Combination of NADPH and Copper Ions Generates Proteinase K-resistant Aggregates from Recombinant Prion Protein*

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Recent studies have demonstrated that the octapeptide repeats of the N-terminal region of prion protein may be responsible for de novo generation of infectious prions in the absence of template. Here we demonstrate that PrP-(23–98), an N-terminal portion of PrP, is converted to aggregates upon incubation with NADPH and copper ions. Other pyridine nucleotides possessing a phosphate group on the adenine-linked ribose moiety (the reduced form of nicotinamide adenine dinucleotide 3′-phosphate, nicotinic acid adenine dinucleotide phosphate, and NADP) were also effective in promoting aggregation, but NADH and NAD had no effect. The aggregation was attenuated by the metal chelator EDTA or by modification of histidyl residues with diethyl pyrocarbonate. The aggregates are amyloid-like as judged by the binding of thioflavin T, a fluorescent probe for amyloid, but do not exhibit fibrillar structures according to electron micrography. Interestingly the aggregates were resistant to proteinase K digestion. Likewise NADPH and zinc ions caused aggregation of PrP-(23–98), but the resulting aggregates were susceptible to degradation by proteinase K. Upon incubation with NADPH and copper ions, the full-length molecule PrP-(23–231) also formed proteinase K-resistant amyloid-like aggregates. Because it is possible that PrP, NADPH, and copper ions could associate in certain tissues, the aggregation observed in this study may be involved in prion initiation especially in the nonfamilial types of prion diseases.

The C-terminal domain of PrP includes residues 126–231, has a globular structure composed of three α-helices and two short β-strands, whereas the N-terminal domain, including residues 23–125, is largely unstructured (1, 2). Residues 60–91 consist of four repeats of an octapeptide sequence, PHGGG-WGQ, and selectively bind copper ions over other divalent metal ions (1, 2). The affinity of this domain for copper ions is in the micromolar range (3). Another copper-binding site including residues His-96 and His-111 has also been reported (4–6). In fact, affinity-purified PrP preparations from mouse and human brain were shown to bind three and seven copper atoms per molecule, respectively (7, 8). PrP purified from cultured cells was found to contain one to four copper atoms depending on the availability of copper in the culture medium (7). In light of these results, it is probable that native PrP in vivo exists in the copper-bound form.

Among the substances able to bind PrP, copper ions can modulate the pathogenesis of prion disease (9–12). However, the influence of copper on prion disease is controversial. A recent study showed that addition of copper ions to culture medium reduces the accumulation of disease-associated PrP isoform (PrPSc) in scrapie-infected neuroblastoma cells (11) and that the onset of prion disease is significantly delayed when scrapie-infected Syrian hamsters are administered a copper salt (11). Other studies reported that administration of a copper chelator delays the onset of disease in scrapie-inoculated mice (12). Copper also promotes the conversion in vitro of aged recombinant mouse PrP, in which Asn-187 is altered to an Asp residue (15), to the proteinase K (PK)-resistant form. Copper may also help recover the infectivity and PK resistance of partially denatured PrPSc (16). These results highlight the multifaceted nature of copper in the pathogenesis of prion disease.

Based on the current understanding of prion formation, prion initiation (i.e., de novo generation of PrPSc from cellular PrP isoform (PrPc) without template) and prion propagation (i.e., conversion of PrPC to PrPSc in the presence of PrPSc template) are two distinct processes. Prion propagation likely does not require the N-terminal domain because its removal from PrPSc by limited protease digestion did not significantly alter infectivity titers (17) and because transgenic mice expressing PrP with a deletion of the octapeptide region on the PrP knock-out background were able to produce infectious prions albeit with increased incubation times and reduced prion titers compared with inoculation with the full-length protein (18–21). However, the N-terminal domain is likely important for efficient formation of protease-resistant PrP as demonstrated by a cell-free experiment in which de novo generation of protease-resistant PrP was examined by mixing a source of protease-sensitive PrP with protease-resistant PrP isolated from the tissue of an infected animal (22, 23). Moreover several types of
familial Creutzfeldt-Jakob disease are characterized by increased numbers of the octarepeat in the N-terminal domain (13, 14), and a recent in vitro study indicates that the number of repeats in the sequence is critical in the self-association of glutathione S-transferase fusion proteins with octarepeat sequences (24). Likewise insertion of octarepeats increases the rate of protease-resistant PrP formation (25). These results support the hypothesis that the N-terminal domain plays a key role in prion initiation. Contrary to these observations, other experiments suggest that in vitro prion initiation does not require the octarepeat region because when fibrils produced with a recombinant N-terminally truncated mouse prion protein (MoPrP-(89–230)) were inoculated intracerebrally into transgenic mice expressing the same protein the mice developed neurological dysfunction (26). A better understanding of the role of the N-terminal region of PrP in prion initiation and propagation will require further studies.

