Biochemical and Biophysical Research Communications **280,** 115–120 (2001)
doi:10.1006/bbrc.2000.4097, available online at http://www.idealibrary.com on IDEAL®

Immunomodulation of the Human Prion Peptide 106–126 Aggregation

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Received November 20, 2000

Site-directed monoclonal antibodies (mAbs) may interact with their antigens, leading to stabilization, refolding, and suppression of aggregation. In the following study, we show that mAbs raised against the peptide 106–126 of human prion protein (PrP 106–126) modulate the conformational changes occurring in the peptide exposed to aggregation conditions. MAbs 3–11 and 2–40 prevent PrP 106–126's fibrillar aggregation, disaggregates already formed aggregates, and inhibits the peptide's neurotoxic effect on the PC12 cells system, while mAb 3F4 has no protective effect. We suggest that there are key positions within the PrP 106–126 molecule where unfolding is initiated and their locking with specific antibodies may maintain the prion peptide native structure, reverse the aggregated peptide conformation, and lead to rearrangements involved in the essential feature of prion diseases.

Key Words: **monoclonal antibody; aggregation; neurotoxicity; prion peptide; immunomodulation.**

Prion diseases involve conversion of the normal cellular prion protein (PrP^C) into the corresponding scrapie isoform (PrP^{Sc}). Spectroscopic measurements demonstrate that the conversion of PrP^C into the scrapie isoform (PrP^{Sc}) involves a major conformational transition, implying that prion diseases, like other amyloidogenic diseases, are disorders of protein conformation. The transition from PrP^C to PrP^{Sc} is accompanied by a decrease in α-helical secondary structure (from 42 to 30%) and a remarkable increase in β-sheet content (from 3 to 43%) (1, 2). This rearrangement is associated with abnormal physicochemical properties, including insolubility in nondenaturing detergents and partial resistance to proteolysis (3).

Previous studies have shown that a synthetic peptide homologous with residues 106–126 of human PrP (PrP106–126) exhibits some of the pathogenic and physicochemical properties of PrP^{Sc}. The peptide shows a remarkable conformational polymorphism, acquiring different secondary structures in various environments. It tends to adopt a β-sheet conformation in buffered solutions, and aggregates into amyloid fibrils that are partly resistant to digestion with protease (4–7). Recently the x-ray crystallographic studies of a complex of antibody 3F4 and its peptide epitope (PrP 104–113) provided a structural view of this flexible region (8). These data suggest that the PrP region including residues 106–126 might be one of the key regions where conformational changes are initiated leading to the conversion of PrP^C to PrP^{Sc}. The identification of classes of sequences that participate in folding-unfolding and/or solubilization-aggregation processes suggests the existence of effective solutions for the prevention of aggregation (9–11).

Binding of high affinity mAbs to such regions may alter the molecular dynamics of the whole protein chain or assembly (12–16). By appropriate selection, mAbs have been found to recognize incompletely folded epitopes and to induce native conformation in partially or wrongly folded protein. Recent studies of Alzheimer's β-amyloid fibrils assembled from the synthetic peptide (βAP) showed that mAbs 6C6 and 10D5 raised against the N-terminal region of the βAP (residues 1–28) can disaggregate βAP fibrils, restore the peptide's solubility and prevent neurotoxic effects (17, 18). In this study, we propose mAbs raised against the peptide 106–126 as modulators of conformational changes occurring in the prion peptide exposed to aggregating conditions, leading to prevention of aggregation and related neurotoxicity on cultivated neural-like cells.

MATERIALS AND METHODS

Preparation of Monoclonal Antibodies against PrP 106–126

Balb/C mice immunized with synthetic peptide corresponding to the sequence of human PrP 106–126 (obtained from Chiron Tech-

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0006-291X/01 $35.00

nologies (Claton Victoria, Australia)) coupled to the larger carrier protein keyhole limpet haemocyanin (KLH) were used for generating monoclonal antibodies following the fusion techniques of Kohler and Milstein (19).

