Purified Scrapie Prions Resist Inactivation by UV Irradiation

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The development of effective purification protocols has permitted evaluation of the resistance of isolated scrapie prions to inactivation by UV irradiation at 254 nm. Prions were irradiated on ice with doses of UV light ranging up to 120,000 J/m². UV dosimetry experiments, performed with *Saccharomyces cerevisiae* plasmid DNA or eucaryotic cells, indicated that under these experimental conditions an incident UV dose of 10 J/m² formed 2 thymine dimers per 5.1 × 10⁶ daltons of eucaryotic cell DNA. The *D*₃₇ values for scrapie prions ranged from 17,000 to 22,000 J/m²; *D*₃₇ values were also determined for virus, viroid, and enzyme controls. The number of pyrimidine dimers formed was correlated with the *D*₃₇ values obtained for irradiated prions and target nucleic acids. The *D*₃₇ value for bacteriophage M13, 6.5 J/m², occurred at a dose that would form 0.56 dimers per target genome; the *D*₃₇ for potato spindle tuber viroid, 4,800 J/m², occurred at a dose that would form about 24 dimers per target viroid. The *D*₃₇ value for an *Eco*RI restriction site, a target of 12 bases, occurred at a dose that would correspond to the formation of 0.89 thymine dimers per target site. The *D*₃₇ value for prions occurred at a dose that would form 1 dimer in every 4 bases of single-stranded target nucleic acid. If the putative scrapie nucleic acid were double-stranded and readily repairable after UV damage, then the prion *D*₃₇ value could reflect a nucleic acid molecule of 30 to 45 base pairs. While the *D*₃₇ value for prions fell within the range of pure protein targets, our experiments cannot eliminate the possibility that a prion contains a small, highly protected nucleic acid molecule.

The slow infectious agent that causes scrapie became a scientific enigma when its extreme resistance to UV and ionizing radiation was first discovered (1, 2). Two decades later, those results continue to be either criticized (11, 23, 32) or ignored. Many investigators believe that the scrapie agent is a virus (11, 23) despite a growing body of evidence to the contrary (3, 8, 19, 24-26). Following the development of purification procedures that yield highly enriched scrapie preparations (28), scrapie infectivity was found to be dependent on a sialoglycoprotein, PrP 27-30 (6). The protein has a relative molecular weight of 27,000 to 30,000, as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (5, 22). PrP 27-30 exhibits extreme resistance to proteolytic degradation, but after denaturation it does become sensitive to enzymatic proteolysis (22). Prion titers have been shown to diminish when PrP 27-30 undergoes denaturation.

In 1982, the term prion was proposed to describe the infectious particles causing scrapie which require a protein for infectivity and are resistant to procedures that selectively modify or hydrolyze nucleic acids (25). Despite investigations designed to detect a scrapie-specific nucleic acid within the infectious particle, no evidence for such a macromolecule has been accrued. With cDNA encoding scrapie PrP 27-30, a PrP-related DNA sequence was found within the genomes of both normal and scrapie-infected hamsters, but no PrP-related nucleotide sequences were detected in extracts prepared from purified prions (24). Although the PrP 27-30 protein is encoded by a host gene rather than by a gene within the infectious particle itself, the possibility remains that prions might contain an as yet undetected polynucleotide which is essential for infectivity.

Sensitivity to UV irradiation is a prominent characteristic of all nucleic acid-dependent organisms and pathogens. Although early studies showed that scrapie prions were highly radiation resistant (2), they were criticized for their use of crude homogenates of infected mouse brains. The studies described here were undertaken for two reasons. First, we examined the effects of UV radiation on infectious prions that were purified 3,000- to 10,000-fold with respect to protein. This level of purity minimizes the likelihood of shielding or protection of prions by contaminating macromolecules. Second, we performed dosimetry experiments in conjunction with these studies to correlate UV dose with the number of thymine dimers formed in DNA targets of known size. We compared the effects of UV irradiation at 254 nm on scrapie prions with those on a filamentous virus (bacteriophage M13), an enzyme (RNase A), and a naked infectious RNA (potato spindle tuber viroid [PSTV]).

