Polymerization of Alzheimer amyloid β peptide (Aβ) into amyloid fibrils is associated with resistance to proteolysis and tissue deposition. Here, it was investigated whether Aβ might be generated as a protease-resistant core from a polymerized precursor. A 100-amino acid C-terminal fragment of the Alzheimer β-amyloid precursor protein (C100), containing the Aβ and cytoplasmic domains, polymerized both when inserted into membranes and after purification. When subjected to digestion using the nonspecific enzyme proteinase K, the cytoplasmic domain of C100 was degraded, whereas the Aβ domain remained intact. In contrast, dissociated C100 polymers were almost completely degraded by proteinase K. Mammalian cells transfected with the human Alzheimer β-amyloid precursor gene contained a fragment corresponding to C100, which needed similar harsh conditions to be dissolved, as did polymers formed by purified C100. Hence, it was concluded that C100 polymers are formed in mammalian cells. These results suggest that the C terminus of Aβ can be generated by nonspecific proteases, acting on a polymerized substrate, rather than a specific γ-secretase. This offers an explanation of how the Aβ peptide can be formed in organelles containing proteases capable of cleaving most peptide bonds.

The Alzheimer amyloid β peptide (Aβ) is the primary constituent of the amyloid deposited in the brain parenchyma and blood vessels of the brain in association with Alzheimer’s disease (AD) (1–4). Aβ is generated through proteolytic processing of Alzheimer β-amyloid precursor protein (β-APP), a transmembrane protein expressed in most mammalian cells (5). Much interest has been focused on the metabolic mechanisms generating Aβ (6). β-APP can be metabolically processed by at least two pathways. The α-secretase pathway cleaves β-APP in the central section of the Aβ domain (7, 8) and thereby precludes amyloidogenesis, whereas the β-secretase pathway generates the free N terminus of intact Aβ (6). The C-terminal β-APP fragment containing the intact Aβ and cytoplasmic domains will hereafter be referred to as C100 (9). Subsequently, both pathways converge, and the β-APP metabolites are cleaved by a protease activity, tentatively named γ-secretase, generating the free C terminus of Aβ and a related, nonamyloidogenic fragment, p3 (10, 11). The enzyme or enzymes catalyzing γ-secretase cleavage have not yet been identified. The enzymatic characteristics of γ-secretase activity include a certain degree of nonspecificity, since the β-APP fragment can be cleaved after either amino acid 40 or amino acid 42 of the Aβ domain (6). In either case, the cleavage site is located within the predicted transmembrane region of β-APP (5) and should therefore be protected by the phospholipid bilayer, raising the possibility that this cleavage may not occur on the C100 substrate as long as it is membrane-inserted.

Several studies suggest that γ-secretase activity is localized to an endosomal or lysosomal compartment (12–14). Lysosomes contain a multitude of proteolytic enzymes (15) and therefore it seems unlikely that C100 in endosomes or lysosomes will encounter protease activities with defined and narrow substrate specificities (i.e. a specific γ-secretase). Instead, C100 will be present in an environment of enzymes cleaving many or most peptide bonds. In an earlier article, it was reported that Aβ acquires protease resistance in association with polymerization (16). It is therefore possible that the Aβ domains of several C100 molecules can interact and generate a “protease-resistant core,” capable of withstanding the nonspecific proteolytic environment of endosomes or lysosomes. In the present study we have investigated whether purified and membrane-associated C100 polymers can serve as a substrate for the generation of Aβ by nonspecific proteolysis, using recombinant C100 and proteases with various substrate specificities.

**EXPERIMENTAL PROCEDURES**

Expression of C100 in Insect Cells—Sf9 cells were grown in Grace’s supplemented insect medium with 10% fetal bovine serum (Life Technologies, Inc.) to a density of 10⁶ cells/ml (in a total volume of 500 ml in a 1,000-ml spinner flask). The cells were then infected with recombinant baculovirus containing the C100 gene generously provided by Dr. Rachael Neve, Harvard University. Four days after infection, the cells were centrifuged at 1,000 × g for 10 min. The pellet was washed with phosphate buffer, pH 6.2, containing 1 mM phenylmethylsulfonyl fluoride (PMSF) and centrifuged again. The harvested cells were stored at −80 °C in aliquots of 10⁶ cells/vial.