Hybridomas were screened for the production of peptide-specific antibodies by enzyme-linked immunosorbent assay (ELISA) using PrP 106–126 as the antigen. Selected monoclonal antibodies were purified from ascetic fluid according to published procedures: IgG molecules on a protein A column (20) and IgM on KaptiveM column. Two mAbs, namely 2–40 and 3–11, were used for further studies. The mAb 3F4 was purchased from Senetek, Ca.

PrP 106–126 Aggregation and Immunocomplexation

In vitro aggregation of peptide 106–126 was induced by incubation of an aqueous solution of PrP 106–126 (10 mg/ml in water) for 7 days at 37°C. The aggregated peptide was incubated either alone or with monoclonal antibodies 2–40, 3–11, or 3F4 at molar ratios specified later.

Modulation of PrP 106–126 Conformation followed by:

1. Electron microscopy. Negatively stained amyloid fibrils were prepared by floating carbon-coated grids with peptide solutions (250 μM in PBS) and air drying. Fibrils of PrP 106–126, either alone or immunocomplexed to mAbs 2–40 and 3–11 (the molar ratios between the mAbs and the peptide were for 2–40: 1:2 and 1:1000, for 3–11: 1:500) for 7 days at 37°C, were negatively stained with aqueous (2% wt/vol) uranyl acetate and then visualized by using a JEOL model 1200 EX electron microscope operated at 80 kV.

2. Thioflavin T (ThT) fluorimetry assay. Prion amyloid fibril formation was quantified by the Thioflavin T (ThT) binding assay which is specific to identify amyloid fibrils (21, 22). The aggregation of the prion peptide was followed using samples of PrP 106–126 (0.5 mg/ml) in PBS incubated for 7 days at 37°C, either alone or with mAbs 3–11, 2–40, and 3F4. The molar ratios between the mAbs and the peptide were for 3F4: 1:5, for 2–40: 1:5, 1:10, 1:100 and 1:1000, for 3–11: 1:500, 1:1000, 1:5000 and 1:10000. Disaggregation of already formed prion amyloid fibrils was measured using samples of PrP 106–126 that were incubated for 7 days at 37°C and then supplemented with the mAbs for an additional 24 h. Fluorescence (emission at 482 nm after excitation at 435 nm) was measured after an addition of the samples to ThT (2 μM in 50 mM glycine, pH 9).

Cytotoxicity Assay of PrP 106–126 Using PC12 Cells

Rat pheochromocytoma PC12 cells were cultured in DMEM supplemented with 8% horse serum, 8% fetal calf serum, 2 mM L-glutamine and 100 units/ml penicillin/streptomycin and incubated at 37°C under 5% CO_2.

For the neurotoxicity assay, cultured PC12 cells were seeded on 96-well plates at a density of 2×10^4 cells/100 μl/well in a serum-free medium supplemented with 2 μM of insulin. Cells were treated for 3 days with 100 μM PrP 106–126 preincubated 7 days at 37°C to induce maximal aggregation of the peptide. Cell viability was assessed by the MTT assay (23). Briefly, MTT was added to the wells to a final concentration of 1 mg/ml and incubated with the cells for an additional 3 h at 37°C. Cell lysis buffer (20% wt/vol SDS in a solution of 50% dimethylformamide, pH 4.7) was added, and the plate was incubated overnight at 37°C. MTT reduction was determined colorimetrically by measuring the optical density (OD) at 550 nm.

Prevention of PrP 106–126 Neurotoxicity

The effect of mAbs on the inhibition of PrP 106–126 neurotoxicity was determined as follows: Monoclonal antibodies 3–11, 2–40, and 3F4 were added (at molar ratios between the mAbs and the peptide of 1:500, 1:10, and 1:10, respectively) for 1 h to samples of 1 mM of the already aggregated peptide (diluted with DMEM). The antibody-peptide mixtures, as well as the aggregated peptide alone, were applied to the cells to a peptide final concentration of 100 μM. Cell viability following a 3 day incubation at 37°C with the aforementioned reaction mixture, was assessed as described above. 100% viability was defined as the value of MTT assay for untreated cells.