MATERIALS AND METHODS

Materials. [³H]dTTP (80 μCi/mmol) was obtained from New England Nuclear Corp., Boston, Mass. Polyethylene glycol 8000 (PEG-8000), RNase A, and 2,3'-cytidine cyclic monophosphate (cCMP) were purchased from Sigma Chemical Co., St. Louis, Mo. *Eco*RI, *Pvu*II, and DNA polymerase I were bought from Bethesda Research Laboratories, Gaithersburg, Md.

Propagation and purification of scrapie prions. Large-scale propagation and purification of hamster-adapted scrapie prions was performed as described previously (28).

Scrapie prion infectivity assay. Prions were assayed in weanling female hamsters (LVG/LAK) (Charles River Laboratories, Wilmington, Mass.) by intracerebral inoculation with 50 μl of sample diluted by a factor of 10 or 100. Scrapie prion titers were determined by measuring the incubation

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time intervals from inoculation to onset of illness and to death (27, 29).

Bacteriophage M13 propagation and isolation. Bacteriophage M13 was cultured and prepared by the method of Hines and Ray (14). The virus was propagated in *Escherichia coli* RL108, an F⁺ Tet^r *recA56* derivative of *E. coli* WA948. M13 was recovered from an overnight culture supernatant by being mixed with 1/10 volume each of 5 M NaCl and 50% PEG-8000. This mixture was incubated at 4°C for 15 min, and the precipitated virus was harvested by centrifugation at $10,000 \times g$ for 20 min. The pelleted virus was resuspended in 1/100 culture volume of 10 mM Tris hydrochloride (pH 8.0)–1 mM EDTA–0.5% sodium dodecyl sarcosinate. A second precipitation with PEG-8000 was performed as just described, and the viral pellet was suspended in 1/100 volume of 10 mM Tris hydrochloride (pH 8.0)–1 mM EDTA. Virus stock produced this way can be stored at 4°C for up to 6 months without a decrease in titer.

Bacteriophage M13 infectivity assay. M13 infectivity was assayed by the plaque assay method of Hines and Ray (14). One-tenth milliliter of the virus sample to be assayed and 0.1 ml of an overnight suspension of host cells were mixed with 3 ml of melted warm (40°C) top agar and poured onto the surface of preprepared plates. Plaques were visible at 18 h and were scored at 24 h. Several different dilutions of each sample were assayed in duplicate. Titers were calculated by averaging the number of plaques on plates containing between 30 and 300 plaques per plate.

RNase A activity. RNase A activity was determined spectrophotometrically by measuring the hydrolysis of cCMP (4). RNase A (50 µg/ml) and cCMP (0.15 mg/ml) were mixed in a total volume of 1 ml of 15 mM sodium citrate–150 mM NaCl, pH 7.0. The absorbance at 284 nm was monitored for 3 to 5 min, and the change in absorbance per minute (dA/dt) was used as a measure of RNase A activity.

Viroid propagation and isolation. PSTV was propagated in tomato plants (*Lycopersicon esculentum* cv. Rutgers). Infected plants were harvested 6 to 8 weeks postinfection, and the viroid was purified as described previously (7).

Viroid infectivity assay. Each irradiated and control sample was diluted 10^{-1} to 10^{-8} , and 20 Rutgers tomato seedlings at the cotyledonary stage were inoculated with each dilution. Cotyledons were dusted with 600-mesh carborundum and then lightly rubbed with inoculum-soaked cotton applicators. Leaves were rinsed with tap water immediately after inoculation. Plants were scored for symptom expression at 2-day intervals starting 12 days after inoculation. Scoring was continued until no additional plants started to display symptoms (usually about 6 weeks postinfection).

Results were evaluated by transforming percent infection values into probits (10) and by graphic determination of 50% infective dose (ID_{50}) values.

Propagation of XP12RO cells. Human DNA repair-deficient (XP12RO) cells from complementation group A of *Xeroderma pigmentosum* were cultured in 24-well culture trays in Eagle minimal essential medium with 10% fetal calf serum supplemented with [³H]dTTP (final concentration, 0.01 µCi/ml). After the cells reached confluence, the medium was removed and the cells were washed twice with phosphate-buffered saline to prepare them for irradiation.

