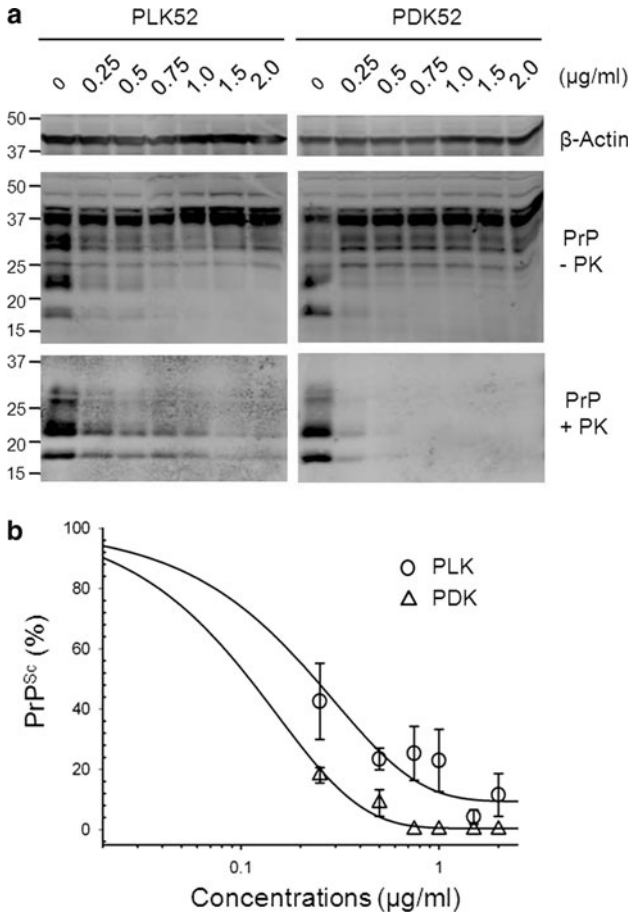

Fig. 2 Elimination of PrP^{Sc} by PLK and PDK from an alternative source. **a** The levels of PrP^{Sc}, total PrP, and β-actin from ScN2a cells incubated with PLK52 or PDK52 (0–2.0 μg/ml) are presented. PrP, prion protein; −/+ PK, digested with or without proteinase K. **b** Densitometry analysis of the PrP^{Sc} levels in a

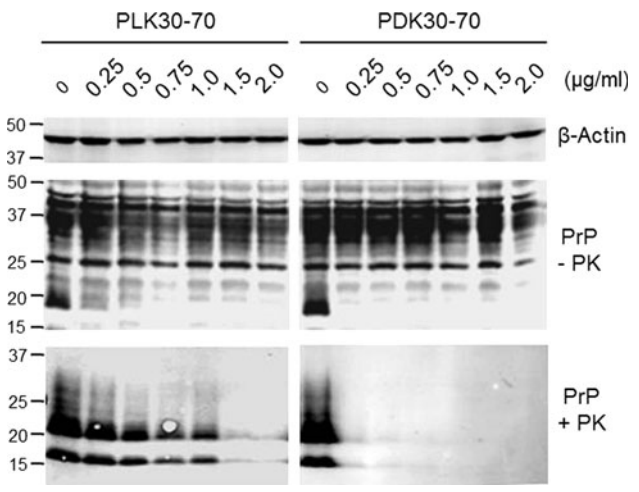

Fig. 3 Anti-prion efficacy of PLK and PDK in SMB cells. The levels of PrP^{Sc}, total PrP, and β-actin from SMB cells incubated with PLK30–70 or PDK30–70 (0–2.0 μg/ml) are shown. PrP, prion protein; −/+ PK, digested with or without proteinase K