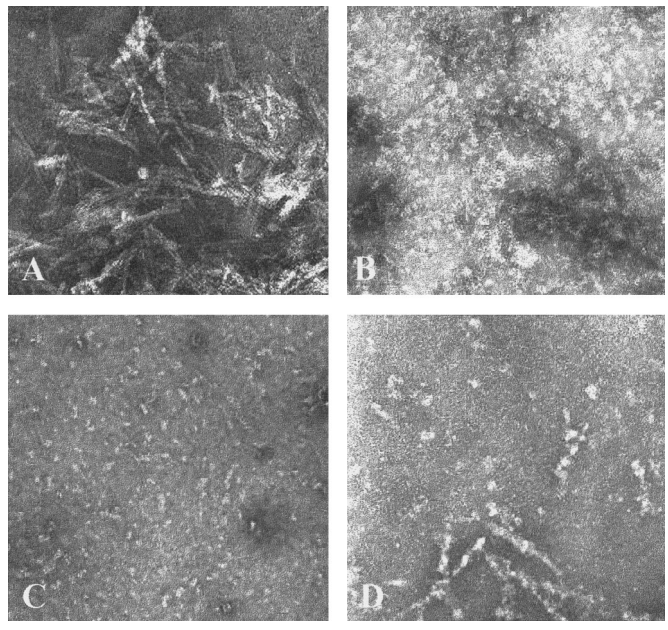

FIG. 1. Modulation of PrP conformation via mAbs as detected by the electron microscopy. PrP 106–126 (250 μM) was incubated for 7 days at 37°C alone (A) or with mAbs 2–40 (B, D) and 3–11 (C). The molar ratios between the mAbs and the peptide were 1:2, 1:500, and 1:1000 (B–D, respectively). Amyloid fibrils were detected by electron microscopy (Magnification $\times$ 50,000).

RESULTS

Isolation of Monoclonal Antibodies against PrP 106–126

Mice immunized with synthetic peptide corresponding to the sequence of human PrP 106–126 coupled to the larger carrier KLH were used for generating monoclonal antibodies against epitopes on this peptide. An array of positive clones detected by ELISA, composed mainly of immunoglobulin M (IgM) molecules, has been isolated though several IgG clones were present as well. In this study we will discuss the anti-aggregating activity of mAbs 3–11 (IgM $\mu\lambda$) and 2–40 (IgG $\gamma 1\kappa$), both of which were revealed to bind the whole prion protein in its cellular form (PrP^C) as well as in its scrapie form (PrP^{Sc}) (data not shown).

Immunomodulation of PrP 106–126 Fibrillar Aggregation followed by Electron Microscopy

Incubation of PrP 106–126 for 7 days at 37°C resulted in amyloid fibril formation, as seen via electron microscopy (Fig. 1A). Preincubation of the peptide with