Thin-layer chromatography of cellular DNA. Radiolabeled DNA was extracted from XP12RO cells with phenol-chloroform and then precipitated with ethanol. The extracted nucleic acid was digested for 1 h at 170 to 180°C in 0.2 ml of 97% formic acid to liberate thymine and dimerized thymine. The bases were chromatographed on Silica Gel G

(Merck & Co., Inc., Rahway, N.J.) thin-layer plates in water-saturated ethyl acetate–*n*-propanol–water (4:1:2, vol/vol/vol). The radioactivity associated with the monomer and dimer forms of thymine was quantitated by liquid scintillation counting.

Plasmid cleavage and radiolabeling. A 9-kilobase fragment from YEp13 (a gift from P. Meecham) was nick-translated with 2.5 mCi of [³H]dTTP and DNA polymerase I per ml (31). Irradiated and unirradiated samples of the labeled DNA were cleaved with *EcoRI* (20-µl reaction volume containing 20 U of enzyme, 37°C, for 5 h). Radiolabeled fragments were separated by agarose gel electrophoresis.

Agarose gel electrophoresis. Radiolabeled plasmid samples were electrophoresed in 5% agarose vertical slab gels in Tris-borate-EDTA buffer by the method of Maniatis et al. (21). Samples were run at 75 V (direct current) for 10 h until the bromophenol blue tracking dye reached the bottom of the gel. The individual gel lanes were sliced into 0.3-mm slices, and the radioactivity was quantitated by liquid scintillation counting.

Irradiation experiments. Samples of M13, RNase A, PSTV, scrapie prions, and mixed samples containing both M13 and prions were diluted to a total volume of 500 µl with 10 mM Tris hydrochloride (pH 8.0)–1 mM EDTA (M13 and PSTV) or with 75 mM NaCl–50 mM sodium citrate, pH 7.0 (RNase A and prions). Samples were placed beneath 5 GE15 germicidal lamps ($e_{\max} = 254$ nm) at a distance of about 5 cm. The doses delivered to the samples were measured with a Spectroline UV dosimeter fitted with a collar to simulate the conformation of the irradiation containers. Irradiated PSTV and prion samples were stored at –70°C until bioassay could be performed; M13 and RNase A samples were held at 4°C until assay. All samples were irradiated on ice to minimize evaporation during prolonged irradiation. Twenty-four-well cell culture plates (Linbro, Inc.) with flat bottoms were used. Each well measured 16 mm in diameter and 18 mm deep. The irradiated liquid samples formed a thin layer with a depth of 2 mm over the bottom of the wells.

XP12RO cells were washed free of serum and irradiated directly in 24-well culture trays at 1.3 J/m² per s (254 nm). Plasmid samples were irradiated in 5 µl of 10 mM Tris hydrochloride (pH 8.0)–1 mM EDTA buffer.

RESULTS

Dosimetry. The incident UV dose that reached irradiated samples was measured in three separate ways: (i) physical dosimetry, (ii) thymine dimer formation within eucaryotic cellular DNA, and (iii) thymine dimer formation within an *EcoRI* restriction site. Physical dosimetry at 254 nm, as measured with a Spectroline UV dosimeter, indicated that the UV dose rate at the bottom of the wells in a 24-well microtiter plate was 6.6 ± 1.5 J/m² per s. Because the equipment was designed for irradiation at longer distances and lower doses over a rotating platform, one major source of variation was the inhomogeneity of the light source at the shorter ranges used. Measurements were taken over several points in the field, and an average value was calculated. This physical dosimetry value was then correlated with the values from the two biological methods to obtain a more precise measure of the UV light striking the biological targets.