immobilized polylysines to inhibit prions in contrast to the solubilized. The levels of PrP^{Sc} from ScN2a cells maintained in PLK-/PDK-coated or uncoated culture dishes were compared. The PrP^{Sc} levels of cells grown in PLK-/PDK-coated culture dishes were substantially lower than that of control cells cultured in uncoated plastic surface (Fig. 4). Interestingly, the levels of prion inhibition were similar despite that cells were maintained in culture dishes coated with different polylysine enantiomers (Fig. 4), indicating no stereoselective mechanism in eliminating PrP^{Sc} from cells under described experimental conditions. PLK and PDK immobilized onto the surface of culture dishes did not affect growth of ScN2a cells either. The growth rate of cells cultured in PLK-/PDK-coated and uncoated culture dishes was identical (data not shown). Together with the results described in Fig. 1, we conclude that anti-prion activity of polylysine enantiomers is maintained regardless of their physical states but stereoselectivity for anti-prion activity is achieved when unimmobilized PLK and PDK exist free within the culture media. In addition, our data suggest that decrease of the PrP^{Sc} level in ScN2a cells facilitated by polylysines is the outcome of a cellular event occurred at the cell surface because immobilized polylysines, which are difficult to be transported into the cells, showed almost equivalent anti-prion activity to free polylysines in culture media.

To elucidate whether polylysine enantiomers stereoselectively control plasminogen-mediated prion propagation, we investigated the efficacy of PLK and PDK to inhibit PrP^{Sc} formation in PMCA supplemented with plasminogen. The results showed that plasminogen increased PrP^{Sc} propagation, as reported previously (Mays and Ryou 2010), and inhibition of plasminogen-mediated PrP^{Sc} propagation by PDK was more robust than that accomplished by PLK (Fig. 5a). An unexpected increase (∼38 %) of the PrP^{Sc} level in the PMCA reaction performed with 0.5 μM plasminogen and 0.04 μM PLK compared to that of the control reaction was dependent on uneven protein concentrations

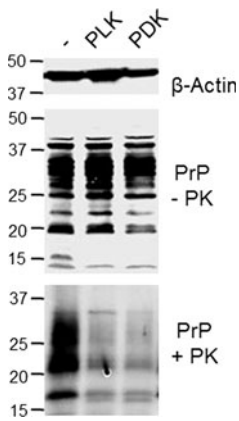

Fig. 4 Anti-prion activity of immobilized PLK and PDK. Inhibition of cellular PrP^{Sc} propagation and enantio-preference were measured in ScN2a cells cultured on immobilized PLK30–70 and PDK30–70. Western blots show the levels of β-actin, total PrP including PrP^{C}, and PK-resistant PrP^{Sc} from ScN2a cells cultured in uncoated (−), PLK-, or PDK-coated plastic culture containers. PrP, prion protein; −/+ PK, digested with or without proteinase K

among samples. The protein concentration of the PMCA reaction performed with 0.5 μM plasminogen and 0.04 μM PLK was ~41 % higher than that of control. Thus, this observation did not conflict with the activity of polylysine to inhibit PrP^{Sc}. The half-maximal effective concentrations (EC_{50}) for anti-prion activity of PLK and PDK were ~180 and ~27 nM, respectively, demonstrating PDK is ~six-fold more efficacious than PLK to inhibit PrP^{Sc} propagation in PMCA (Fig. 5b). This proposes that enantio-preference toward PDK to suppress plasminogen-mediated PrP^{Sc} propagation leads to differential activity of polylysine enantiomers to inhibit prions under cellular conditions. These results are in contrast to a recently proposed mechanism that interaction of polylysines and PrP is responsible for anti-prion activity of polylysines (Xu et al. 2011). Moreover, the authors were not able to observe that binding of polylsine enantiomers to PrP is selective.

To confirm interaction of polylysines with plasminogen, we investigated complex formation of polylysines and plasminogen. PLK and PDK with increasing concentrations were individually mixed with purified plasminogen to form complexes and the resultant complexes were analyzed in native polyacrylamide gels. As the concentrations of plasminogen increased, the migration of plasminogen was marginally retarded, while detection of the complexes failed when the high concentrations of polylysines were maintained in the binding reaction with plasminogen owing to disappearance of the bands representing the complexes (Fig. 6). Because mix of plasminogen and polylysines did not result in precipitation in binding reactions (data not shown), this result indicates that polylysines directly interact with plasminogen. The result also leads to considering a possibility that failure of detecting the complexes formed at the high polylysine concentrations is due to the large size or the net positive charges of the complexes, both of which in turn prevent the complexes to enter the gel. It is of interest that the ability of plasminogen to form a complex with polylysine enantiomers was not obviously different (Fig. 6). Nonetheless, complex formation of polylysines with plasminogen bolsters the mechanistic possibility we have proposed.