Gradient Centrifugation of Infected Cells—Infected and harvested...
cells (one vial, 10^6 cells) were homogenized in a Dounce homogenizer with 2 ml of 5 mM Hepes buffer, pH 7.4, containing 1 mM PMSF. The homogenate was centrifuged at 500 g for 5 min. The supernatant was loaded on a 10–30% sucrose gradient with 5 ml Hepes, pH 7.4, and centrifuged in a swinging bucket rotor (Beckman SW 40 Ti) at 125,000 g for 1.5 h. The fractions (100 µl each) were collected and the 70% sucrose fraction was aspirated from the top of the tubing. A 100-µl aliquot of each fraction was subjected to SDS-PAGE and immunoblotting.

**Purification of C100**—Infected and harvested cells (two vials, 2 × 10^6 cells) were homogenized in a Dounce homogenizer with 2 ml of 5 mM Hepes buffer containing 1 mM PMSF. The homogenate was centrifuged at 500,000 g for 20 h with 200 µl of sucrose corresponding to 23–30% sucrose, were diluted with TBS, pH 7.4, and centrifuged in a fixed angle rotor (Beckman TLA 40). The resulting pellet was washed with 300 µl of methanol/200 µl of chloroform for 1 h. After addition of 100 µl of methanol, the sample was centrifuged for 30 min at 125,000 g. The pellet was washed with 100 µl of methanol with continuous stirring. The sample was centrifuged for 30 min at 125,000 g, and the supernatant was injected on a Superose 12 size exclusion column and eluted with 70% formic acid. Fractions were collected and analyzed by SDS-PAGE and immunoblotting using 6E10 and 369 as primary antibodies. The fractions with the highest content of C100 were pooled and aliquoted prior to the formation of the mobile phase in a vacuum centrifuge.

**Digestion of Purified C100 with Proteases**—Purified C100 was incubated in Tris-buffered saline (TBS) containing 50 mM NaCl and 50 mM Tris-HCl, pH 7.4, at 37°C for 96 h. The incubated fractions were centrifuged with 0, 10, 100, or 1000 µg of protease K/ml for 5 h. Purified C100 was also incubated on 4, 48, and 170 h prior to digestion with protease K. In the same experiment, polymerized C100 (48 h incubation) was dissolved in 70% formic acid with continuous stirring for 2 h, lyophilized, and digested with protease K (100 µg/ml). The polymerized and digested samples were centrifuged at 22,000 × g, and 170 µl of the supernatant was replaced with an equal volume of 2 mM PMSF, 9 µl urea, and 50 mM Tris, pH 10. After 3 days with continuous stirring, the samples were analyzed by SDS-PAGE and immunoblotting.

**Digestion of Membrane-bound C100 with Proteases**—The fractions from the gradient centrifugation with the highest C100 content, corresponding to 23–30% sucrose, were dialyzed with TBS, pH 7.4, and centrifuged at 100,000 × g for 1 h to remove the sucrose. The pellet was suspended in the same buffer, aliquoted, and incubated at 37°C in a shaking water bath overnight. Two volumes of either trypsin (150 µg/ml in 50 mM ammonium bicarbonate buffer, pH 7.8), or protease K (50 µg/ml in 50 mM TBS, pH 7.4) were added to the samples. After 5 h at 37°C, the samples were analyzed by SDS-PAGE and immunoblotting.

**Digestion of the Cytoplasmic Domain of C100 with Proteases**—A synthetic peptide corresponding to amino acids 48–99 in C100 was a gift from Dr. Andrew J. Czernik (Rockefeller University). The peptide was incubated at 100 µM in TBS, pH 7.4, for 1 week. Protease K was added to a final concentration of 50 µg/ml. After 5 h at 37°C, the samples were analyzed by SDS-PAGE and immunoblotting.