The first biological method measured thymine dimer formation in eucaryotic cellular DNA. XP12RO cells were cultured in 24-well culture trays, and the DNA was labeled with [³H]dTTP. The cells were irradiated, and the extracted nucleic acid was analyzed by one-dimensional thin-layer

FIG. 1. Correlation of UV dose with thymine dimer formation. XP12RO cells were grown in 24-well culture plates in Eagle minimal essential medium supplemented with 0.01 μ Ci of [3 H]dTTP. After the cells reached confluence, the medium was removed and the labeled cells were washed free of unincorporated thymidine with phosphate-buffered saline before irradiation. Cellular DNA was extracted after irradiation, and the nucleotide bases were liberated by digestion in 0.2 ml of 97% formic acid at 170 to 180°C for 1 h. One-dimensional thin-layer chromatography on silica gel plates (water-saturated ethyl acetate-*n*-propanol-water) was used to separate dimerized from undimerized thymine. The exact amount of dimerized thymine found in each sample was quantitated by liquid scintillation counting. The results are expressed as the percentage of 3 H incorporated into thymine dimers plotted against UV dose. The incident dose at 254 nm at the bottom of the culture wells was 6.6 ± 1.5 J/m 2 per s, as measured with a Spectroline UV dosimeter.

chromatography after formic acid digestion to liberate thymine and dimerized thymine. The amounts of tritium label associated with the monomer and dimerized forms of thymine were quantitated by liquid scintillation counting. Figure 1 shows the relationship between the measured UV dose at 254 nm and the percentage of total incorporated tritium label found in thymine dimers. At a dose of 100 J/m 2 , 0.43% of the incorporated thymidine label was found in dimer form. This gave a dimer yield of 0.0043%/J per m 2 . This means that for each joule per square meter of incident UV light, 0.0043 thymine dimers were formed for each 100 thymines present, or 2 dimers formed in every 46,500 thymines. Assuming that eucaryotic DNA normally contains about 30% thymine, we can calculate that 2 dimers were formed in every 1.55×10^5 bases or for every 5.1×10^7 daltons (assuming a molecular size of 330 daltons per nucleotide). A dose of 10 J/m 2 would then be expected to form 2 dimers for every 5.1×10^6 daltons of DNA.

The second method of correlating physical dosimetry with the UV effect on a biological target used the fact that the *Eco*RI restriction site contains adjacent thymines (Fig. 2, inset). Figure 2 shows the pBR322-derived yeast plasmid, YEp13, that was used as the target DNA. This plasmid contains three *Eco*RI sites and generates four fragments after complete *Eco*RI cleavage. The plasmid was irradiated in solution at UV doses of 3,186 and 5,310 J/m 2 . The irradiated plasmid was restricted first with *Pvu*II and then with *Eco*RI, and the fragments were then nick-translated with [3 H]dTTP. This cleavage sequence yielded four *Eco*RI fragments, three of which (fragments A, B, and C) were retained on a 5% agarose gel.

Figure 3 shows the radioactivity profiles of the gel lanes obtained when electrophoresis was performed on irradiated and unirradiated plasmid samples. Panel A is the profile associated with the unirradiated intact plasmid. The intact, uncleaved plasmid was found in gel slices 9 and 10 (peak PL). After the unirradiated plasmid was cleaved with *Eco*RI, the profile seen in panel B was obtained. The small peak at slices 9 and 10 (PL) was produced by residual intact plasmid (or fragment A-B-C). The first major peak (peak 1) of radioactivity at slices 12 and 13 probably contained an incompletely cleaved section of the plasmid composed of fragments B and C (B-C) or B, C, and D (B-C-D). This would represent cleavage of either one or two of the three *Eco*RI sites that flank these fragments. The middle peak (peak 2), at slice 20, probably contained a mixture of an A-B fragment and fragment C (or C-D). All of these fragments result from cleavage of all of the possible flanking sites. The third peak (peak 3), at slices 28 and 29, probably represented a completely cleaved mixture of fragments A and B. This peak would also represent complete cleavage of the two possible cleavage sites flanking fragments A and B. The smallest fragment, D, was not retained on the gel.

Panels C and D show the profiles obtained after exposing the plasmid to UV doses of 3,186 and 5,310 J/m 2 , respectively. In both cases, the amount of tritium label associated with peaks 2 and 3 decreased. The highest dose resulted in a dramatic decrease in the size of the second peak and in the complete disappearance of the third peak.