Although many studies describe the in vitro conversion of the C-terminal domain into amyloid fibrils (26–32), there have been few studies aimed at investigating the formation of amyloid fibrils with the N-terminal domain. Human PrP-(23–144) (HuPrP-(23–144)), containing the Y145Stop variant of Gerstmann-Sträussler-Scheinker disease, has recently been tested for amyloid formation (33, 34). HuPrP-(23–144) spontaneously polymerized into highly ordered, PK-resistant fibrils. The truncated proteins HuPrP-(23–124), HuPrP-(23–126), HuPrP-(23–134), and HuPrP-(23–137) did not develop these characteristics (33), suggesting that residues 138–141 are required for this conversion. In the present study we focused on the in vitro conversion of a recombinant PrP encompassing residues 23–98 (PrP-(23–98)). PrP-(23–98) contains four highly conserved copper-binding octarepeats (residues 60–91, PHGGGWGQ) (PrP-(23–98)). PrP-(23–98) contains four highly conserved copper-binding octarepeats (residues 60–91, PHGGGWGQ) and one partial repeat (residues 92–98, GGTHNQ) that can bind another copper atom (6). We found that PrP-(23–98) spontaneously aggregated upon incubation with NADPH and copper or zinc ions and that the aggregates formed with NADPH and copper were resistant to proteolysis by PK. The same phenomena were observed with the full-length recombinant PrP encompassing residues 23–231 (PrP-(23–231)). The data presented here suggest that the aggregation observed in this study may be involved in prion initiation.

**EXPERIMENTAL PROCEDURES**

**Antibodies**—Mouse monoclonal antibody SAF 32 recognizing the octarepeat region of PrP and mouse monoclonal antibody SAF 84 recognizing epitope 160–170 of hamster PrP were obtained from Cayman Chemical Co. Both antibodies react with human and mouse PrP.

**Preparation of PrP-(23–98) and PrP-(23–231)**—The expression and purification of human recombinant PrP-(23–98) and mouse recombinant PrP-(23–231) were performed as described previously (35, 36). The concentration of PrP-(23–98) and PrP-(23–231) was determined by absorbance at 280 nm and using the molar extinction coefficients of 36,700 and 62,160 M$^{-1}$ cm$^{-1}$, respectively (37). Diethyl pyrocarbonate (DEPC)-modified PrP-(23–98) was prepared as follows. Mixtures containing 50 mM Mes (pH 7.2), 50 μM PrP-(23–98), and 1 mM DEPC were incubated at 25 °C for 1 h followed by dialysis at 4 °C for ~18 h against 5 mM Mes (pH 7.2). The number of modified histidyl residues was estimated at three based on a molar extinction coefficient at 240 nm of 3,200 M$^{-1}$ cm$^{-1}$ for the modified compound (38).

**Aggregation Assay**—Mixtures (120 μl) containing 50 mM Mes (pH 7.2) and 10 μM PrP-(23–98) or 2 μM PrP-(23–231) were incubated at 25 °C for 30 s in the presence of metal ions (1–40 μM). Pyridine nucleotide (10–500 μM) was then added to induce aggregation. Aggregation was followed by measuring turbidity at 550 nm. EDTA, l-histidine, or glycine was included in the initial mixtures when tested in this assay.

To evaluate aggregate formation, samples were centrifuged at 18,800 × g for 10 min, and the concentration of protein in the resulting supernatants was determined using a Coomassie protein assay reagent (Pierce). The pellets were washed three times with 50 mM Mes buffer (pH 7.2), suspended in 100 mM Mes buffer (pH 7.2) containing 6 M urea, and placed in an ultrasonic bath for 10 min. The amount of copper and NADPH in the dissolved samples was measured using an atomic absorption spectrophotometer (AA-6800, Shimadzu, Kyoto, Japan) and a spectrophotometer (with a molar extinction coefficient at 340 nm of 6,300 M$^{-1}$ cm$^{-1}$), respectively.