We, then, measured the particle size of plasminogen–polylysine complexes to assess dimension of the complex. DLS measurements revealed that the average particle sizes of the plasminogen–PLK and plasminogen–PDK complexes were ~14 and ~18 nm, respectively, which were 1.4- to 1.8-fold greater than that of the control (plasminogen alone) (Fig. 7). Considering the molecular weights of polylysines and plasminogen, the complexes form compact particles in a nanoscale. This result reconfirms binding of plasminogen and polylysines, and suggests their tight binding, as implied by the relatively small particle size. Reminiscent to the result shown in Fig. 6, it is difficult to address that there is a difference in the particle size of plasminogen formed with either polylysine enantiomer. This indicates that the enantio-preference is unexplainable through measurement of the particle size.

Because the difference in the formation of the polylysine-plasminogen complex is not the only feasible mechanism to explain the enantio-preference, it remains to investigate other plausible scenarios. We envision that stability of the plasminogen–PDK complex is greater than that of the plasminogen–PLK complex, which will result in enantio-preference toward PDK in inhibiting plasminogen-

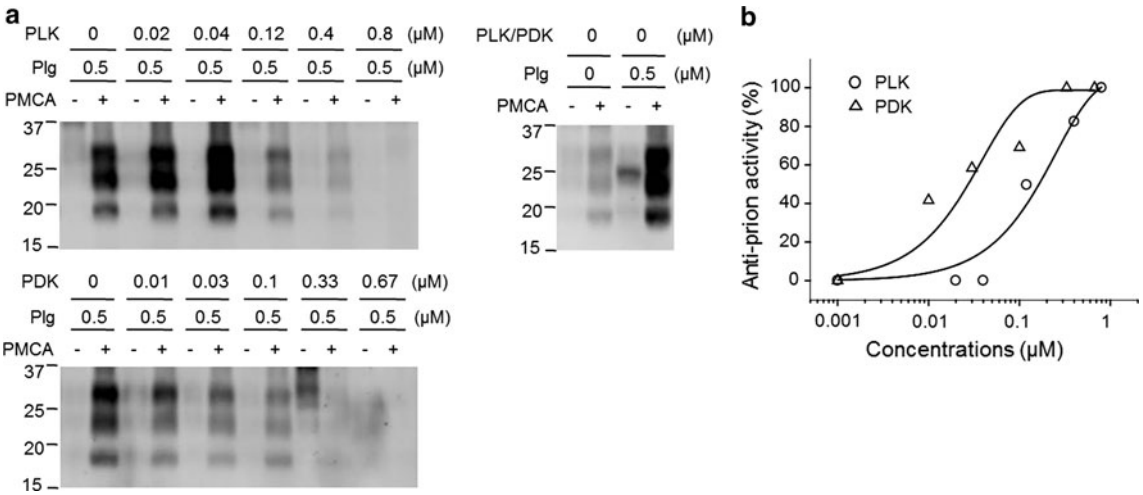

Fig. 5 Enantio-preference toward PDK in plasminogen-assisted in vitro PrP^{Sc} propagation assay. **a** PMCA performed with purified human Glu-plasminogen (Plg) in the presence or absence of PLK300 and PDK300. **b** Anti-prion activity of PLK300 and PDK300. The decrease of PrP^{Sc} levels, representatively shown in a, was converted to the activity of prion inhibition referencing the values of control (no PLK and PDK) and complete PrP^{Sc} removal (0.8 μM for PLK and 0.67 μM for PDK) as 0 and 100 %, respectively. Anti-prion activity was plotted as a function of the PLK and PDK molar concentrations