**SDS-PAGE and Immunoblotting**—Gradient gels, 5–18%, were run in a Tris-Tricine buffer system (17). Laemmli sample buffer containing 6 µl urea was added to the samples. After boiling for 5 min, 80 µl of sample was applied to the gel. After 3.5 h at 70 mA, the gel was either stained with Coomassie Brilliant Blue or electroblotted overnight to a nitrocellulose membrane (0.2-µm pore size, Schleicher & Schuell). The membranes were incubated with monoclonal antibodies 6E10 and 4G8 (mouse), directed against residues 5–10 and 18–21 in Aβ, respectively, and polyclonal antibody 369 (rabbit), recognizing epitopes located in the C terminus of C100.8 Horseradish peroxidase-conjugated secondary antibodies and chemiluminescence detection (Amersham) were used to visualize bound primary antibodies.

**Electron Microscopy**—To determine the intracellular localization of C100, infected cells were fixed in 2% formaldehyde/0.1% glutaraldehyde in phosphate-buffered saline, pH 7.3, dehydrated in graded ethanol, and embedded in LR White. Thin sections were immunogold stained with 369 as primary antibody and anti-rabbit IgG conjugated to 10 nm colloidal gold particles as secondary antibody. Purified C100 was incubated with 0.2 µl primary antibody. One sample was digested with protease K (50 µg/ml) for 5 h, and one was not digested. The samples were centrifuged for 1 h at 125,000 × g, and the pellet was triturated in water (Milli-Q, Waters). A 5-µl sample of this suspension was placed on a grid covered with a carbon-stabilized Formvar film. After 0.5–1 min, excess fluid was adsorbed on a filter paper, and the grids were air dried. Negative staining was made with 2% uranyl acetate in water. For immunostaining, the grids were incubated on droplets of Tris-buffered saline, pH 7.4, containing 0.1% bovine serum albumin to block nonspecific binding. The grids were then exposed to primary antibodies 6E10 and 369, washed, and exposed to secondary antibodies conjugated to colloidal gold particles of different sizes (anti-mouse IgG conjugated to 10 nm of colloidal gold particles and anti-rabbit IgG conjugated to 20 nm of colloidal gold particles). Both primary and secondary antibodies were dissolved in TBS, pH 7.4, with 0.1% bovine serum albumin, which was also used to wash the samples after exposure to antibodies.

**Extraction of C100 from Chinese Hamster Ovary (CHO) Cells**—CHO cells were stably transfected with p-APP751sw (18). Approximately 40 × 10^6 cells were homogenized in a Dounce homogenizer with 1 ml of 0.3 M sucrose in 20 mM Tris, pH 7.3. The homogenate was centrifuged for 5 min at 500 × g. The supernatant was centrifuged for 1.5 h at 125,000 g in a Beckman TLA rotor. The resulting pellet was suspended in 1% SDS in 20 mM Tris, 7.4, and the centrifugation was repeated. The supernatant was lyophilized, and the pellet was delipidated with methanol/chloroform. A solution of 9 µl urea/100 mM Tris, pH 10, was added to the pellet. After 1 day with continuous stirring, Laemmli sample buffer was added to the sample. The lyophilized SDS extract was dissolved in Laemmli sample buffer supplemented with urea. The samples were then subjected to SDS-PAGE followed by immunoblot analysis using 6E10 or 369 as the primary antibody.

**RESULTS**

**Proteolytic Digestion of Polymerized C100 Yields Peptides Indistinguishable from Synthetic Aβ**—Previously, it was shown that polymerization of synthetic Aβ fibrils leads to resistance to proteolytic degradation (16). We therefore investigated whether purified C100, which readily polymerizes and forms fibrils (9, 28), also develops protease resistance in association with fibril formation.