**Thioflavin T (ThT) Fluorescence Measurement**—Mixtures (500 μl) containing 50 mM Mes (pH 7.2), 20 μM PrP-(23–98), 80 μM CuCl$_2$ or 80 μM ZnSO$_4$, and 500 μM NADPH with or without EDTA (500 μM) were incubated at 25 °C for 5 min, and then ThT (final concentration of 20 μM) was added. The fluorescence spectrum was recorded on a spectrofluorometer (RF-5300PC, Shimadzu) with excitation at 450 nm, excitation bandwidth of 5 nm, and emission bandwidth of 10 nm.

**Electron Microscopy**—Negative staining was performed on collodion-coated 400-mesh copper grids. Samples were adsorbed for 30 s, stained with freshly filtered 3% uranyl acetate, dried, and then viewed in a JEOI JEM 1220 electron microscope (Tokyo, Japan) at 80 kV at a standard magnification of 50,000.

**PK Resistance Assay**—The degradation of preformed aggregates was monitored by measuring turbidity at 550 nm. In some experiments, mixtures (120 μl) containing 50 mM Mes (pH 7.2), 500 μM NADPH, 40 or 10 μM CuCl$_2$, and 10 μM PrP-(23–98) or 2 μM PrP-(23–231) were incubated at 25 °C for 30 min, and then one-half of each sample was incubated with PK (weight ratio of PK to PrP, 1:10) (Roche Applied Science) at 37 °C for 1 h in the presence or absence of EDTA (500 μM). Digestion was terminated by addition of phenylmethylsulfonyl fluoride (Roche Applied Science) to a final concentration of 1 mM. Digested samples were separated by SDS–PAGE and then electrophoresed onto Hybond ECL nitrocellulose membranes (Amersham Biosciences). The membranes were blocked with 0.5% (w/v) nonfat dry milk in Tris-buffered saline containing 0.1% Tween 20 at room temperature for 1 h and then incubated with primary antibody (0.2 μg/ml antibody SAF 32 or 0.2 μg/ml antibody SAF 84) in Tris-buffered saline containing 0.1% Tween 20 and 1% bovine serum albumin for 1 h with shaking at room temperature. Membranes were washed with Tris-buffered saline containing 0.1% Tween 20 and then incubated with the appropriate horseradish peroxidase-conjugated anti-IgG secondary antibody (Chemicon International) (diluted 1:5,000...
in Tris-buffered saline containing 0.1% Tween 20 and 1% bovine serum albumin) at room temperature for 1 h. The blots were developed using Western blot detection reagent (Amer-

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sham Biosciences), and the results were recorded with a charge-coupled device camera (Lumino Capture AF-650, ATTO Co. Ltd., Tokyo, Japan).

The aggregates of PrP-(23–98) formed with NADPH and copper ions were dialyzed at 4 °C for ~18 h against two changes of 5 mM Mes buffer (pH 7.2) and centrifuged at 18,800 × g for 10 min at 4 °C. Pellets were suspended in a solution containing 2% Sarkosyl and 2% SDS and dissolved by sonication in an ultrasonic bath for 10 min. After the sample was centrifuged at 18,800 × g for 10 min at 4 °C, the amount of copper and NADPH in the resulting supernatant was measured. The supernatant was diluted with water, and the diluted sample (containing 0.06% Sarkosyl and 0.06% SDS) was digested with PK as described above. PK resistance was assessed by visualization of undigested protein by Western blot.

RESULTS

NADPH and Copper Ions Cause Aggregation of PrP-(23–98)—It has been reported that metal ions, such as copper, zinc, and manganese, modulate aggregation of a synthetic peptide comprising residues 106–126 of PrP (39) and protease resistance of full-length PrP (15, 40, 41). To investigate whether the N-terminal domain of the PrP protein has the potential to aggregate in the presence of metal ions, we tested the ability of copper ions to aggregate PrP-(23–98), an N-terminal region of PrP. When PrP-(23–98) (10 μM) was incubated with 40 μM CuCl2 in 50 mM Mes buffer (pH 7.2) at 25 °C for 5 min, only a slight increase in turbidity at 550 nm was observed (Fig. 1A, cross E). Unexpectedly further addition of NADPH (500 μM) to the mixture caused marked aggregation (Fig. 1A, solid circle). Because incubation of PrP-(23–98) with NADPH caused only a slight increase in turbidity (Fig. 1A, open circle) and because the metal chelator EDTA prevented the aggregation (data not shown), it is clear that the combination of NADPH and copper ions is important for this process. The aggregates formed were large enough to be col-

