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Decrease of Protease-Resistant PrP^{Sc} Level in ScN2a Cells by Polyornithine and Polyhistidine

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Based on previous studies reporting the anti-prion activity of poly-L-lysine and poly-L-arginine, we investigated cationic poly-L-ornithine (PLO), poly-L-histidine (PLH), anionic poly-L-glutamic acid (PLE) and uncharged poly-L-threonine (PLT) in cultured cells chronically infected by prions to determine their anti-prion efficacy. While PLE and PLT did not alter the level of PrP^{Sc}, PLO and PLH exhibited potent PrP^{Sc} inhibition in ScN2a cells. These results suggest that the anti-prion activity of poly-basic amino acids is correlated with the cationicity of their functional groups. Comparison of anti-prion activity of PLO and PLH proposes that the anti-prion activity of poly-basic amino acids is associated with their acidic cellular compartments.

Keywords: Prion, polyornithine, polyhistidine, cationic amino acid polymer

Prion diseases are fatal, progressive neurodegenerative conditions in humans and animals [1]. The normal cellular form of prion protein (PrP^C) is conformationally changed to the pathogenic isoform of prion protein (PrP^{Sc}), which is the sole component of prion agents [1]. Accumulation of PrP^{Sc} in the brain results in neuronal damage and subsequent cell death, leading to degeneration of the central nervous system [2].

Unfortunately, there is no treatment available for prion diseases [3]. Among a number of attempts to discover effective anti-prion agents, a group of studies reported that cationic compounds exhibit potent activity in inhibiting prions [4–14]. In particular, our group demonstrated that poly-L-lysine (PLK) suppresses PrP^{Sc} propagation in various systems, including the cell-free, cultured cell, and mouse models of prion diseases [10, 11]. In the following study, we showed that poly-D-lysine, an enantiomer of PLK, retains greater anti-prion potency as well as cytotoxicity than PLK [9, 14]. Similarly, we also found poly-L-arginine (PLR) inhibits PrP^{Sc} more efficiently in cultured cells in which prions of different origins propagate [12]. Furthermore, nanostructures made of oligo-L-arginine showed comparable anti-prion activity to PLR, while reducing the cytotoxicity level [13].

Poly-L-ornithine (PLO) is a cationic polymer composed of L-ornithine, a metabolic intermediate of L-arginine. Like PLK and PLR, PLO has been used as a DNA transfection agent into mammalian cells and a medium to attach cells onto the culture containers [15, 16]. Poly-L-histidine (PLH) is comprised of L-histidine, which is responsible for most of the buffering competence of proteins in the physiological pH array due to its pKa value. By the same reason, PLH is a pH-responsive polymeric carrier and has been used as an endosomal pH targeting agent [17].

In this study, we investigated the anti-prion efficacy of constitutive and conditional cationic amino acid polymers, PLO and PLH, respectively, in cultured cells with permanent prion infection, together with PLR previously shown to exhibit potent anti-prion activity [12]. To confirm the relationship between anti-prion activity and the cationic property of amino acid polymers, poly-L-glutamic acid (PLE), an anionic poly-amino acid, and poly-L-threonine (PLT), a polar but electrically uncharged poly-amino acid, were also examined for anti-prion efficacy.