The data obtained from the second and third peaks were used to correlate physical dosimetry with the fraction of *Eco*RI fragments remaining after irradiation. The first peak was an unsatisfactory choice for this type of analysis because the B-C fragments (resulting from cleavage of two sites) cannot be distinguished from the B-C-D fragment (resulting from cleavage of only one site) or from material forming the trailing shoulder seen in the peak of uncleaved plasmid. Figure 4 shows the data from the second and third peaks expressed as the fraction of *Eco*RI fragments remaining plotted against measured UV dose. The curves resulting

FIG. 2. pBR322-derived plasmid containing *S. cerevisiae* chromosomal DNA. The pBR322-derived plasmid YEp13 contains a 9-kilobase insert of yeast chromosomal DNA and three *Eco*RI sites. The sizes of the four fragments generated by *Pvu*II and subsequent *Eco*RI digestion are shown. Points of cleavage are indicated. The insert shows the sequence of an *Eco*RI restriction site.

FIG. 3. Electrophoretic profiles of nick-translated YEp13 plasmid DNA. YEp13 plasmid DNA was irradiated in solution in a total volume of 5 μ l in 24-well culture trays. The irradiated plasmid was first cleaved with *Pvu*II to yield the 9-kilobase fragment and subsequently cleaved with *Eco*RI. The resulting *Eco*RI fragments were tritium-labeled by nick translation as described in the text. The labeled restriction fragments were subjected to electrophoresis on 5% agarose gels with Tris-borate-EDTA buffer. The individual gel lanes were sliced into 0.3-mm slices, and the radioactivity in each slice was quantitated by liquid scintillation counting. (A) Radioactivity profile seen with intact unirradiated plasmid (uncleaved control); (B) profile seen with unirradiated plasmid following complete *Pvu*II and *Eco*RI cleavage (cleaved control); (C) profile of plasmid cleaved after exposure to a dose of 3,186 J/m²; (D) profile of plasmid cleaved after exposure to a dose of 5,310 J/m². In all panels, the direction of migration is from left to right.

from both the second and third peaks were colinear, indicating that the site flanking the fragments in both peaks responded to UV radiation in the same way. The D_{37} (the dose at which 37% of the original activity remained) for an *Eco*RI site obtained from these curves was 2,750 J/m².

The dosimetry calibration from the XP12RO cells indicated that a dose of 10 J/m² formed 2 thymine dimers per 5.1×10^6 daltons of DNA. Therefore, a dose equivalent to the D_{37} for an *Eco*RI site should form about 550 dimers per 5.1×10^6 daltons or 550 dimers per 1.5×10^4 bases. Thus, at a dose equivalent to the D_{37} , about 1 thymine dimer was formed in every 27 bases. Each fragment generated was flanked by two *Eco*RI sites; each site was composed of 2×6 base pairs (24 bases). The D_{37} , therefore, occurred at a dose that produced 0.89 thymine dimers per target *Eco*RI site.

UV irradiation of virus, viroid, and protein controls. Several different types of biological target systems were chosen for comparison with scrapie prions. Because prions have been considered by some investigators to be a filamentous virus (11), the filamentous bacteriophage M13, a single-stranded DNA virus, was included in these studies. RNase A was chosen as a model of a biologically active protein, and PSTV was included as an example of a biologically active RNA viroid. These viral molecules allowed us to include both DNA and RNA controls, although the photochemistry of these nucleic acids is very similar (34).

Figure 5 shows the effect of UV radiation on these target systems. M13 has a single-stranded DNA genome of 6,410

bases, which one would expect to be quite sensitive to UV light. As shown in Fig. 5A, M13 infectivity decreased markedly as a result of irradiation. The titers dropped by 10^{10} PFU/ml at doses of less than 300 J/m². The D_{37} for M13 was found to be 6.5 J/m². This agrees well with published values of 9.9 J/m² (30).

The dosimetry experiments allowed us to calculate the number of thymine dimers formed after a UV dose of 6.5 J/m². If 10 J/m² forms 2 thymine dimers per 5.1×10^6 daltons of DNA, then at the D_{37} for M13, 1.3 thymine dimers would be formed in 5.1×10^6 daltons of M13 DNA, or 1.0 thymine dimer in 11,446 bases. This would be equivalent to forming 0.56 thymine dimers per target genome of 6,410 bases.