Purified C100 was allowed to polymerize by incubation in TBS for 96 h before the addition of proteinase K, an enzyme capable of cleaving most peptide bonds (19). The samples were then subjected to SDS-PAGE, followed by immunoblot analysis using 6E10 or 369 as primary antibody. The 6E10 epitope is located in the N terminus (amino acids 70–75 and 90–95) of C100. Polymerized C100 could not be completely dissolved under the conditions used, although the bulk of the material migrated as a monomer at ~12 kDa (calculated molecular mass, 11.3 kDa). In addition to this band, a number of high molecular mass species, corresponding to polymers of various sizes, were seen (Fig. 1, lane 1). The antibodies used bind with higher avidity to polymers than to monomers, and the amount of polymers is therefore likely to be

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overrepresented in the immunoblots.3 Proteinase K (10 μg/ml) treatment of polymerized C100 cleaved the protein and generated several fragments with molecular masses lower than that of native C100 (Fig. 1, lane 2). Addition of higher concentrations of proteinase K yielded a 6E10-reacting species with a molecular mass indistinguishable from that of synthetic Aβ (Fig. 1, compare lanes 3 and 4 with 5). The Aβ-specific antibody 4G8, recognizing amino acids 18–21 in Aβ, yielded an essentially identical pattern (data not shown). Analysis with antibody 369 showed that all material corresponding to the cytoplasmic domain of C100 had been degraded by the proteinase K (100 and 1000 μg/ml) treatment (data not shown).

**Polymerization of C100 Is Required for Generation of Aβ**—In the next series of experiments, it was investigated whether polymerization of C100 is required for the generation of Aβ in a reaction catalyzed by a nonspecific protease. Purified C100 was incubated in TBS at 37 °C for the indicated periods (Fig. 2). To determine whether the polymerization and protease resistance is a reversible reaction, one polymerized aliquot was treated with formic acid, which dissociates the fibrils, and lyophilized prior to digestion. After addition of proteinase K, the samples were incubated for 5 h. The reaction was then stopped, and the samples were dissolved and analyzed by immunoblotting (Fig. 2). The nondigested sample showed a smearlike pattern, indicating that the fibrils had not been completely dissolved (Fig. 2, lane 1). Nonpolymerized C100 was almost completely digested, and no Aβ-like species were detected (Fig. 2, lane 3), indicating that monomeric C100 cannot serve as a substrate for the generation of Aβ by nonspecific proteolysis. This is in agreement with previous results using monomeric Aβ as a substrate (16). Polymerized C100 that had been treated with formic acid to dissociate the fibrils was not totally digested, and a faint band in the Aβ region (~4 kDa) was detected (Fig. 2, lane 2). The difference between this sample and the nonpolymerized sample may be due to the fact that the fibrils formed were not completely dissociated in formic acid. The polymerized and proteinase K-treated samples gave a strong signal in the Aβ region, i.e. ~4 kDa (Fig. 2, lanes 4 and 5).

**Electron Microscopy of C100 Fibrils and Infected Cells**—Purified and polymerized C100, digested with proteinase K or nondigested, were adsorbed to grids, negatively stained with uranyl acetate, and examined by electron microscopy. Like Aβ, C100 formed fibrils with a diameter of about 5–8 nm, typically arranged in tight bundles (Fig. 3, A and B). Immunogold staining, with 6E10 and 369 as primary antibodies, revealed that the fibrils contained epitopes located both in the Aβ and the cytoplasmic domains of C100 (Fig. 3D). Thus, it was concluded that the fibrils consisted of intact C100. Electron microscopic examination of the proteinase K-treated C100 fibrils revealed a fibrillar structure, indistinguishable from that of genuine Aβ (Fig. 3, compare A and C). Moreover, immunogold staining

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3 L. O. Tjernberg, J. Näsund, and C. Nordstedt, unpublished observations.
revealed that the proteinase K-treated C100 fibrils remained reactive with 6E10 but not with 369 (data not shown). Hence, it is concluded that nonspecific proteolysis of C100 can generate an Aβ species electrophoretically, immunologically, and morphologically equivalent to purified Aβ.