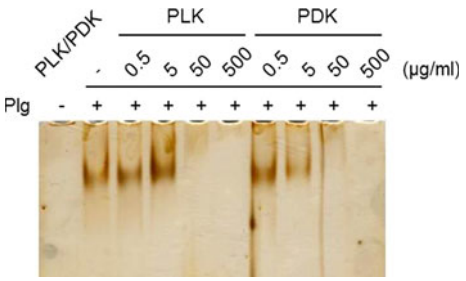

Fig. 6 Detection of the plasminogen–polylysine complexes in native polyacrylamide gels. Purified human Glu-plasminogen (Plg, 0.5 μM) complexed with PLK52 and PDK52 (0–500 μg/ml) was analyzed under non-denaturing conditions and visualized by silver staining. The first lane represents the control sample composed of no plasminogen and mixture of PLK and PDK (500 μg/ml each)

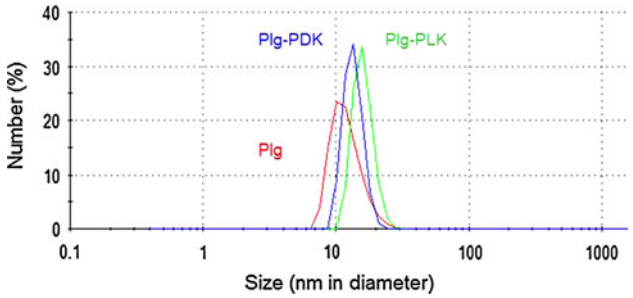

Fig. 7 The size distribution of plasminogen–polylysine particle. The complex was formed as described in the section "Materials and methods" using purified human plasminogen and PLK52/PDK52. Particle size (nm in diameter) distribution was plotted as a function of percentage particle numbers. Data was obtained by DLS measurement

mediated prion propagation. Previously, Hatton and Regoeczi (1975) showed that recovery of plasminogen [Type I fraction (Sodetz et al. 1972), which is deduced to include Glu-plasminogen, the plasminogen species used in this study] is greater from L-lysine-conjugated resin than from D-lysine-conjugated resin, suggesting that biochemical dissociation of plasminogen from PDK could be more difficult than that of plasminogen from PLK. Alternatively, preferential degradation of PLK may lead to enantio-specific anti-prion activity of PDK. Stability of free PDK in solution against proteolytic degradation by cellular proteases can be greater than that of PLK (Tsuyuki et al. 1956; Banecerraf and Levine 1964). This will result in a longer half-life of PDK, more complex formed between plasminogen and PDK, and differential anti-prion activity by polylysine enantiomers. Detection of these differences does not appear to be monitored by complex formation between polylysine and plasminogen. Xu et al. (2011) proposed differential degradation of polylysine enantiomers by cellular proteases, although experimental approaches were based on indirect determination of PLK degradation using flow cytometry that measured the level of PDK binding on the cells and a critical control experiment such as the binding of polylysine to the cells without PrP expression, which can guarantee to state the specific interaction of polylysines with PrP, is missing.

Lastly, we investigated a possibility whether an unequal ability of PLK and PDK to destabilize PrP^{Sc} is the mechanism responsible for enantio-preference in inhibiting prions since the role of cationic dendrimers in PrP^{Sc} destabilization was previously proposed (Supattapone et al. 1999, 2001; Lim et al. 2010). Incubation of prion-infected brain homogenate with PLK and PDK at a low concentration (1 μg/ml) did not result in decreased PrP^{Sc} levels at both neutral and acidic pH conditions (Fig. 8). At pH 5–7, the PrP^{Sc} levels remained unchanged in the samples incubated with either PLK or PDK compared to control. Although the PrP^{Sc} levels determined after incubation with PLK and PDK at pH 4 were substantially lower than those of samples tested at pH 5–7, such decreases did not appear to be caused by the function of polylysines because the samples incubated with or without polylysines showed the same level of PrP^{Sc}. It appears that acidic pH alters the level of PrP^{Sc} as shown in the control reaction incubated with no PLK or PDK. Our in vitro assay demonstrated no alteration of PrP^{Sc} and enantio-specificity in destabilizing PrP^{Sc} by polylysine enantiomers under described experimental conditions. These results suggest that, at least in low concentrations of polylysine enantiomers, destabilization of PrP^{Sc} by PLK and PDK is an unlikely mechanism that elicits observed anti-prion activity and enantio-preference. Although this idea supports the fact that PLK and PDK exert anti-prion activity by controlling biological phenomena at the cell surface, not within the acidic cellular compartments, it requires a careful consideration to generalize inability of PLK and PDK to destabilize preexisting PrP^{Sc} at an acidic pH because the outcomes of similar experiments with PLK and PDK at high concentrations (10–100 μg/ml), in which immediate aggregation occurred, were inconclusive.

