Properties of Scrapie Prion Protein Liposomes*

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Purified scrapie prions contain one identifiable macromolecule, PrP 27–30, which polymerizes into rod-shaped amyloids. The rods can be dissociated with retention of scrapie infectivity upon incorporation of PrP 27–30 into detergent-lipid-protein complexes (DLPC) as well as liposomes. As measured by end-point titration, scrapie infectivity was increased >100-fold upon dissociating the rods into liposomes. The incorporation of PrP 27–30 into liposomes was demonstrated by immunoelectron microscopy using colloidal gold. Detergent extraction of prion liposomes followed by chloroform/methanol extraction resulted in the reappearance of rods, indicating that this process is reversible. Scrapie prion infectivity in rods and liposomes was equally resistant to inactivation by irradiation at 254 nm and was unaltered by exposure to nucleases. A variety of lipids used for producing DLPC and liposomes did not alter infectivity. Fluorescently labeled PrP 27–30 in liposomes was used to study its entry into cultured cells. Unlike the rods which remained as large fluorescent extracellular masses, the PrP 27–30 in liposomes rapidly entered the cells and was seen widely distributed within the interior of the cell. PrP 27–30 is derived by limited proteolysis from a larger protein designated PrPSc which is membrane bound. PrPSc in membrane fractions was solubilized by incorporation in DLPC, thus preventing its aggregation into amyloid rods. The functional solubilization of scrapie prion proteins in DLPC and liposomes offers new approaches to the study of prion structure and the mechanism by which they cause brain degeneration.

Scrapie is a transmissible, degenerative neurological disease of sheep and goats. The clinical signs of neurologic dysfunction become manifest after a prolonged incubation period (Eklund et al., 1967; Gajdusek, 1977; Prusiner, 1982b). The scrapie agent, in contrast to viruses, resists inactivation by procedures that modify or hydrolyze nucleic acids (Alper et al., 1967; Diener et al., 1982; Prusiner, 1982); however, it is sensitive to reagents which modify proteins (Prusiner et al., 1981; McKinley et al., 1983; Prusiner et al., 1983). The requirement of a protein for infectivity and the unusual properties of the scrapie agent prompted the introduction of the term “prion” to denote this novel class of infectious pathogens (Prusiner, 1982, 1987; Diener, 1987). The only identifiable macromolecule of purified preparations of scrapie prions is a protein, designated PrP 27–30 (Bolton et al., 1982; Prusiner et al., 1982; McKinley et al., 1983). This protein is derived by proteinase K digestion from a larger molecule of apparent molecular weight 33,000–35,000, denoted PrPSc (Oesch et al., 1986; Barry et al., 1986; Meyer et al., 1986). Both PrP 27–30 and PrPSc are membrane bound, aggregate into rod-shaped polymers upon detergent extraction, and can be purified as aggregates (Barry et al., 1986; Meyer et al., 1986).

Recently, we described the functional solubilization of purified PrP 27–30 from the rod-shaped polymers into detergent-lipid-protein complexes (DLPC) and liposomes (Gabizon et al., 1987). PrP 27–30 in rods was mixed with phosphatidylcholine and 2% sodium cholate, vortexed, and sonicated to form DLPC. The 100,000 × g supernatant of this mixture contained most of the PrP 27–30 demonstrating that the protein had been solubilized. After removing the detergent by dialysis, PrP 27–30 was found incorporated into liposomes. Both the DLPC and liposomes exhibited scrapie prion infectivity.

In this report, we extend our initial observations on prion DLPC and liposomes. The properties of these novel forms of the prion are described and compared to those previously reported for the rods which possess the properties of amyloid (Prusiner et al., 1983; McKinley et al., 1986). In contrast to liposomes which are closed spherical vesicles, DLPC are small fragments of these phospholipid vesicles. Because of their small size, the DLPC were used for all studies except those where detergents interfered with the experimental protocols such as immunoelectron microscopy and cell fusion. In these studies, liposomes were formed upon removal of the detergent by dialysis. The formation of rods, DLPC, and liposomes is a reversible process with all forms being infectious. These properties of prions are unlike those of viruses and are most compatible with a one-component system.

MATERIALS AND METHODS

1 The abbreviations used are: DLPC, detergent-lipid-protein complex; PC, phosphatidylcholine; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; FITC, fluorescein isothiocyanate; Sarkosyl, sodium dodecyl sarcosinate; PMSF, phenylmethylsulfonyl fluoride.