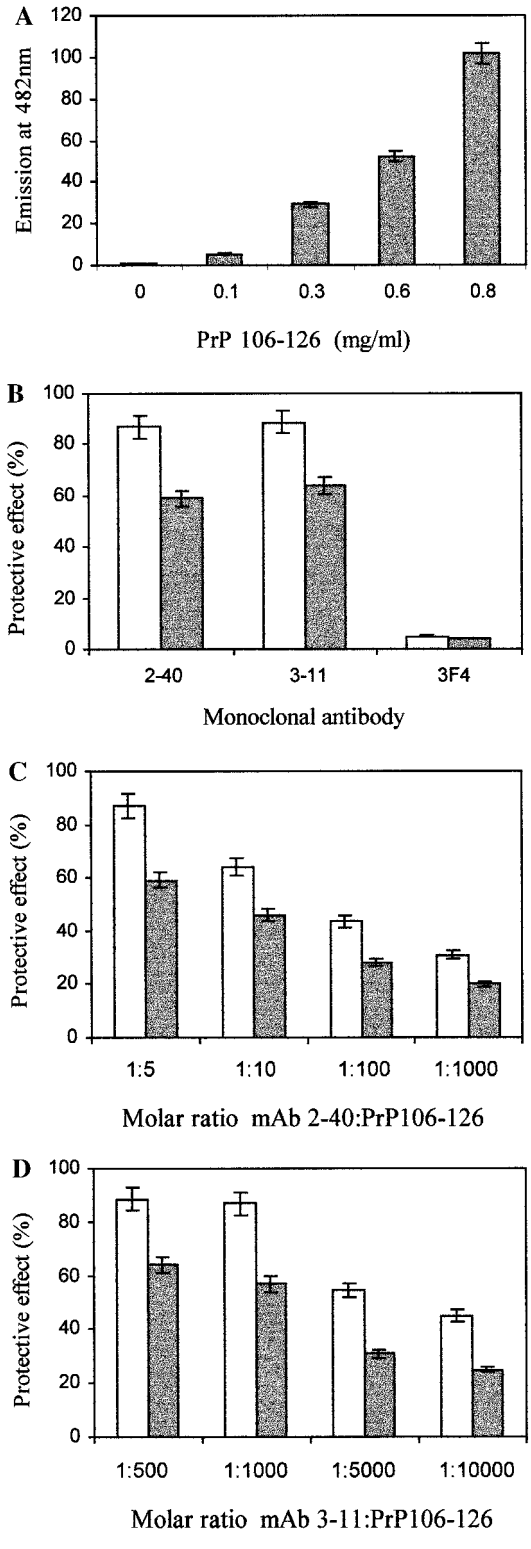

FIG. 2. Modulation of PrP conformation via mAbs as detected by the ThT assay. (A) Increasing concentrations of PrP 106–126 (0–0.8 mg/ml) were incubated for 7 days at 37°C. Fibril formation was assessed by the ThT binding assay. (B) PrP 106–126 (0.5 mg/ml) was incubated for 7 days at 37°C alone (1) or with mAbs 2–40, 3–11, and 3F4 (2, 3, and 4, respectively). The antibodies were added either before the exposure to 37°C (white bars) or after, for an additional 24 h (grey bars). The molar ratios between mAbs 2–40, 3–11, 3F4, and the peptide were 1:5, 1:500, and 1:5, respectively. Fibril formation was assessed by the ThT binding assay. (C, D) PrP 106–126 (0.5 mg/ml) was incubated for 7 days at 37°C with increasing dilutions of mAbs 2–40 (C) and 3–11 (D). The antibodies were added either before the exposure to 37°C (white bars) or after, for an additional 24 h (grey bars). The molar ratios between the mAbs and the peptide were for 2–40: 1:5, 1:10, 1:100, and 1:1000, for 3–11: 1:500, 1:1000, 1:5000, and 1:10000.

mAbs 2–40 and 3–11 interfered with the fibrillar aggregation (Figs. 1B and 1C) in a dose dependant manner. As shown in Fig. 1D at low molar ratios between the mAb 2–40 and the peptide, amyloid fibrils are still detected but at a lower extent compared to the aggregated peptide alone.

Prevention of Amyloid Fibril Formation as Measured by ThT Assay

Incubation of PrP 106–126 at 37°C at different concentrations led to a dose dependent amyloid fibrillar aggregation, as measured by the Thioflavin T binding fluorescence assay (Fig. 2A). The concentration of PrP 106–126 at 0.5 mg/ml was chosen for further studies.

MAbs 3–11 and 2–40 prevent PrP 106–126 fibrillar aggregation and reverse the aggregated form to a nonamyloid conformation. A significant decrease in amyloid formation (up to 88% prevention) occurred after the preincubation of PrP 106–126 at 37°C in the presence of mAbs 3–11 and 2–40, supporting the electron microscopy results.