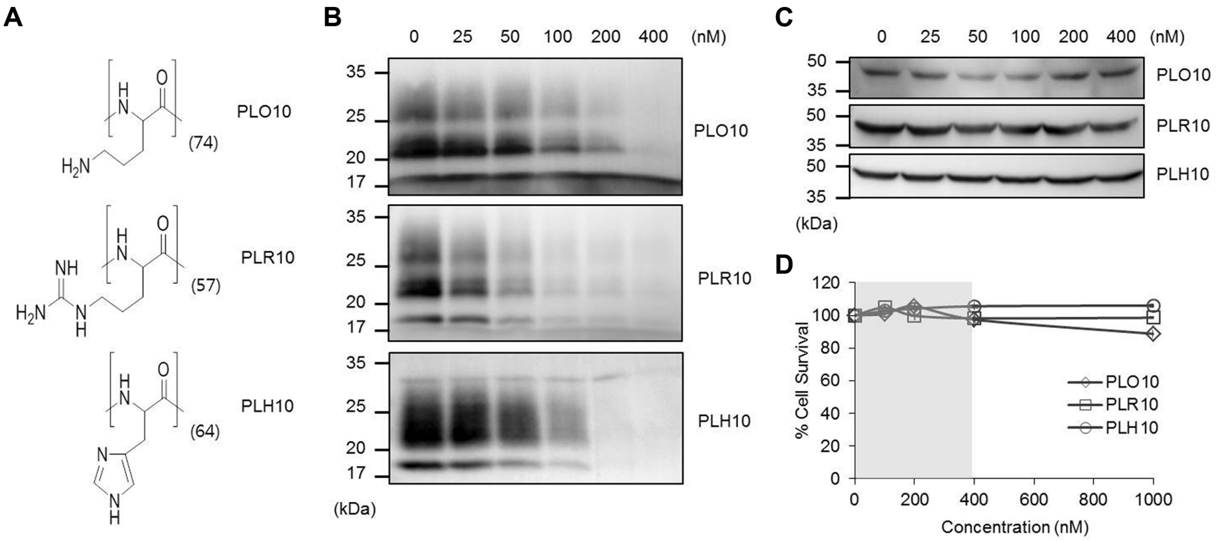

Fig. 1. Anti-prion efficacy of PLO10, PLH10, PLR10 in ScN2a cells.

(**A**) Structures of PLO10, PLH10, PLR10. The numbers within the parentheses indicate repeated unit counts. (**B**) Western blots of PK-resistant PrP^{Sc} in ScN2a cells incubated with 0–400 nM PLO10, PLH10, and PLR10. (**C**) Western blots of βIII tubulin in ScN2a cells incubated with 0–400 nM PLO10, PLH10, and PLR10. (**D**) Survival of ScN2a cells incubated with 0–1,000 nM PLO10, PLH10, and PLR10. The shaded box represents the concentration range used for efficacy tests in Panel B. Survival rates at each data point were obtained from the average of triplicate assays and the error bars indicate the standard deviation. Western blotting and cytotoxicity assays were confirmed by at least more than three independent experiments.

Poly-amino acids used in this study, PLO10, PLH10, PLR10, PLE22.5, and PLT22.5 (Figs. 1A and 2A), were purchased from Sigma-Aldrich (USA). The average molecular mass (kDa) of these polymers was shown as suffix numbers in their names. To measure the anti-prion activity of poly-amino acids, the culture of ScN2a cells [18], incubation of cells with amino acid polymers, and assays to examine the levels of PrP^{Sc}, a biochemical marker for prion

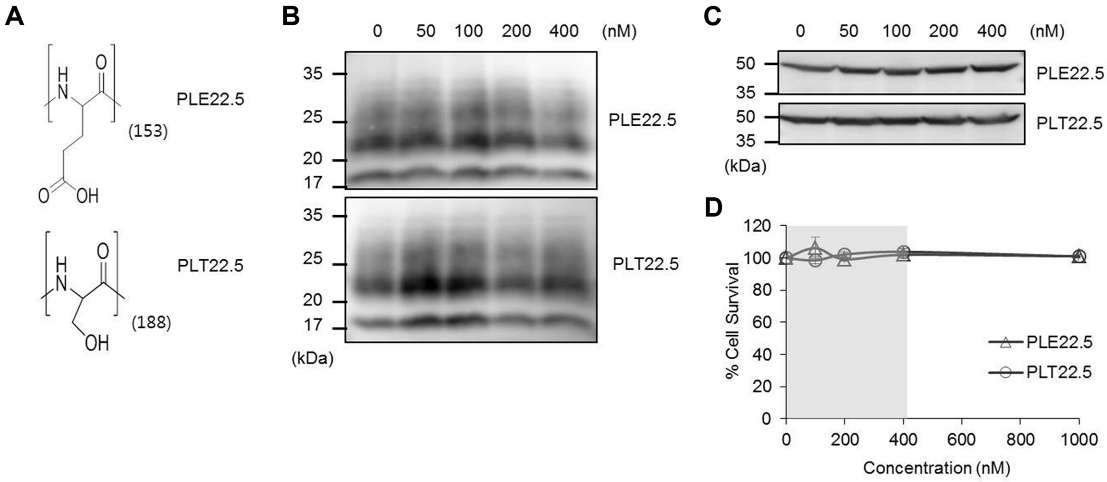

Fig. 2. Anti-prion efficacy of PLE22.5 and PLT22.5 in ScN2a cells.

(**A**) Structures of PLE22.5 and PLT22.5. The numbers within the parentheses indicate repeated unit counts. (**B**) Western blots of PK-resistant PrP^{Sc} in ScN2a cells incubated with 0–400 nM PLE22.5 and PLT22.5. (**C**) Western blots of βIII tubulin in ScN2a cells incubated with 0–400 nM PLE22.5 and PLT22.5. (**D**) Survival of ScN2a cells incubated with 0–1,000 nM PLE22.5 and PLT22.5. The shaded box represents the concentration range used for efficacy tests in Panel B. Survival rates at each data point were obtained from the average of triplicate assays and the error bars indicate the standard deviation. Western blotting and cytotoxicity assays were confirmed by at least more than three independent experiments.