2 Portions of this paper (including "Materials and Methods," part of "Results," Figs. 2–8, and Tables I and II) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are included in the microfilm edition of the Journal that is available from Waverly Press.

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RESULTS

The studies described in a previous communication (Gabizon et al., 1987) and the Miniprint section of this paper have used PrP 27–30 which is purified as insoluble rod-shaped polymers. Subsequent PrP 27–30 disaggregation without denaturation was accomplished by a combination of detergent and phospholipids which led to the formation of DLPC. PrP 27–30 is derived from a larger molecule, PrPk, during purification which utilizes limited proteinase K digestion. This digestion hydrolyzes the N-terminal 67-amino acid residues (Prusiner et al., 1984a; Basler et al., 1986). PrPsc is a membrane-bound protein which polymerizes upon detergent extraction (Meyer et al., 1986).

A protocol was developed to prevent polymerization of PrPsc into rods upon detergent extraction of membranes by adding phospholipids. Microsomal membranes from scrapie-infected or normal hamster brain were extracted with 2% (w/v) sodium dodecyl sarcosinate (Sarkosyl) in the presence or absence of phosphatidylcholine and then subjected to ultracentrifugation. The supernatant fluids (Fig. 1, lanes 1–6) and pellets (lanes 7–12) were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis followed by immunoblotting with PrP 27–30 antisera (Fig. 1). Since PrP 27–30 antisera reacts with both PrPsc and PrPC, we used proteinase K digestion to distinguish these two proteins. All odd-numbered lanes were digested with proteinase K under conditions where PrPsc is converted to PrP 27–30 and PrPC is hydrolyzed prior to denaturation in 2% sodium dodecyl sulfate at 100 °C. Addition of phospholipid during detergent extraction results in the solubilization of PrPsc (Fig. 1, lanes 1, 2, 7, and 8) instead of aggregation (lanes 3, 4, 9, and 10). On the other hand, PrPC is solubilized by detergent alone (lanes 5, 6, 11, and 12).

The detergent solubilization of PrPC bound to membranes from uninfected hamster brains was included as a control (supernatant fluid, lanes 5 and 6; pellet, lanes 11 and 12). Digestion of PrPsc by proteinase K is shown in lane 5.

The formation of DLPC by addition of Sarkosyl and phosphatidylcholine to scrapie-infected microsomal membranes did not alter the scrapie infectivity titer. The microsomal membrane fraction had a titer of 10^8.3 ID50 units/ml; after detergent extraction in the presence of phospholipid followed by ultracentrifugation, the titer of the supernatant fraction was 10^8.2 ID50 units/ml.

DISCUSSION

For many years, investigators reported the membrane-bound nature of the scrapie agent but were unable to solubilize the infectious entity (Hunter, 1972, 1979; Hunter et al., 1974). In fact, the intimate association of scrapie infectivity with membranes gave rise to the “membrane hypothesis” (Hunter et al., 1967) and to the notion that the scrapie agent was inseparable from cellular membranes, and thus, could not be purified (Hunter et al., 1971). Subsequently, purification schemes, based upon the detergent-induced polymerization of scrapie prions into rod-shaped amyloids, were developed by us (Prusiner et al., 1980b, 1981, 1982, 1983) and modified by others (Diringer et al., 1983a; Hilmert and Diringer, 1984; Hope et al., 1986).

Although the rod-shaped scrapie prion polymers could be dissociated, this required denaturation of the component protein, PrP 27–30 or PrPsc, until recently. Denaturation of PrP 27–30 was registered by conversion from a prion with a sensitive form which is accompanied by a loss of scrapie infectivity; this diminution of infectivity greatly impeded characterization of the prion particle (Bolton et al., 1984). Dissociation of the prion rods by a combination of detergent and phospholipid was a significant advance since infectivity was retained under these conditions (Gabizon et al., 1987).

We now report the extraction of PrPsc and scrapie infectivity from membranes directly into DLPC without aggregation into rods as an intermediate step (Fig. 1). The development of a protocol for solubilization of scrapie prions should facilitate the development of new methods for purification and characterization of these novel infectious pathogens.

The number of hypotheses which can be seriously considered to explain the molecular structure of the infectious scrapie agent have been significantly constrained by recent studies (Diener et al., 1982; Oesch et al., 1985; Carlson et al., 1986; Gabizon et al., 1987; Prusiner, 1987; Westaway et al., 1987). The studies reported here and elsewhere have made the possibility that scrapie is caused by a virus seem remote and have forged a strong case for PrPsc being an integral and necessary component of the infectious particle (Diener et al., 1982; McKinley et al., 1983; Oesch et al., 1985; Gabizon et al., 1987; Diener, 1987).