![FIGURE 1. Aggregation of PrP-(23–98) is induced by NADPH and copper ions. A, turbidimetry measured by absorbance at 550 nm. A complete mixture (120 μl) containing 10 μM PrP-(23–98), 40 μM CuCl2, and 500 μM NADPH in 50 mM Mes buffer (pH 7.2) was incubated at 25 °C. The arrow indicates the last addition of reagent, NADPH or copper. C, complete system minus CuCl2; E, complete system minus NADPH. B, precipitation of aggregates by centrifugation. The reaction was carried out for 10 min in the complete system (closed bar) or in the absence of copper (open bar) as specified in A. Mixtures were centrifuged at 18,800 × g for 10 min, and the concentration of protein in the supernatant was determined. Error bars are S.D. of three independent experiments. *, significantly different by unpaired Student’s t test from the experiment with the complete system minus CuCl2, p < 0.01. D, pH dependence. The reaction conditions were the same as in A except pH was changed as indicated. Error bars are S.D. of three independent experiments. D, enhancement of ThT fluorescence. A complete mixture (500 μl) containing 50 mM Mes (pH 7.2), 20 μM PrP-(23–98), 80 μM CuCl2, and 500 μM NADPH was incubated at 25 °C for 5 min, and then ThT was added to the mixture for fluorescence measurement. Because the scattering of light by the aggregates and a slight increase in ThT fluorescence due to its interaction with NADPH disturbed the measurement, these components were subtracted from the recorded spectrum. As a control (C), the complete mixture minus copper was subjected to the same procedure. Error bars are S.D. of three independent experiments. E, electron micrographs of aggregates. Aggregation of PrP-(23–98) was carried out in the same complete system as in A. Electron micrographs of negatively stained aggregates were taken at 50,000× magnification. The size of the scale bar is 50 nm.]
Aggregation of N-terminal Domain of PrP

It has been reported that PrP interacts with metal ions other than copper (2–4). Therefore, we examined whether zinc or manganese ions initiate aggregation under the same conditions as in Fig. 1. Zinc induced aggregation of PrP-(23–98), whereas manganese had no effect (Fig. 2A). Similarly the aggregates formed in the presence of NADPH and zinc ions also seemed amyloid-like as judged by binding of ThT (Fig. 2B), and round particles were observed by electron microscopy (data not shown). Dose-response experiments for copper and zinc ions showed that the rate of aggregation plateaued at a lower concentration of copper (5–10 μM) than for zinc (>40 μM) (Fig. 2C). To examine the structural requirement for NADPH, we tested other pyridine nucleotides for the aggregation of PrP-(23–98). Nicotinamide adenine dinucleotide 3'-phosphate (an analogue of NADPH with a phosphate group bound to 3'-OH of the adenine-linked ribose moiety) and nicotinic acid adenine dinucleotide phosphate (NAADP) were equally effective; NADP was also effective but to a lesser extent. However, NADH and NAD had no effect (Fig. 2D). Other compounds containing a phosphate group, such as α-D-glucose 1-phosphate and AMP, did not induce aggregation (data not shown). Thus, the phosphate group on the adenine-linked ribose moiety is essential for the observed effect, and the reduced state of the pyridine moiety is not an absolute requirement.

We next examined the amount of NADPH and copper bound to the aggregates formed with PrP-(23–98) (10 μM) and CuCl2 (40 μM). When NADPH was added at 50 μM, the precipitated aggregates contained 0.93 ± 0.03 mol of NADPH and 1.93 ± 0.05 mol of copper/mol of protein. Even when the NADPH concentration was increased 10-fold, the aggregates contained the same amount of NADPH and copper (0.94 ± 0.02 mol of NADPH and 1.99 ± 0.02 mol of copper/mol of protein). It is clear that NADPH and copper strongly bind to PrP-(23–98) in the aggregates.

Participation of Histidine Residues of PrP-(23–98) in Aggregation—To investigate the participation of histidine residues of PrP-(23–98) in the binding of copper, we examined the effect of chemical modification of the protein with DEPC, which covalently binds to the histidine residues in the octarepeat region, rendering the binding of metal ions inefficient (42). The aggregation of PrP-(23–98) induced by NADPH and copper or zinc ions was attenuated by treatment with DEPC (Fig. 3A). Furthermore, 1-histidine markedly inhibited the aggregation of PrP-(23–98) (Fig. 3B) as was the case for copper binding to the octarepeat region (43). The effect of glycine was negligible. These results indicate that the octarepeat region participates in binding of copper to PrP-(23–98).