replication, were performed as described previously [10, 12]. Initially, 4 × 10^6 cells were seeded in culture dishes (100 mm) and cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum, 1% penicillin-streptomycin, and 1% Glutamax. Cell culture reagents were purchased from Invitrogen (Carlsbad, USA). Cells were incubated at 37°C, 5% CO_2 and saturated humidity. As seeded cells attached on the surface of culture dishes, various concentrations of amino acid polymers were added to the culture media. Incubation lasted for six days and on the fourth day media were replaced with the fresh culture media containing polymers. Then, cell lysate was prepared in 1 ml cell lysis buffer (20 mM Tris, pH 8.0; 0.5% Nonidet P-40; 0.5% sodium deoxycholate; 150 mM NaCl). Cell lysate (~30 µg of protein) was analyzed to measure the levels of total PrP and βIII tubulin loading controls by western blotting using anti-PrP antibody 6D11 (Covance, Dedham, USA) and anti- βIII tubulin antibody (R&D System, Minneapolis, USA). For proteinase K (PK)-resistant PrP^{Sc} preparation, cell lysate (2 mg of protein) was incubated with PK (20 µg/ml) for 1 h at 37°C and centrifuged for 1 h at 16,000 ×*g* at 4°C. PrP^{Sc} in the pellet was subjected to analysis. Protein bands in western blots were visualized using ECL Prime Detection Reagents (Amersham, GE Healthcare, Piscataway, USA) and detected by G:Box Chemi XR5 system (Syngene, Cambridge, UK). The viability of ScN2a cells incubated with amino acid polymers was measured using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay protocol described previously [10, 12]. Briefly, ScN2a cells in a 24-well culture plate were seeded and incubated with amino acid polymers in the same manner as described earlier. Then, cells were incubated with DMEM containing 0.5 mg/ml MTT (Sigma-Aldrich, USA) for an additional 3 h. MTT formazan products were extracted with 0.05 N HCl-isopropanol and quantified through colorimetric readouts at 570 nm using an Infinite M200Pro Multimode Reader (Tecan, Männedorf, Switzerland).

To measure anti-prion efficacy of PLO and PLH in comparison to PLR, ScN2a cells were incubated with various concentrations of PLO10, PLH10, and PLR10 and the level of PK-resistant PrP^{Sc} was monitored. Western blot analysis showed that both PLO10 and PLH10 effectively decreased the level of PrP^{Sc} in ScN2a cells in a concentration-dependent manner (Fig. 1B). The level of loading control, βIII tubulin, remained constant (Fig. 1C). The dose responsiveness to inhibit PrP^{Sc} propagation by the low concentrations of PLO10 and PLH10 was less sensitive than by the corresponding concentrations of PLR10. This indicates that efficiency of PrP^{Sc} inhibition varies for different cationic poly-amino acids, presumably due to the functional group of each amino acid. This suggests that the guanidinium groups in PLR are more potent than the amine groups in PLO to inhibit PrP^{Sc} propagation. Unlike constitutively cationic PLO and PLR, PLH conditionally becomes cationic under acidic local environment owing to protonation of the imidazole ring of histidine, which occurs at pH below its pKa (~6.0). Hence, anti-prion activity exerted by PLH suggests that inhibition of PrP^{Sc} propagation by cationic poly-amino acids is facilitated in the acidic subcellular compartments, presumably within the endosomes or lysosomes known to be the subcellular organelles where PrP^{Sc} is converted from PrP^{C} and accumulated as aggregates, respectively [19]. The results of cytotoxicity tests for PLO10, PLH10, and PLR10 showed that these amino acid polymers were, overall, not toxic (Fig. 1D). The concentrations of PLO10, PLH10, and PLR10, displaying effective anti-prion activity, were at non-toxic concentrations. This suggests that anti-prion activity achieved by PLO10, PLH10, and PLR10 was attributed to inhibitory activity of the polymers, but not to the death of prion-infected cells caused by their toxic effect.

To authenticate the correlation of cationic property of PLO, PLR, and PLH to anti-prion activity, anionic poly-amino acids PLE22.5 and electrically uncharged poly-amino acids PLT22.5 were examined to determine whether they affect the level of PrP^{Sc} in ScN2a cells. Incubation of cells with PLE22.5 and PLT22.5 did not change the level of PK-resistant PrP^{Sc} (Fig. 2B). The level of loading control, βIII tubulin, and cell survival were not affected by PLE22.5 and PLT22.5 (Figs. 2B and 2C). These results indicate that amino acid polymers with negative or no charges are not able to inhibit PrP^{Sc} propagation.

In conclusion, anti-prion activity exhibited by PLO, PLR and PLH is attributed to the cationicity of poly-amino acids. It appears that inhibition of PrP^{Sc} propagation by basic amino acid polymers is facilitated in the acidic subcellular organelles.

Conflict of Interest

The authors have no financial conflicts of interest to declare.

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