Our studies show that morphological transformation of the scrapie PrP isoform from a rod-shaped polymer to a liposome is accompanied by a significant increase in infectivity. This increase in biological activity is generally >10-fold as assessed by incubation time bioassay and ~100-fold as reported here as measured by end-point titration (Fig. 3). That PrP 27–30 is actually transferred into the liposomes during disruption of the rods is well documented by immunoelectron microscopy. Fig. 2 shows that the liposomes acquire antigens which bind PrP 27–30 antisera as registered by the attachment of colloidal gold second antibodies. The dispersion of PrP 27–30 was indirectly demonstrated by fusion of the prion liposomes to cultured cells (Fig. 8). Fluorescein isothiocyanate (FITC)-labeled rods were seen as clumps adjacent to cells while prion

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Fig. 1. Western blot of detergent-extracted scrapie and cellular PrP isoforms in the presence or absence of phosphatidylcholine. Brain microsomal membranes from scrapie-infected or uninfected controls were isolated and extracted with 2% (w/v) Sarkosyl in the presence (+) and absence (−) of phosphatidylcholine (PC). The extracts were centrifuged in a Type 50 Beckman ultracentrifuge rotor at 170,000 x g for 30 min at 4 °C. Supernatant (S) and pellet (P) fractions were separated and analyzed by electrophoresis into an 15% polyacrylamide gel according to the conditions of Laemmli (1970). Prior to electrophoresis, selected fractions were digested (+) with proteinase K (PK) in order to distinguish the scrapie PrP isoform from the cellular one. After electrotransfer from the electrophoretic gel to nitrocellulose, the blot was stained with rabbit antiserum raised against purified hamster brain PrP 27–30. Molecular weight markers are given in kilodaltons.
lipoosomes fused to cells resulted in the transfer of fluorescent PrP 27-30 to the interior of the cells. It will be of interest to learn whether cultured cells can be more efficiently infected with prions using lipoosomes rather than rods.

In our initial study of prion phospholipid vesicles, we reported the resistance of scrapie infectivity in DLPC and lipoosomes to inactivation by nucleases and Zn++ (Gabizon et al., 1987) in accord with other studies using purified prion rods (Bellinger-Kawahara et al., 1987b). Those results have been extended by adding nucleases to the rods and then dissociating the rods into DLPC in the presence of the nucleases (Table I). Again, no change in scrapie infectivity was found. Irradiation at 254 nm of the DLPC produced an inactivation curve virtually identical to that observed for the rods in the experiment reported here and earlier studies (Fig. 4) (Bellinger-Kawahara et al., 1987a). The resistance of scrapie infectivity to inactivation by irradiation at 254 nm in preparations of purified prion rods suggests that if prions contain a nucleic acid, it will be ~5 bases in length for a single-stranded molecule or 30-45 base pairs for a double-stranded molecule (Bellinger-Kawahara et al., 1987a). The validity of these earlier studies is strengthened by the observations reported here; the same results were obtained after prions were transformed from rods into DLPC. The D values for prion DLPC is in good agreement with values reported two decades earlier for murine scrapie agent in brain homogenates (Alper et al., 1967).

In contrast to resistance of prion rods and DLPC to procedures that modify nucleic acids, prolonged exposure of the rods and DLPC to proteolytic digestion resulted in a significant decrease in scrapie infectivity (Fig. 5). Inactivation of prion rods and DLPC by proteinase K was both a function of the time of digestion and the concentration of protease as reported earlier for both partially purified (Prusiner et al., 1981) and more extensively purified samples (McKinley et al., 1983). Our experiments clearly demonstrate that the resistance of PrP 27-30 to enzyme-catalyzed proteolysis is an intrinsic property of the scrapie PrP isoform and not a consequence of its polymerization into amyloid rods.

We do not understand the molecular basis for solubilization of PrPSc by a combination of detergent and phospholipid. No detergent alone or in combination with other detergents has been identified which solubilizes the majority of either PrPSc or scrapie infectivity (Millon and Manning, 1979; Prusiner et al., 1978, 1980a, 1980b, 1984b; Diringer et al., 1983b). Recent studies on the aspartate receptor show a similar phenomenon where a combination of detergent and phospholipid solubilized this protein in a biologically active form while detergents alone did not (Bogonez and Koshland, 1985). Many transport proteins must be solubilized in the presence of lipids in order to isolate and reconstitute them in a functionally active state (Maron et al., 1979; Barzilai et al., 1984; Braiman et al., 1987; Moriyama and Nelson, 1987).