When the antibodies were added to already formed aggregates of PrP 106–126 more than 60% of fibrils are disaggregated under the experimental conditions employed. MAb 3F4 exhibited poor ability both in inhibiting fibril formation and in their disaggregation (Fig. 2B). The inhibition of fibril formation and their disaggregation were related to the antibody's concentration and to the epitope location (Figs. 2B–2D). The protective activity of mAb 3–11 is exhibited at lower concentrations compared to mAb 2–40. This behaviour may be partly explained by the multi-valency of mAb 3–11 being an IgM. The protective effect percentage was calculated in the following way:

$$\%\ \text{Protective effect} = 100 - \frac{\text{Emission 482 nm (PrP 106–126 incubated with Ab)}}{\text{Emission 482 nm (PrP 106–126 incubated alone)}} \times 100.$$

Prevention of PrP 106–126 Toxicity in a PC12 Cell Culture Model

Since the major target organ for scrapie agent is the nervous system, an *in vitro* neuronal model system was

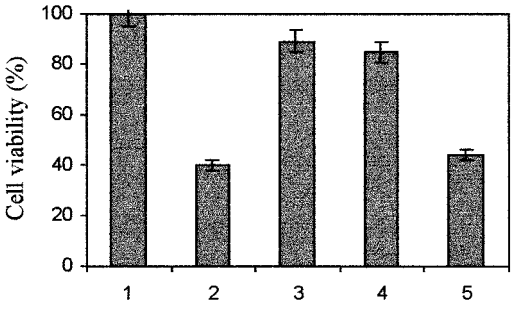

FIG. 3. Protective effect of antibodies on PrP peptide neurotoxicity. PC12 cells were seeded on 96 well plates in a DMEM medium supplemented with 2 mM insulin 2 mM L-glutamine and 100 units penicillin/streptomycin. Cells were subjected to the following treatments for 3 days: 1. Positive Control, untreated cells. 2. 100 μM PrP 106–126 preincubated for 7 days at 37°C. 3, 4, 5. A mixture of mAbs 3–11, 2–40, and 3F4, respectively and the aggregated peptide preincubated for 1 h before applied on cells. Following the above treatments cell viability was assessed by MTT assay.

used to analyze PrP toxicity. The rat pheochromocytoma PC12 cells—a neuron-like cloned tumor cell line (24)—served as a model for detection of protective molecules against the toxicity induced by PrP fibrils.

PrP 106–126 was found to be toxic to PC12 cells in a dose dependant manner, related to its conformational state. Cell viability considerably decreased, (as detected by MTT assay), when cells were exposed for 3 days to a preincubated PrP 106–126. Under the described conditions, cell viability reduced to 40% at 100 μM of PrP 106–126 (Fig. 3).

The cytoprotective effect of mAb 3–11 and 2–40 is shown in Fig. 3. mAbs 3–11 and 2–40 were shown to inhibit death of cells induced by 100 μM PrP 106–126. The viability of cells treated with a mixture of either antibody and the peptide was 85–89%, comparing to a 40% survival of cells treated with the peptide alone (the antibodies without the peptide had no affect on cell viability). The antibodies' protective effect was apparently related to the specific epitope on the PrP 106–126 molecule, since no evident protection was demonstrated by mAb 3F4 (44% viability) which binds only the PrP^{C} form (25).

DISCUSSION

High affinity ligands bind to the native conformation of proteins involved in some amyloidoses and inhibit the conformational changes required for amyloid fibril formation (17, 18, 26, 27). The possibility of involvement of PrP amyloid in the pathogenesis of nerve cell degeneration and glial cell reaction led to the identification of PrP sequences that are important to amyloid formation. A fragment of PrP consisting of amino acids 106–126 was found to be toxic to rat hippocampal neurons (6), to mouse cortical and cerebellar cells (28, 29), and to be particularly highly fibrillogenic (4). The fibrils were partially resistant to proteases digestion and exhibited properties of *in situ* amyloid (4, 5). Synthetic peptides corresponding to this region of PrP exhibit considerable conformational flexibility consistent with the α-helix to β-sheet transition (4) similar to that of PrP^{C} transformed into PrP^{Sc}. The conformational plasticity of this region is further emphasized by the findings that two distinct prion strains exhibit different sites of proteolytic cleavage within this region (30, 31).