Figure 5B shows the effect of UV light on PSTV. This small infectious RNA (molecular size, 1.27×10^5 daltons) is much more resistant to UV inactivation than M13. The D_{37} for PSTV inactivation was close to 4,800 J/m². This value agrees well with the value of 5,000 J/m² obtained by Diener et al. (9). The D_{37} of 4,800 J/m² correlates with a dose that would form about 1 uridine dimer per 15 bases or 24 pyrimidine dimers per target viroid molecule.

The pure protein target, RNase A, proved quite resistant to UV inactivation (Fig. 5C). RNase A enzymatic activity diminished slowly only after high doses of radiation. The D_{37} for RNase A enzymatic activity was calculated to be 11,800 J/m². This value is within the expected range for enzyme targets (see Table 3).

UV irradiation of scrapie prions. We examined the resistance of several different preparations of purified prions to

UV radiation (Fig. 6). No significant change in infectivity could be detected until the samples received doses of more than 12,000 J/m². The titer of an unirradiated control sample that was incubated in a culture tray on ice for the duration of the experiment did not diminish, indicating that no decrease in titer resulted from the lengthy incubation time. The irradiation curves obtained yielded D_{37} values ranging from 17,000 to 22,000 J/m², with an average value of 20,000 J/m².

The possibility was considered that some component in the purified prion samples might be protecting these particles from the effects of UV light and thereby increasing their apparent UV resistance. No evidence for such a chromophore was found when we compared the UV sensitivity of M13 bacteriophage in the absence and presence of purified scrapie prions (Table 1). The M13 titer decreased to 1% of control levels when irradiated either alone or in the presence of prions at a dose of 10 J/m². Irradiation with a dose of 30 J/m² gave similar results. These data indicate that neither prions nor contaminants present in purified gradient fractions protect M13 bacteriophage from damage by UV irradiation at 254 nm.

DISCUSSION

In 1967, Alper and co-workers reported their studies on UV irradiation at 254 nm of the scrapie agent in 10% mouse brain homogenates clarified by low-speed centrifugation (1). No inactivation was seen at UV doses up to 5,000 J/m². However, exposure of bacteriophage T3 in a 10% mouse brain homogenate under the same conditions showed almost 100% inactivation after an incident dose of 200 J/m². Even those target systems with efficient nucleic acid repair mech-

FIG. 4. Fraction of *Eco*RI fragments remaining correlated with UV dose. Data obtained from the middle and smallest peaks shown in Fig. 3 were used to correlate the fraction of *Eco*RI fragments remaining after irradiation with the incident UV dose. Symbols: ○, values obtained from peak 3 (slice 28); □, values obtained from peak 2 (slice 20).

FIG. 5. UV irradiation of M13, PSTV, and RNase A. Ten-microliter samples of each were diluted to 500 μ l with 75 mM NaCl-50 mM sodium citrate, pH 7.0. Samples were irradiated in 24-well culture trays on ice. (A) M13. Following irradiation, all samples were held at 4°C until infectivity assays could be performed. (B) PSTV. Samples were frozen and held at -70°C until bioassay. (C) RNase A. Enzymatic activity assays for RNase A (100 μ g/ml) were performed as described in Materials and Methods. RNase A activity is plotted as the change in absorbance with time (dA/dt).

anisms (*Micrococcus radiodurans* and transforming DNA in a host-repair system) showed marked UV effects at the dose level given to the scrapie agent.

Latarjet et al. extended these findings in 1970 when they demonstrated that irradiation of the scrapie agent at 254, 267, and 280 nm generated essentially the same dose-response curves (19). At all three wavelengths, 180,000 J/m² was required to inactivate 99.9% of the prion infectivity. Over the years, proposed D_{37} values for the effect of UV light on the scrapie agent have ranged from 21,500 to 42,000 J/m² (1, 8, 13). The highest D_{37} of 42,000 J/m² was reported by one of us (25). From the dosimetry studies described here, it is now clear that this value is too high by a factor of 2; it was calculated incorrectly due to an error in determining

FIG. 6. UV irradiation of scrapie prions. Ten-microliter samples of scrapie prions were diluted to 500 μ l with 75 mM NaCl–50 mM sodium citrate, pH 7.0. Samples were irradiated on ice in 24-well culture trays and then held at -70°C until they could be assayed. These samples typically contained 15 μ g of protein per ml and 10^8 ID₅₀ of prions per ml. Prion titers were determined by the incubation time interval assay as described in Materials and Methods. Each sample was assayed in four hamsters. The open circles represent the individual data points, and the bars represent the standard error.