The studies presented here and elsewhere (Meyer et al., 1986; Gabizon et al., 1987) suggest that a complex equilibrium may exist between the membrane-bound and rod polymer forms of PrPSc. This putative equilibrium appears to be influenced by the relative concentrations of detergent and lipid. Detergent extraction of membranes from scrapie-infected brains results in the formation of rods. The rods can be dissociated by phospholipids in the presence of detergent; from the resulting DLPC, the rods can be reformed by raising the detergent level (Fig. 6). In its most simple representation, the system behaves as if it is an equilibrium between the membrane-bound and rod forms of PrPSc that can be described by the following equation:

$$K = \frac{[\text{PrP}^{\text{Sc}} \cdot \text{lipid}]}{[\text{detergent}]}$$

where PrPSc, is membrane-bound PrPSc, K is the equilibrium constant, and PrPSc is the amyloid rod form of PrPSc.

A process similar to that described by the above equation appears to occur in vivo where aggregated PrPSc found in scrapie-infected brains in the form of amyloid filaments is probably a consequence of cell death which releases PrPSc into the extracellular space where it polymerizes into filaments (Bendheim et al., 1984; DeArmond et al., 1985). Even in advanced disease, most of the PrPSc appears to be membrane bound (Meyer et al., 1986, DeArmond et al., 1987), suggesting that the membrane form of PrPSc may be important with respect to transmitting disease from cell to cell (Hay et al., 1987b; Prusiner and DeArmond, 1987).

While PrPSc has been shown to be localized almost exclusively to the external surface of cells where it is anchored by a phosphatidylinositol glycolipid (Stahl et al., 1987), the topology of PrPSc is under investigation. Studies on PrP 27-30 have shown that, like PrPSc, it contains a phosphatidylinositol glycolipid. Interestingly, PrP contains a hydrophobic domain of sufficient length to span the membrane (Bazan, 1987). This domain was found buried within the lipid bilayer in cell-free translation studies (Hay et al., 1987a). A second form of PrP presumed to be secretory was found within the interior of microsomal vesicles used in cell-free translation studies (Hay et al., 1987b). Although some portion of PrP 27-30 is on the surface of the liposomes (Fig. 2), the topology of PrP 27-30 or PrPSc within liposome membranes remains to be established.

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REFERENCES

Alper, T., Cramp, W. A., Haig, D. A., and Clarke, M. C. (1967) Nature 214, 764-766

Barry, R. A., McKinley, M. P., Bendheim, P. E., Lewis, G. K., DeArmond, S. J., and Prusiner, S. B. (1985) J. Immunol. 135, 603-612

Barry, R. A., Kent, S. B. H., McKinley, M. P., Meyer, R. K., DeArmond, S. J., Hood, L. E., and Prusiner, S. B. (1986) J. Infect. Dis. 155, 848-854

Barzilai, A., Spanier, R., and Rahmimoff, H. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 6521-6525

Beaser, K., Oesch, B., Scott, M., Westaway, D., Wälchi, M., Groth, D. F., McKinley, M. P., Prusiner, S. B., and Weissmann, C. (1986) Cell 46, 417-428

Bazan, J. F., Fletterick, R. J., and Prusiner, S. B. (1987) Nature 325, 58-63

Bellinger-Kawahara, C., Cleaver, J. E., Diener, T. O., and Prusiner, S. B. (1987a) J. Virol. 61, 159-166

Bellinger-Kawahara, C., Diener, T. O., McKinley, M. P., Groth, D. F., and Prusiner, S. B. (1987b) Virology 160, 271-274

Bendheim, P. E., Barry, R. A., DeArmond, S. J., Stites, D. P., and Prusiner, S. B. (1984) Nature 310, 418-421

Bogonez, E., and Koshland, D. E. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 4891-4895

Bolton, D. C., McKinley, M. P., and Prusiner, S. B. (1982) Science 218, 1509-1511

Bolton, D. C., McKinley, M. P., and Prusiner, S. B. (1984) Biochemistry 23, 5898-5906

Braiman, M. S., Stern, L. J., Chao, B. H., Khorana, H. G. (1987) J. Biol. Chem. 262, 9271-9276

Carlson, G. A., Kingsbury, D. T., Goodman, P. A., Coleman, S.,
Materials and Methods

Preparation of Scrapie Prion Proteins Liposomes

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Immunoprecipitation of Prion Proteins and Liposomes

Scrapie prion protein (PrP) was immunoprecipitated from brain extracts of scrapie-infected animals using a polyclonal antibody raised against amino acid residues 231-235 of the C-terminal end of the prion protein sequence.