Copper Ions Modulate the Protease Resistance of PrP-(23–98)—We next tested whether degradation of the aggregates occurred by incubation with EDTA, PK, or a combination of both. When EDTA was added to reaction mixtures containing the aggregates that had been formed with NADPH and copper ions, a slight decrease in turbidity was observed (Fig. 4A, square, arrow **; ΔA_550/5 min = 0.13). Further addition of PK caused a marked decrease in turbidity (square, arrow **; ΔA_550/5 min = 0.02 mol of copper/mol of protein).
Aggregation of N-terminal Domain of PrP

FIGURE 3. The role of histidine residues of PrP-(23–98) in aggregation. A, chemical modification with DEPC. DEPC-treated PrP-(23–98) was prepared as described under “Experimental Procedures.” The experimental conditions were the same as in Fig. 1A. Open bars, untreated PrP-(23–98); hatched bars, PrP-(23–98) treated with DEPC. Error bars are S.D. of three independent experiments. * indicates significantly different by unpaired Student’s t test from the experiment with untreated PrP-(23–98), p < 0.01. B, effect of L-histidine and glycine on the aggregation of PrP-(23–98). The conditions were the same as in Fig. 1A except the indicated amounts of L-histidine or glycine were incubated for 5 min before the addition of NADPH. Cont, complete system without addition of the amino acids; Nil, incubation with PrP-(23–98) only.

FIGURE 4. Resistance of the aggregates to EDTA and PK. The mixture (120 μl) containing the aggregates (containing 10 μM PrP-(23–98)) produced with NADPH and copper (A) or zinc (B) ions were incubated at 25 °C for 1 min; 5 μl of an EDTA solution (●), a final concentration of 500 μM or 5 μl of water (○) was added at the point marked with arrow *, and PK (weight ratio of PK to PrP-(23–98), 1:10) was added at the point marked with arrow **. The turbidity at 550 nm was measured.

FIGURE 5. PK digestion of PrP-(23–98) aggregates. A, the aggregates were prepared by incubation with NADPH and copper ions (complete system) for 30 min under the same conditions as described in Fig. 1A. One half of each sample was digested with (+) or without (−) PK at 37 °C for 1 h. Digestion was performed using a weight ratio of PK to PrP of 1:10 in the presence (+ EDTA) or absence (− EDTA) of EDTA. After SDS-PAGE, PrP-(23–98) was detected by immunoblotting with antibody SAF 32. Molecular masses in kDa are indicated on the left. − Cu2+ − NADPH, PrP-(23–98) alone; − NADPH, complete system minus CuCl2. B, the aggregates formed as in A were dialyzed and then digested with (+) or without (−) proteinase K in 50 mM Mes buffer (pH 7.2) containing 0.06% Sarkosyl and 0.06% SDS at 37 °C for 60 min. Digestion was performed using a weight ratio of PK to PrP of 1:10 in the presence (+ EDTA) or absence (− EDTA) of EDTA. After SDS-PAGE, PrP-(23–98) was detected by immunoblotting with antibody SAF 32. Molecular masses in kDa are indicated on the left.

It has been reported that PrP acquires PK resistance when incubated with copper ions (15, 40, 41). This was confirmed with PrP-(23–98) in our study. Mixtures were processed under the same conditions as those described in Fig. 1A and were subjected to PK digestion and analysis by Western blot. When PrP-(23–98) was incubated with copper ions, a weak signal from a covalently bound dimer appeared in addition to the primary monomeric band (Fig. 5A, lane 3). Both forms of the protein became resistant to PK digestion (Fig. 5A, lane 4), which was almost completely suppressed by EDTA (Fig. 5A, lane 10). When incubation was performed in the presence of NADPH and copper ions, polymerization of the protein was observed as indicated by formation of higher polymers, and all forms of the protein in the aggregates were PK-resistant (Fig. 5A, lanes 7 and 8). In contrast, the aggregates formed with NADPH and zinc ions were almost completely digested with PK (data not shown). The aggregates formed with NADPH and copper ions became sensitive to PK in the presence of EDTA (Fig. 5A, lane 12). When weakly bound copper ions and NADPH were removed from the aggregates by dialysis, we noticed that the aggregates clumped together. The dialyzed aggregates contained 2.26 ± 0.26 mol of copper and 0.69 ± 0.07 mol of NADPH/mol of protein and were also resistant to PK (Fig. 5B, lane 2), but they were susceptible to proteolysis in the presence of EDTA (Fig. 5B, lane 4). These results indicate that copper and zinc ions differentially influence the formation of aggregates of PrP-(23–98).