the incident UV dose. D_{37} values for prions irradiated at 254 nm varied between 17,000 and 22,000 J/m² in the studies reported here. The imprecision of the hamster bioassay appears to account for this range of values, which compare favorably with the value of 24,000 J/m² obtained by Alper et al. for prions irradiated in crude homogenates of murine brain and assayed by endpoint titrations in mice (1). One criticism of these earlier experiments with UV irradiation is that those studies were performed on crude brain homogenates, since effective purification protocols were unavailable. Investigators could not determine whether the extreme resistance of prions to UV irradiation reflected the effects of shielding of the prions by contaminants, interactions between parts of some as yet unknown complex, energy transference phenomena, or the unusual macromolecular composition of the prion.

The experiments described here attempted to resolve some of these questions. The dosimetry experiments reported here allowed us to make correlations between incident dose at 254 nm and the expected number of thymine dimers formed in target DNA. The experiments which used XP12RO cell DNA as the target molecule indicated that a dose of 10 J/m² formed 2 thymine dimers for every 5.1×10^6 daltons of eucaryotic DNA. With this information, we were able to detect the formation of 0.89 thymine dimers within a target site of 24 bases of DNA.

The irradiation data from these experiments demonstrate that scrapie prions are highly resistant to UV irradiation and that they behave more like pure proteins than like viruses or viroids. The use of highly purified prions for these studies minimized the possibility of shielding or protection of the putative scrapie prion nucleic acid by contaminating mate-

rials found in crude brain homogenates. The addition of purified prions to M13 samples failed to protect the bacteriophage from inactivation by UV light (Table 1). These results provide evidence that the apparent UV resistance of scrapie prions is a fundamental property of the pathogen itself. However, we cannot distinguish whether this resistance to inactivation by UV irradiation is due to the molecular structure of the prion itself or to highly efficient cellular repair processes.

Shielding of a hypothetical prion nucleic acid by aggregation or by a unique protein coat is unlikely to be the cause for such a high D_{37} . Aggregation of infectious particles would produce a multitarget structure whose inactivation curve would have exhibited a shoulder at low doses (35), which was not observed in our experiments. Shielding by a protein coat is also an unlikely cause for the high D_{37} because free DNA in the form of transforming factor has a D_{37} which is not significantly different from that of whole phage particles (34). Also, DNA inside mammalian nuclei, shielded by chromatin and the cytoplasm, shows a pyrimidine dimer yield that is within a factor of 2 of the same DNA irradiated in vitro (20). In addition, the protein concentration of the purified prion samples irradiated in our studies was 15 μ g/ml, which is lower by a factor of approximately 1,000 than that used in early studies with scrapie-infected mouse brain homogenates (1). Yet the D_{37} values for purified prions and those found in homogenates were the same.

UV doses equivalent to D_{37} values for both M13 phage and the *Eco*RI fragments give approximately 1 dimer per target genome (Table 2). Such values are not unexpected, since M13 is a single-stranded DNA bacteriophage and its genome is not readily repairable after UV cleavage. The *Eco*RI sites were contained within yeast plasmid double-stranded DNA, but the electrophoretic assay used to assess thymine dimer formation at *Eco*RI sites does not allow for any biological repair. On the other hand, double-stranded nucleic acid molecules inside cells do generally undergo repair after UV damage; this results in their increased resistance to inactivation by UV irradiation. PSTV is a single-stranded RNA molecule but exhibits extensive base pairing; its D_{37} corresponds to approximately 24 uracil dimers per genome. Polyomavirus contains a double-stranded DNA genome; its D_{37} corresponds to approximately 16 dimers per genome (25). The D_{37} for scrapie prions is approximately 20,000 J/m², and this value can be used to estimate the size of a hypothetical nucleic acid under two alternate assumptions. If the putative scrapie prion nucleic acid is not subject to significant repair after UV irradiation like the single-stranded M13 DNA, then the D_{37} for prions would correspond to a genome of 4 bases. On the other hand, if the putative scrapie prion

TABLE 1. Purified scrapie prions did not alter inactivation of M13 bacteriophage by UV irradiation at 254 nm

UV dose at 254 nm (J/m ²)	Titer (PFU/ml)	
	M13	M13 + prions
0	1.0×10^{12}	ND ^a
10	1.0×10^{10}	1.9×10^9
30	5.9×10^5	1.2×10^6

^a ND, Not determined.