Liposome Formation

Liposomes were formed by the sonication of a solution containing phosphatidylethanolamine and cholesterol.

To assess the stability of the liposomes, a portion of the liposome suspension was aliquoted and stored at 4°C for one week before analysis.

Results

Liposomes were formed from the sonicated liposome suspension and were found to be stable for at least one week at 4°C.

Discussion

The results of this study suggest that liposomes can be used as a model system for the study of prion protein interactions in vivo.

Conclusion

In conclusion, the data presented in this study provide evidence for the involvement of cholesterol in the formation of prion protein liposomes and support the hypothesis that cholesterol may play a role in the pathogenesis of scrapie.

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Properties of Scrapie Prion Protein Liposomes

RESULTS

Immuno-electron microscopy was used to demonstrate the incorporation of PrP 27-30 into lipid vesicles. Prior liposomes or rods were prepared and treated for immunoelectron microscopy as described under Materials and Methods. PrP 27-30 concentration in the two samples was 30 mg/ml, and PC concentration was 50 mg/ml whereas indicated. (A) Rods stained with anti PrP antibody. (B) Rods stained without PrP antibody. (C) Liposomes stained with PrP antibody. (D) Liposomes stained without PrP antibody.

Incubation time biosassays have generally shown a 10-fold increase in scrapie infectivity upon dissociation of the rods into SLPC or liposomes (Dubois et al., 1984). End-point titration showed an increased titers of 10-fold upon conversion of the rods into liposomes (Fig 3). The titers for the formation into liposomes were the same as (10 ± 2) LD50 units/ml as determined by plaque end- point assay. The results indicate that infectivity results from dispersed dispersion of the infectious particles upon liposome formation from scrapie prion aggregates.

Fig 3. End-point titration of scrapie prion rods and liposomes. SLPC containing 30 mg/ml PC and 30 mg/ml PrP 27-30 as well as rods containing the same concentration of protein were diluted in 10-fold sequential dilutions in PBS and inoculated into hamsters (5 animals per sample) for infectivity determination. Rods (O--O); SLPC (D--D).

Disaggregation of the rods gave us an opportunity to search for a "hidden" and protected nucleic acid that may have a role in the scrapie infectivity process. In earlier studies enzyme digestion did not produce nucleic acids without effect (Ghita et al., 1984). In the experiments described here, nucleases were present during the formation of the SLPC. No change in scrapie agent titers of the rods or SLPC was detected upon digestion for 24 h with Phase 1 or Phase 2 nuclease or sRNA nuclease (Table I).

Table I: Scrapie infectivity resistance to procedures that hydrolyze or modify nucleic acid

| Nucleic digestion of rods | Log titer (LD50/ml ± S.E.) |
|--------------------------|---------------------------|
| No digestion              | 4.0 ± 0.3                 |
| Nuclease A, 100 mg/ml    | 4.1 ± 0.7                 |
| Nuclease B, 100 mg/ml    | 6.8 ± 0.7                 |
| Micrococcus, 100 mg/ml   | 7.6 ± 0.3                 |

The kinetics of inactivation of scrapie infectivity by UV irradiation yield an exponential survival curve characteristic of a single-hit process. UV inactivation was the same for the SLPC and rod preparations (Fig. 4). The D50 values for the SLPC and rods were 27 and 25.7 μm, respectively. Yields previously determined were 6.7 ± 2.2 kJ/m2 for purified prion rods (Bollinger-Rassmuss et al., 1982).

Fig. 4. Inactivation by UV irradiation of scrapie prion rods and liposomes. PrP 27-30 in rods and SLPC containing 30 mg/ml protein and 50 mg/ml PC were prepared as described under Materials and Methods, and irradiated with 254 nm UV. Inactivation of PrP 27-30 in SLPC allowed us to test two hypotheses. First, the depletion of infectivity was dependent on the concentration of the PrP 27-30 in the liposomes. Second, the infection of scrapie infectivity less and 500 mg/ml protein K the size of digestion at 37°C with 100 mg/ml of proteinase K, the titer fell for both SLPC and rods decreased by >50% in 4 h, with 500 mg/ml of proteinase K and the titer decreased by >50% in 4 h.