The data presented show that site-directed antibodies may prevent the conversion of the prion peptide to a toxic conformation and disaggregate the fibrillar structure, abolishing its toxicity. Locking this peptide with mAbs led to a considerable protective effect against aggregation as measured by ThT and MTT assays and detected by electron microscopy. We have found that mAbs 3–11 and 2–40 decrease significantly the peptide fibrillar aggregation, reverse the aggregated form to a nonamyloid conformation, and decrease the peptide toxicity on PC12 cells.

The availability of the mAbs 3–11 and 2–40 epitopes for antibody binding when PrP peptide is either in solution or is an aggregate suggests that this epitope is involved in aggregation process and may act as a regulatory site controlling both the solubilization and disaggregation process of PrP peptide. These features remain to be determined on the whole PrP protein level.

The NMR structure of the full length human prion protein (PrP 23–228) shows that the ~100 amino acids of the amino terminal half (residues 23–124) have a random disorganized structure. Epitopes towards the N-terminus of the protein, that are largely cryptic in PrP^{Sc}, are exposed in PrP^{C} suggesting that the major conformational changes required for the acquisition of prion infectivity occur in the N-terminal portion of the protein. The highly flexible region could provide the plasticity required for the conformational transition of PrP^{C} to PrP^{Sc} perhaps by template assisted formation of β-structure. The region could prove to be important in lowering the activation barrier for the conversion of PrP^{C} into PrP^{Sc} (2, 32–34). This is consistent with studies with recombinant monoclonal antibodies that show that epitopes located at the N-terminus but not the C-terminus of PrP^{Sc} are buried, whereas epitopes throughout native PrP^{C} are accessible, suggesting that the amino terminus of PrP^{C} is the part of the protein that changes most profoundly during the conversion to PrP^{Sc} (35). It is also suggested that the 29–125 region of the highly homologous Syrian hamster PrP stabilizes the conformational ensemble of helix B between residue 187 and 193, a region with importance in PrP^{C}/PrP^{Sc} recognition (32).

Understanding the mechanism and molecular details of the toxic conformational conversion of amyloidogenic protein may be of great importance in developing approaches towards the prevention and treatment of such diseases. Intervention in the for-

mation of amyloid fibrils may be a common strategy to prevent the development of different conformational diseases. Appropriate mAbs may interact at such strategic sites where protein unfolding is initiated, thereby stabilizing the protein and preventing further conformational changes and avoiding pathological aggregation.

ACKNOWLEDGMENTS

This work was supported by The Center for the Study of Emerging Diseases. We would like to thank Rela Koppel for her technical help.