Copper Ions Modulate the Protease Resistance of Full-length PrP-(23–231)—We next tested whether full-length PrP-(23–231) undergoes aggregation like PrP-(23–98). The turbidity increased when PrP-(23–231) was incubated with NADPH and copper ions (Fig. 6A). The percentage of the protein collected...
by centrifugation was 40% of the total, which was considerably less than the amount collected when using PrP-(23–98) (Fig. 1B; ~100%). This may be due to the fact that a lower concentration of PrP-(23–231) was used in this experiment (Fig. 6B). Aggregation was almost completely inhibited in the presence of EDTA (data not shown). The aggregates were spherical in shape like those of PrP-(23–98) as observed by electron microscopy (Fig. 6C). NADP and NAADP were less effective than NADPH in the induction of aggregation, and zinc ions had no effect (Fig. 6D).

Like PrP-(23–98), PrP-(23–231) was converted to aggregates that were resistant to digestion with PK. A 23-kDa band representing undigested protein was detected by Western blot (Fig. 7, lane 1). No change was observed when PrP-(23–231) was incubated with copper ions alone except for the appearance of two minor bands at 17–19 and 46 kDa (lane 3). These bands became prominent upon incubation with NADPH and copper ions (lane 5). In contrast, when NADP and NAADP were used instead of NADPH, the intensity of these bands remained the same as when copper ions alone were used (lanes 7 and 9), indicating that a reduction-oxidation reaction of copper was involved. After digestion with PK, a 14-kDa fragment was produced when PrP-(23–231) was incubated with copper ions alone (lane 4). The aggregates formed with NADPH and copper ions were PK-resistant (lane 6) as indicated by the remaining signals on the Western blot. However, the aggregates formed with NADP or NAADP and copper ions were less resistant to PK (lanes 8 and 10).

**DISCUSSION**

It is known that sulfated glycan and RNA enhance the rate of template-dependent conversion of PrP<sup>C</sup> into the aggregated form of the protein (44–46) and that DNA provokes a conversion of PrP<sup>C</sup> into a β-sheet rich isoform (47). Our present study indicates that NADPH and copper or zinc ions also facilitate the conversion of PrP<sup>C</sup> into aggregates. For example, the presence of NADPH and copper or zinc ions polymerized PrP-(23–98) into amyloid-like aggregates. The effect of copper or zinc ions may be mediated by their binding to the octarepeat region of the N-terminal domain. NADPH may also bind to the N-terminal region, possibly to the same site as RNA (45). The aggregates formed with copper ions were resistant to digestion with PK, but the aggregates formed with zinc ions were not. The former aggregates were more stable than the latter, which were degraded by PK treatment in the absence of EDTA (Fig. 4B). The difference may be due to a higher affinity of copper for the octarepeat region than that for zinc (3, 4). Our findings are unique in that a part of the N-terminal domain formed PK-resistant aggregates even though they were not fibrils. This contrasts with recent data that suggest residues 138–141 are essential for in vitro fibril formation of the entire N-terminal domain extending to residue 141 (HuPrP-(23–141)) (33). Similar to the aggregation of PrP-(23–98), we observed that the full-length protein PrP-(23–231) was converted into a PK-resistant form in the same experimental setting (NADPH plus copper ions). Here the conformational change induced in the N-terminal region might have caused a conversion of the C-terminal region into the PK-resistant form. It is tempting to speculate that prion initiation in the nonfamilial types of prion diseases occurs in this fashion. For the PrP-
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FIGURE 7. PK digestion of PrP-(23–231) aggregates produced with pyridine nucleotides and copper ions. The aggregates were prepared by incubation with CuCl₂ with either of the indicated pyridine nucleotides for 30 min at 25 °C under the conditions described in Fig. 6A, and then one-half of each sample was incubated with (+) or without (–) PK (weight ratio of PK to PrP, 1:10) at 37 °C for 1 h. After SDS-PAGE, PrP-(23–231) was detected by immunoblotting with antibody SAF 84. Molecular masses in kDa are indicated on the left.
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