The incorporation of PrP 27-30 into lipid vesicles is a reversible process. Detergent extraction of the liposomes followed by chloroform/methanol extraction allowed the rods to reform while retaining infectivity (Fig. 5). The reformation of the rods was performed under conditions similar to those used to form the rods initially during prion purification. Liposomes were subjected to detergent extraction with sodium-deoxycholate (SDC) in order to produce a resulting extract that was precipitated with PEG-8000 and the pellet collected by centrifugation. Removal of the resulting lipid and detergent was accomplished by extracting the mixture with chloroform/methanol (1:2). A high molar ratio of approximately 10:1 for detergent/lipid seems to be required for optimal rod formation. Omitting the detergent treatment prior to organic solvent extraction drastically diminished the extent of rod formation, although some rods could be seen after organic solvent extraction alone.

Fig. 5. Inactivation of scrapie prion infectivity in rods and liposomes by proteinase K digestion. PrP 27-30 at 30 mg/ml either in rods or as SLPC containing 10 mg/ml Triton-X (pH 7.4). 0.5 ml was dialyzed in buffer containing 100 mg/ml proteinase K for increasing periods of time at 37°C. The reaction was stopped by the addition of 1.5 ml PBS. The samples were then incubated for infectivity measurement. SLPC, 100 mg/ml proteinase K (O--O); rods, 100 mg/ml proteinase K (O--O). The incorporation of PrP 27-30 into lipid vesicles is a reversible process.
Properties of Scrapie Prion Protein Liposomes

In an effort to increase further the level of scrapie prion infectivity, we examined a variety of phospholipids in place of the neutral PC. Some of the lipids examined altered the level of scrapie infectivity including negatively charged lipids such as phosphatidylserine or anionic lipid phosphatidylethanolamine (Pagan and Weintraub, 1989) (Table 1). Our results suggest that liposomes with PC and liposomes in a dispersion process rather than a charge effect on a binding site or a specific lipid interaction with some hypothetical receptor.

| Lipid Composition | Log titer (SD, mean ± S.E.) |
|-------------------|-----------------------------|
| Redo              | 7.2 ± 0.2                   |
| Redo + Na KCl     | 7.5 ± 0.3                   |
| PC                | 8.3 ± 0.2                   |
| PC + PS (8:2)     | 8.3 ± 0.1                   |
| PS + GM (8:2)     | 7.5 ± 0.2                   |
| PC + PS (1:9)     | 8.5 ± 0.1                   |
| PC + GM (8:2)     | 8.3 ± 0.2                   |

*Redo were dispersed with different mixtures of lipids. The molar ratio of protein to lipid (1:100) was constant. The lipids were dried down and then the lipids were then inoculated for scrapie bioassay.

Fig. 7. FITC labeled Pof 27-30. Pof 27-30 in redo was labeled with FITC as described in Materials and Methods. The labeled protein (FITC) was electrophoresed into a 14% polyacrylamide gel and visualized under UV light. Afterwards, the same gel was silver stained. FITC labeling of Pof 27-30 did not alter the scrapie prion titer as described in the text. Horizontal line denotes position of Pof 27-30.

Fig. 8. Fusion of liposomes containing fluorescent Pof 27-30 with neuroblastoma cells: Neuroblastoma (N1E) cells were incubated with FITC-labeled Pof 27-30 polymerized into redo or dispersed within liposomes. Polyethylene glycol (PEG 1500-1000) was added to promote fusion of the redo or liposomes to the cells. After removal of the PEG, the cells were left to recover overnight and were then examined in a Leica fluorescence microscope with phase-contrast capability. (A) Phase-contrast of cells fused with FITC-Pof 27-30 in liposomes; (B) Panel A viewed under UV light; (C) Phase-contrast of cells incubated with FITC-Pof 27-30 in redo; (D) Panel C viewed under UV light.

REFERENCES

Wolinska, E. (1984). J. Appl. Physiol. 56, 1897.

Pagan, E., and Weintraub, J. S. (1989). Ann. Rev. Biochem. 59, 435-468.

Peusner, G. B., Cochran, S. P., Grath, D. F., Dowey, D. E., Bowman, K. A., and Marziner, R. M. (1982). J. Am. Acad. Dermatol. 20, 635-648.

Weintraub, E. (1986). Anal. Biochem. 153, 30-34.