The intracellular localization of C100 in Sf9 cells infected with recombinant baculovirus containing DNA coding for C100 was examined with immunogold staining of thin sections of fixed and embedded cells. The bulk of the immunoreactivity was confined to a complex system of membranes found in both the cytoplasm and the nucleus (Fig. 3), indicating that recombinant C100 is indeed inserted in membranes.

**Proteolytic Digestion of Polymerized C100 in Cell Membranes**—Like polymerized synthetic Aβ (16), the Aβ region of polymerized C100 was apparently partly resistant to proteolytic degradation. An intriguing possibility was therefore that C100 inserted into cell membranes can aggregate and thereby develop protease resistance. It was therefore investigated whether membrane-inserted C100 can polymerize, similar to the purified protein. For this purpose, a membrane preparation from Sf9 cells, infected with baculovirus-containing C100 DNA, was subjected to SDS-PAGE and immunoblot analysis with antibodies 6E10 and 369. Purified, monomeric C100 was loaded on the gel as a reference. The C100 protein from a crude membrane preparation displayed a smearlike pattern, strongly suggesting that C100 had formed polymers of different sizes (Fig. 4, lane 1), whereas the purified protein migrated as a single species with an apparent molecular mass of ~12 kDa (calculated molecular mass, 11.3 kDa) (Fig. 4, lane 2). It is unlikely that the polymers were formed after the addition of sample buffer, which contained a highly denaturing chaotrope (urea) and detergent (SDS), since purified monomeric C100 did not form polymers when it was analyzed under the same conditions. Hence, it is concluded that C100 can polymerize while still inserted in cell membranes.

It was then examined whether C100 inserted in the membrane of transfected cells can serve as a substrate for the generation of Aβ, analogous to purified and polymerized C100. A membrane fraction from Sf9 cells infected with recombinant baculovirus containing C100 DNA was incubated overnight in TBS with or without the zwitterionic detergent CHAPS. CHAPS was added to dissolve potential membrane vesicles, thereby making both the N and C termini of C100 accessible to the protease. The CHAPS molecules act by hydrophobic interaction with the transmembrane region of C100, possibly substituting for the phospholipid bilayer, and the transmembrane region is thereby still shielded from proteases. After digestion with proteinase K for 5 h, the samples were analyzed by immunoblotting (Fig. 5). As seen in Fig. 5, lane 3, a fragment with a slightly higher apparent molecular mass than Aβ, ~6 kDa, was formed. The addition of CHAPS did not alter the apparent molecular mass of the proteolytic fragment generated by proteinase K (Fig. 5, lanes 2 and 4). A cleavage at lysine 53 (which is the first amino acid residue C-terminal to the predicted transmembrane region of C100) would generate a 5.9-kDa fragment. The detected species corresponds in molecular mass and immunoreactivity to such a fragment, indicating that the Aβ domain of C100 can form a protease-resistant core while still inserted in membranes, and that the transmembrane region is shielded from proteolysis by phospholipids and CHAPS. The experiment was repeated using trypsin instead of proteinase K. Trypsin cleaves C-terminally to lysine and arginine residues, a putative cleavage site being lysine 53. A cleavage after this residue should generate a 5.9-kDa fragment, provided that putative tryptic cleavage sites within the Aβ domain of C100 (i.e. Arg5, Lys16, and Lys28) were protease resistant. A 6E10 immunoreactive fragment with the same apparent molecular mass as the fragment generated by proteinase K was detected (data not shown). It is therefore concluded that C100 inserted in membranes can polymerize and develop protease resistance, and that dissolution of the phospholipid bilayer is necessary to generate the free C terminus of Aβ.

**The Cytoplasmic Domain of C100 Forms Non-Protease-resistant Fibrils**—To investigate the role of the cytoplasmic domain of C100 in fibril formation, a synthetic peptide corresponding to this part of C100 was incubated under the same conditions as purified C100. When the incubated peptide was analyzed by electron microscopy, fibrils similar to those generated from Aβ and C100 were found (data not shown). Thus, the cytoplasmic domain of C100 may be involved in the polymerization of C100, since this region can form fibrils per se. After digestion with proteinase K, no immunoreactivity was found when the sample was analyzed by SDS-PAGE followed by immunoblotting with 369 as a primary antibody. Apparently, the fibrils formed from the cytoplasmic domain of C100 were, in agreement with our other experiments, not protease-resistant.