TABLE 2. Pyrimidine dimer yield per genome size after irradiation at 254 nm

Target	D_{37} (J/m ²)	Dimer yield/genome ^a
M13 bacteriophage	6.5	0.56 TT (6,410)
<i>Eco</i> RI site	2,750	0.89 TT (24)
PSTV	4,800	24 UU (359)
RNase A	11,800	NA ^b
Scrapie prions	20,000	1 TT or UU (4 or 30 to 45) ^c

^a Calculations based on the yield of dimers at the D_{37} . Genome size is given in parentheses (in bases).

^b NA, Not applicable.

^c Four bases for a single-stranded genome or 30 to 45 base pairs for a double-stranded genome.

TABLE 3. Inactivation of viruses, viroids, prions, and enzymes by irradiation at 254 nm

Particle	D_{37} (J/m ²)	Reference
Bacteriophages		
T2	4	18
M13	6.5	This paper
M13	9.9	30
S13	20	15
$\phi\chi 174$	20	15
Rous sarcoma virus	150	12
Polyomavirus	240	17
Friend leukemia virus	500	16
Murine leukemia virus	1,400	19
EcoRI site	2,750	This paper
PSTV	5,000	9
PSTV	4,800	This paper
Fumarase	3,100	13
Glutamic dehydrogenase	4,700	13
RNase A	11,800	This paper
Scrapie prions (purified)	17,000–22,000	This paper
Scrapie prions (in 10% brain homogenate)	24,000	19
Urease	30,000	13
Malic dehydrogenase	41,000	13

nucleic acid does undergo significant repair after UV irradiation, like the extensively base-paired PSTV or the double-stranded polyomavirus, then the D_{37} for prions would correspond to a genome of 30 to 45 base pairs.

The data for prions was compared with D_{37} values obtained for a wide variety of bacteriophage, animal virus, and enzyme targets (Table 3). All of the bacteriophages listed had low D_{37} values, about 5 to 20 J/m². The D_{37} values for animal viruses were somewhat higher, ranging from 150 to 1,400 J/m² (12, 16, 17, 19). Pure proteins, as expected, demonstrated the highest D_{37} , ranging from 3,100 to 41,000 J/m² (13, 19). The D_{37} obtained for prions clearly fell within the protein range.

Several other studies are noteworthy with respect to the possibility that prions contain a nucleic acid molecule. First, the exceptionally high D_{37} for prions suggests that if prions do contain a nucleic acid, it is quite small. Nucleic acid hybridization studies have shown that prions do not contain a gene encoding the prion protein (24). Second, early studies on the resistance of scrapie prions to inactivation by ionizing radiation by Alper et al. gave a target size from 100,000 to 150,000 daltons for the infectious particle (2). Ionizing radiation target size analyses on highly purified prions have indicated a target molecular size of 50,000 to 100,000 daltons (C. Bellinger-Kawahara, E. Kempner, T. O. Diener, and S. B. Prusiner, manuscript in preparation). Third, additional experiments with highly purified preparations of prions show that they resist inactivation by hydroxylamine treatment, zinc-catalyzed hydrolysis, psoralen photoadduct formation, nuclease digestion (C. Bellinger-Kawahara, T. O. Diener, D. F. Groth, and S. B. Prusiner, manuscript in preparation), and sonication (22a). All of these procedures modify or hydrolyze nucleic acids and were effective in inactivating control viruses and viroids, as previously reported in studies on less-purified preparations of prions (8, 25).

The data reported here uphold the argument that scrapie prions are fundamentally different from viruses and viroids. The high D_{37} for prion inactivation by UV irradiation at 254 nm cannot eliminate the possibility that a small polynucleotide exists, but they do demonstrate that if one exists, it is quite small.

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