REFERENCES

1. Caughey, B. W., Dong, A., Bhat, K. S., Ernst, D., Hayes, S. F., and Caughey, W. S. (1991) Secondary structure analysis of the scrapie-associated protein PrP 27–30 in water by infrared spectroscopy. *Biochemistry* **30,** 7672–7680.
2. Pan, K. M., Baldwin, M., Nguyen, J., Gasset, M., Serban, A., Groth, D., Mehlhorn, I., Huang, Z., Fletterick, R. J., Cohen, F. E., and Prusiner, S. B. (1993) Conversion of alpha-helices into beta-sheets features in the formation of the scrapie prion proteins. *Proc. Natl. Acad. Sci. USA* **90,** 10962–10966.
3. Meyer, R. K., McKinley, M. P., Bowman, K. A., Braunfeld, M. B., Barry, R. A., and Prusiner, S. B. (1986) Separation and properties of cellular and scrapie prion proteins. *Proc. Natl. Acad. Sci. USA* **83,** 2310–2314.
4. Selvaggini, C., De Gioia, L., Cantu, L., Ghibaudi, E., Diomede, L., Passerini, F., Forloni, G., Bugiani, O., Tagliavini, F., and Salmona, M. (1993) Molecular characteristics of a protease-resistant, amyloidogenic, and neurotoxic peptide homologous to residues 106–126 of the prion protein. *Biochem. Biophys. Res. Commun.* **194,** 1380–1386, doi:10.1006/bbrc.1993.1977.
5. Tagliavini, F., Prelli, F., Verga, L., Giaccone, G., Sarma, R., Gorevic, P., Ghetti, B., Passerini, F., Ghibaudi, E., Forloni, G., Salmona, M., Bugiani, O., and Frangione, B. (1993) Synthetic peptides homologous to prion protein residues 106–147 form amyloid-like fibrils *in vitro*. *Proc. Natl. Acad. Sci. USA* **90,** 9678–9682.
6. Forloni, G., Angeretti, N., Chiesa, R., Monzani, E., Salmona, M., Bugiani, O., and Tagliavini, F. (1993) Neurotoxicity of a prion protein fragment. *Nature* **362,** 543–546.
7. De Gioia, L., Selvaggini, C., Ghibaudi, E., Diomede, L., Bugiani, O., Forloni, G., Tagliavini, F., and Salmona, M. (1994) Conformational polymorphism of the amyloidogenic and neurotoxic peptide homologous to residues 106–126 of the prion protein. *J. Biol. Chem.* **269,** 7859–7862.
8. Kanyo, Z. F., Pan, K. M., Williamson, R. A., Burton, D. R., Prusiner, S. B., Fletterick, R. J., and Cohen, F. E. (1999) Antibody binding defines a structure for an epitope that participates in the PrPC→PrPSc conformational change. *J. Mol. Biol.* **293,** 855–863, doi:10.1006/jmbi.1999.3193.
9. Silen, J. L., and Agard, D. A. (1989) The alpha-lytic protease pro-region does not require a physical linkage to activate the protease domain *in vivo*. *Nature* **341,** 462–464.
10. Frenkel, D., Ballas, M., and Solomon, B. (1998) N-terminal EFRH sequence of Alzheimer's β-amyloid peptide represents the epitope of its anti-aggregating antibodies. *J. Neuroimmunology* **88,** 85–90.
11. Horiuchi, M., and Caughey, B. (1999) Specific binding of normal prion protein to the scrapie form via a localized domain initiates its conversion to the protease-resistant state. *EMBO J.* **18,** 3193–3203.
12. Frauenfelder, H., Petsko, G. A., and Tsernoglou, D. (1979) Temperature-dependent X-ray diffraction as a probe of prote Nature 1989 Oct 5;341(6241):462–4 in structural dynamics. *Nature* **280,** 558–563.
13. Blond, S., and Goldberg, M. (1987) Partly native epitopes are already present on early intermediates in the folding of tryptophan synthase. *Proc. Natl. Acad. Sci. USA* **84,** 1147–1151.
14. Karplus, M., and Petsko, G. A. (1990) Molecular dynamics simulations in biology. *Nature* **347,** 631–639.
15. Carlson, J. D., and Yarmush, M. L. (1992) Antibody assisted protein refolding. *Biotechnology* **10,** 86–89.
16. Solomon, B., and Schwartz, F. (1995) Chaperone-like effect of monoclonal antibodies on refolding of heat-denatured carboxypeptidase A. *J. Mol. Recognit.* **8,** 72–76.
17. Solomon, B., Koppel, R., Hanan, E., and Katzav, T. (1996) Monoclonal antibodies inhibit *in vitro* fibrillar aggregation of the Alzheimer beta-amyloid peptide. *Proc. Natl. Acad. Sci. USA* **93,** 452–455.