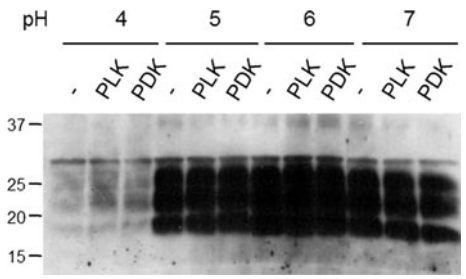

Fig. 8 The effect of PLK and PDK in destabilizing preexisting PrP^{Sc}. The level of PrP^{Sc} measured at various pH in the in vitro PrP^{Sc} destabilization assay. Western blot shows the levels of PK-resistant PrP^{Sc} from the prion-infected brain homogenate incubated with (1 μg/ml) or without (−) PLK30–70 and PDK30–70

Conclusions

We demonstrate that PDK is more efficacious than PLK to inhibit multiple strains of scrapie prions. Furthermore, we found that prion propagation can be inhibited below the toxic levels of polylysine enantiomers. In vitro prion propagation assay with polylysine enantiomers suggests that a superior inhibitory activity of PDK in plasminogen-mediated prion propagation explains the enhanced anti-prion efficacy of PDK in cultured cells with chronic prion infection. Detailed mechanism for enantio-specific anti-prion activity of PDK remains open for further discussion. We emphasize that our study proposes an alternative suggestion that polylysines exert anti-prion effect through the interaction with plasminogen as compared to the interaction of PrP. Our study suggests an option to improve polylysine-based therapy for prion diseases, although additional preclinical and clinical investigations in the future remain to be performed. Because therapy for prion diseases is not available (Ryou 2011), application of the stereochemical principle proposes a unique strategy to advance development of potential anti-prion therapy.

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Conflict of interest The authors declare that we have no conflict of interest.

References

Arnold LJJ, Dagan A, Gutheil J, Kaplan NO (1979) Antineoplastic activity of poly(L-lysine) with some ascites tumor cells. Proc Natl Acad Sci USA 76:3246–3250

Bae Y, Fukushima S, Harada A, Kataoka K (2003) Design of environment-sensitive supramolecular assemblies for intracellular drug delivery: polymeric micelles that are responsive to intracellular pH change. Angew Chem Int Ed Engl 42(38): 4640–4643

Banecerraf B, Levine BB (1964) The relationship between in vivo and in vitro enzymatic degradabilityof hapten-polylysine conjugates and their antigenicities in guinea pigs. J Exp Med 120:955–965

Butler DA, Scott MRD, Bockman JM, Borchelt DR, Taraboulos A, Hsiao KK, Kingsbury DT, Prusiner SB (1988) Scrapie-infected murine neuroblastoma cells produce protease-resistant prion proteins. J Virol 62:1558–1564

Clarke MC, Haig DA (1970) Evidence for the multiplication of scrapie agent in cell culture. Nature 225:100–101

David JT (1997) Stereoselectivity of drug action. Drug Discov Today 2(4):138–147

Greenfield N, Fasman GD (1969) Computed circular dichroism spectra for the evaluation of protein conformation. Biochemistry 8:4108–4116

Hatton MW, Regoeczi E (1975) The relevance of the structure of lysine bound to Sepharose for the affinity of rabbit plasminogen. Biochim Biophys Acta 379(2):504–511