**CHO Cells Stably Transfected with β-APP751sw Generate SDS-insoluble C100 Fragments**—To investigate whether C100 generated from full-length β-APP exists in an aggregated form, CHO cells were stably transfected with β-APP751sw (18). The Swedish mutation was chosen, since it produces approximately five times more Aβ than the wild-type gene and, according to the present hypothesis, therefore should produce more C100 polymers. A postnuclear supernatant from CHO cells, stably
transfected with β-APP751Sw, was centrifuged at 125,000 × g for 1 h. The resulting pellet was extracted with 1% SDS in TBS and centrifuged once more. The supernatant was lyophilized, and the pellet was dissolved in 9 M urea at pH 10 for 24 h. After addition of urea to the SDS-extracted sample and SDS to the urea-extracted sample, Laemmli sample buffer was added, and the samples were subjected to SDS-PAGE. Immunoblot analysis, using 6E10 or 369 as primary antibody, showed a remarkable difference between the urea-extracted sample and the SDS extract (Fig. 6). No immunoreactivity could be detected in the SDS extract, either with 6E10 or with 369 as the primary antibody. The urea treated sample displayed, with 6E10 as the primary antibody, one intense band in the C100 region. The same band was detected with 369, suggesting that the detected species corresponds to C100. A fragment with slightly lower molecular mass was also detected with antibody 369, probably corresponding to the C-terminal fragment of β-APP generated by α-secretase cleavage, the Esch fragment (7). The absence of immunoreactive fragments in the SDS extract indicates that C100 generated from β-APP751Sw is tightly bound and, in accordance with our earlier results, probably occurs as a polymer.

**DISCUSSION**

Here, we have presented evidence that Aβ can be generated from polymerized, but not from nonpolymerized, C100 by the nonspecific proteolytic activity of proteinase K.

C100, a metabolic fragment generated through β-secretase cleavage of β-APP (13, 20–23), can polymerize and form fibrils similar to those of Aβ (24). In our experiments, polymerization occurred both when the protein was inserted in cell membranes and after purification of the protein. A reasonable explanation is that C100 polymerizes through interaction between Aβ domains, since Aβ readily forms fibrils (25). The cytoplasmic domain of C100 also forms fibrils per se, and this interaction may enhance the ability of C100 to form fibrils.

Polymerization of synthetic Aβ is associated with increased resistance of the molecule to proteolytic enzymes with varying substrate specificities in vitro (16). It has also been shown that aggregates of synthetic Aβ 1–42 internalized by cultured human skin fibroblasts are stable for several days and co-localizes with late endosomes and lysosomes (26). Therefore, we wanted to investigate whether the Aβ domains in C100 could form “protease-resistant cores” through a similar mechanism. Intracellular proteolysis in endosomes and lysosomes involves several endoproteases and exoproteases with various substrate specificities (15, 27). Since it would be difficult to mimic this combination of enzymes in a reconstituted system, we decided to use proteinase K, a bacterial protease with a wide substrate specificity, cleaving all natural peptide bonds (19). This enzyme has previously been suggested to be capable of generating Aβ-like peptides from β-APP (28). Digestion of polymerized C100 with proteinase K rendered a fragment chromatographically, immunologically, and morphologically indistinguishable from that of synthetic Aβ. These results demonstrate that: (i) polymerization of C100 is associated with formation of a protease-resistant core of tightly bound Aβ domains; and (ii) an enzyme cleaving all natural peptide bonds can substitute for the putative γ-secretase. When C100 was digested in cell membranes, in the presence or absence of detergent, a species with slightly higher molecular mass than Aβ was formed. The transmembrane domain of C100 is apparently shielded from proteolysis by phospholipids or detergent. However, the phospholipid membrane can be degraded in lysosomes, exposing the transmembrane domain of the protein to proteases. The proposed model is described schematically in Fig. 7.