18. Solomon, B., Koppel, R., Frankel, D., Hanan-Aharon, E. (1997) Disaggregation of Alzheimer beta-amyloid by site-directed mAb. *Proc. Natl. Acad. Sci. USA* **94,** 4109–4112.
19. Kohler, G., and Milstein, C. (1975) Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* **256,** 495–497.
20. Harlow, E., and Lane, D. (1988) *In* Antibodies, A Laboratory Manual, Chapter 8, Cold Spring Harbor Laboratory.
21. Naiki, H., Higuchi, K., Hosakawa, M., and Takeda, T. (1989) Fluorometric determination of amyloid fibrils *in vitro* using the fluorescent dye, thioflavin T1. *Anal. Biochem.* **177,** 244–249.
22. LeVine, H., III (1993) Thioflavine T interaction with synthetic Alzheimer's disease beta-amyloid peptides: Detection of amyloid aggregation in solution. *Protein Sci.* **2,** 404–410.
23. Hansen, M. B., Nielsen, S. E., and Berg, K. (1989) Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. *J. Immunol. Methods* **119,** 203–210.
24. Greene, L. A., and Tischler, A. S. (1976) Establishment of a noradrenergic clonal line of rat adrenal pheochromocytoma cells which respond to nerve growth factor. *Proc. Natl. Acad. Sci. USA* **73,** 2424–2428.
25. Safar, J., Wille, H., Itri, V., Groth, D., Serban, H., Torchia, M., Cohen, F. E., and Prusiner, S. B. (1998) Eight prion strains have PrP(Sc) molecules with different conformations. *Nat. Med.* **4,** 1157–1165.
26. Blaney, J. M., Jorgensen, E. C., Connolly, M. L., Ferrin, T. E., Langridge, R., Oatley, S. J., Burridge, J. M., and Blake, C. C. F. (1982) Computer graphics in drug design: molecular modeling of thyroid hormone-prealbumin interactions. *J. Med. Chem.* **25,** 785–790.
27. Miroy, G. J., Lai, Z., Lashuel, H. A., Peterson, S. A., Strang, C., and Kelly, J. W. (1996) Inhibiting transthyretin amyloid fibril formation via protein stabilization. *Proc. Natl. Acad. Sci. USA* **93,** 15051–15056.
28. Brown, D. R., Hernis, J., and Kretzschmar, H. A. (1994) Mouse cortical cells lacking cellular PrP survive in culture with aneurotoxic PrP fragment. *Neuroreport* **5,** 2057–2060.
29. Brown, D. R., Herms, J. W., Schmidt, B., and Kretzschmar, H. A. (1997) Different requirements for the neurotoxicity of fragments of PrP and β-amyloid. *Eur. J. Neurosci.* **9,** 1162–1169.
30. Bessen, R. A., and Marsh, R. F. (1994) Distinct PrP properties suggest the molecular basis of strain variation in transmissible mink encephalopathy. *J. Virol.* **68,** 7859–7868.
31. Telling, G. C., Parchi, P., DeArmond, S. J., Cortelli, P., Montagna, P., Gabizon, R., Mastrianni, J., Lugaresi, E., Gambetti, P., and Prusiner, S. B. (1996) Evidence for the conformation of the pathologic isoform of the prion protein enciphering and propagating prion diversity. *Science,* **274,** 2079–2082.
32. Donne, D. G., Viles, J. H., Groth, D., Mehlhorn, I., James, T. L., Cohen, F. E., Prusiner, S. B., Wright, P. E., and Dyson, H. (1997) Structure of the recombinant full-length hamster prion protein

PrP(29–231): The N-terminus is highly flexible. *Proc. Natl. Acad. Sci. USA* **94,** 13452–13457.

33. Riek, R., Hornemann, S., Wider, G., Glockshuber, R., and Wuthrich, K (1997) NMR characterization of the full-length recombinant murine prion protein, mPrP(23–231). *FEBS Lett.* **413,** 282–288.
34. Zahn, R., Liu, A., Luhrs, T., Riek, R., von Schroetter, C., Lopez Garcia, F., Billeter, M., Calzolai, L., Wider, G., and Wuthrich, K. (2000) NMR solution structure of the human prion protein. *Proc. Natl. Acad. Sci. USA* **97,** 145–150.
35. Peretz, D., Williamson, R. A., Matsunaga, Y., Serban, H., Pinilla, C., Bastidas, R. B., Rozenshteyn, R., James, T. L., Houghten, R. A., Cohen, F. E., Prusiner, S. B., and Burton, D. R. (1997) A conformational transition at the N-terminus of the prion protein features in formation of the scrapie isoform. *J. Mol. Biol.* **273,** 614–622, doi:10.1006/jmbi.1997.1328.