Langeland N, Moore LJ, Holmsen H, Haarr L (1988) Interaction of polylysine with the cellular receptor for herpes simplex virus type 1. J Gen Virol 69(6):1137–1145

Lim Y-b, Mays CE, Kim Y, Titlow WB, Ryou C (2010) The inhibition of prions through blocking prion conversion by permanently charged branched polyamines of low cytotoxicity. Biomaterials 31(8):2025–2033

Luo D, Saltzman WM (2000) Synthetic DNA delivery systems. Nat Biotech 18(1):33–37

Mays CE, Ryou C (2010) Plasminogen stimulates propagation of protease-resistant prion protein in vitro. FASEB J 24(12): 5102–5112

Mays CE, Kang H-E, Kim Y, Shim SH, Bang J-E, Woo H-J, Cho Y-H, Kim J-B, Ryou C (2008) CRBL cells: establishment, characterization and susceptibility to prion infection. Brain Res 1208:170–180

Mays CE, Titlow W, Seward T, Telling GC, Ryou C (2009) Enhancement of protein misfolding cyclic amplification by using concentrated cellular prion protein source. Biochem Biophys Res Commun 388(2):306–310

Park SH, Raines RT (2004) Fluorescence gel retardation assay to detect protein–protein interactions. Methods Mol Biol 261:155–160

Prusiner SB (1998) Prions. Proc Natl Acad Sci USA 95:13363–13383

Ryou C (2007) Prions and prion diseases: fundamentals and mechanistic details. J Microbiol Biotechnol 17(7):1059–1070

Ryou C (2010) Transmissible spongiform encephalopathy. In: Saleh M (ed) Molecular aspects of infectious diseases. Nova Science Publisher, Hauppauge, pp 151–172

Ryou C (2011) Prion diseases. Encyclopedia of Life Sciences. Wiley, Chichester. doi:10.1002/9780470015902.a0000428.pub2

Ryou C, Mays CE (2010) Prions. In: Saleh M (ed) Molecular aspects of infectious diseases. Nova Science Publisher, Hauppauge, pp 129–149

Ryou C, Legname G, Peretz D, Craig JC, Baldwin MA, Prusiner SB (2003) Differential inhibition of prion propagation by enantiomers of quinacrine. Lab Invest 83:837–843

Ryou C, Titlow WB, Mays CE, Bae Y, Kim S (2011) The suppression of prion propagation using poly-L-lysine by targeting plasminogen that stimulates prion protein conversion. Biomaterials 32(11):3141–3149

Saborio GP, Permanne B, Soto C (2001) Sensitive detection of pathological prion protein by cyclic amplification of protein misfolding. Nature 411:810–813

Shima S, Matsuoka H, Iwamoto T, Sakai H (1984) Antimicrobial action of epsilon-poly-L-lysine. J Antibiot 37(11):1449–1455

Sodetz JM, Brockway WJ, Castellino FJ (1972) Multiplicity of rabbit plasminogen: physical characterisation. Biochem 11(24):4451–4458

Supattapone S, Nguyen H-OB, Cohen FE, Prusiner SB, Scott MR (1999) Elimination of prions by branched polyamines and implications for therapeutics. Proc Natl Acad Sci USA 96(25):14529–14534

Supattapone S, Wille H, Uyechi L, Safar J, Tremblay P, Szoka FC, Cohen FE, Prusiner SB, Scott MR (2001) Branched polyamines cure prion-infected neuroblastoma cells. J Virol 75:3453–3461

Tsuyuki E, Tsuyuki H, Stahmann MA (1956) The synthesis and enzymatic hydrolysis of poly-D-lysine. J Biol Chem 222: 479–485

Xu Z, Adrover M, Pastore A, Prigent S, Mouthon F, Comoy E, Rezaei H, Deslys JP (2011) Mechanistic insights into cellular alteration of prion by poly-D-lysine: the role of H2H3 domain. FASEB J 25(10):3426–3435

Yavin E, Yavin Z (1974) Attachment and culture of dissociated cells from rat embryo cerebral hemispheres on polylysine-coated surface. J Cell Biol 62(2):540–546