It was also demonstrated that most of the C100 produced by β-APP751Sw-transfected CHO cells was SDS-insoluble. The C100 produced by these cells could, like C100 polymers formed from purified C100 or C100 in Sf9 cells, be dissolved in 9 M urea at pH 10. The SDS insolvibility is also characteristic for the Aβ fibrils found in Alzheimer’s disease brains (29). These findings indicate that the C100 molecules produced by the β-APP751Sw-transfected cells are tightly associated, probably in polymeric form. If present in human brain cells, such polymers could be partially degraded, leaving a protease-resistant core of polymeric or oligomeric Aβ.

An important and obvious question is whether Aβ can be formed through nonspecific proteolysis not only in the artificial systems described here but also in intact cells. A recent study (30) demonstrated that Aβ secreted from cells was present as soluble oligomers of various size. The oligomers were detectable after separation under denaturing conditions in SDS-PAGE, indicating that they were joined by strong bonds. It can be speculated that the oligomers had been formed prior to γ-secretase cleavage and secretion in a reaction similar to the one described here, rather than from monomeric Aβ in the cell media. When β-APP-transfected human kidney cells are treated with aggregated Aβ 1–42, the accumulation of stable,

![Fig. 6. Extraction of C100-like material from mammalian cells.](image)

*Left panel* CHO cell membranes extracted with urea or SDS as described under “Experimental Procedures” and purified C100 were separated by SDS-PAGE. Following electrophoresis, immunoreactive material was visualized using the Aβ region-specific antibody 6E10. *Right panel*, the same samples as in A, but here immunoreactivity was visualized using antibody 369, which is specific for the cytoplasmic region of β-APP. Filled arrowhead, C100; open arrowhead, the Esch fragment.

![Fig. 7. Proposed model of generation of the Aβ peptide in cells.](image)

When β-APP is cleaved by β-secretase, en route to or at the plasma membrane, C100 is generated (1). A fraction of these molecules bind to each other by interactions between their Aβ domains and possibly also between their cytoplasmic domains. Thereby, protease-resistant Aβ cores are formed (2). Bound and free C100 molecules are transported to an organelle, in which they are exposed to a multitude of proteolytic enzymes (3). Nonresistant parts of the molecules are degraded (4), whereas the protease-resistant Aβ cores remain intact and subsequently are secreted from the cells (5).
insoluble amyloidogenic aggregates is stimulated (31). One possible explanation to this finding is that the Aβ domains in endogenously produced C100 bind to internalized Aβ 1–42 and thereby form protease-resistant cores. The fact that an intact fragment corresponding to C100 lacking the Aβ domain has yet not been detected in cells may also be an argument against the idea that the γ-secretase is a specific enzyme (see Ref. 21).

Apparently, nonspecific protease activity cleaving any peptide bond can serve as γ-secretase, if the substrate has the right higher order structure. This model suggests that Aβ can be formed in organelles such as endosomes and lysosomes, containing a multitude of proteases covering a broad spectrum of substrate specificities (15, 27). Low pH promotes formation of Aβ fibrils in vitro (32). Through a similar mechanism, the acidic environment in late endosomes and lysosomes may enhance the formation of C100 fibrils with a protease-resistant core corresponding to Aβ.

The mechanism for Aβ formation proposed here may have pharmacological implications. It has been suggested that protease inhibitors capable of inhibiting β- and γ-secretase activity may be used to reduce Aβ production in vivo and, therefore, are candidate drugs for the treatment of Alzheimer’s disease-associated amyloidosis (3). Considering the present model, it would not be possible to find a selective γ-secretase inhibitor. Alternatively, a molecule capable of antagonizing C100 polymerization would prevent the generation of a protease-resistant core of Aβ, thereby preventing amyloidogenesis in Alzheimer’s disease.

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