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A novel substrate for analyzing Alzheimer's disease γ-secretase

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Abstract Proteolytic processing of Alzheimer's disease amyloid precursor protein (APP) by β-secretase leads to A4CT (C99), which is further cleaved by the as yet unknown protease called γ-secretase. To study the enzymatic properties of γ-secretase independently of β-secretase, A4CT together with an N-terminal signal peptide (SPA4CT) may be expressed in eukaryotic cells. However, in all existing SPA4CT proteins the signal peptide is not correctly cleaved upon membrane insertion. Here, we report the generation of a mutated SPA4CT protein that is correctly cleaved by signal peptidase and, thus, identical to the APP-derived A4CT. This novel SPA4CT protein is processed by γ-secretase in the same manner as APP-derived A4CT and might be valuable for the generation of transgenic animals showing amyloid pathology.

Key words: Signal peptidase; γ-Secretase; Alzheimer's disease; Amyloid precursor protein; Protein transport

1. Introduction

Alzheimer's disease (AD) γ-secretase is a so far unidentified protease activity that is of particular interest because it catalyzes the last step in the generation of the β-amyloid peptide (Aβ), which is the main proteinaceous component of the amyloid plaques found in the brain of patients with AD [1,2]. Aβ is a ~4 kDa peptide which is proteolytically derived from the larger amyloid precursor protein (APP) [3].

In order to be cleaved by γ-secretase, APP first needs to be proteolytically processed by one of the protease activities termed α- and β-secretases (Fig. 1, for reviews see [4,5]). β-Secretase has not yet been identified, whereas there is recent evidence that α-secretase is a member of the ADAM family of proteases. Among these family members tumor necrosis factor-α converting enzyme and ADAM10 possess α-secretase activity [6–8].

The α- and β-secretases cleave APP within its ectodomain at a short distance from the transmembrane domain. This yields secretory APP (α-APPs or β-APPs), comprising most of the N-terminal ectodomain of APP, and the remaining membrane-bound C-terminal fragments p3CT (C83) and A4CT (C99), respectively. A4CT and p3CT are further cleaved by γ-secretase in the middle of their putative transmembrane domains, but it is not clear whether the cleavage occurs while A4CT and p3CT are still inserted into the membrane or after release of both proteins from the membrane. The γ-secretase cleavage generates the C-terminal fragment p7, which is believed to be rapidly degraded, and the N-terminal peptide fragments Aβ and p3, which are secreted by cultured cells. The γ-secretase cleaves mainly after residue 40 of A4CT and partly after residue 42, thus generating the peptide $A\beta_{40}$ and to a lesser extent $A\beta_{42}$ [9–11]; the same C-termini have been observed for the p3 peptides [12,13].

So far, γ-secretase activity has been well characterized ([14] and references therein), but has not yet been identified, although presenilin 1, a polytopic membrane protein, has recently been suggested to be γ-secretase [15,16].

Currently, no in vitro assay system is available that would allow the direct analysis of the enzymatic properties of γ-secretase. Thus, the usual way to study γ-secretase activity is to express APP in eukaryotic cells and to evaluate its processing to Aβ. This approach is problematic in that it requires the cleavage of APP by both β- and γ-secretase. Thus, to study γ-secretase activity independent of β-secretase cleavage, several groups directly expressed the A4CT protein, in which the β-secretase cleavage has been anticipated compared with full-length APP (Fig. 1, [17–19]). This A4CT may be cleaved either directly by γ-secretase (Fig. 1) or first by α- and then by γ-secretase, generating Aβ and p3, respectively. Moreover, the directly expressed A4CT is processed to $A\beta_{42}$ and $A\beta_{40}$ in the same way as full-length APP [13]. To ensure the correct insertion of A4CT into the membrane of the endoplasmic reticulum, a signal peptide (SP) was fused to the N-terminus of A4CT, which is cleaved during membrane insertion by signal peptidase (Fig. 1, SPA4CT, and [20]). However, the signal peptide was never directly fused to the A4CT sequence. Instead, additional amino acids were used to link the signal peptide and A4CT: either the dipeptide LE (leucine and glutamic acid) (Fig. 1, [21,22]), which directly follows the signal peptide within the APP sequence, or only the single amino acid leucine (L) [10].

Thus, after signal peptidase cleavage, the artificial A4CT has a modified N-terminus compared with the A4CT derived from the natural APP (Fig. 1). While this does not seem to influence the γ-secretase cleavage of A4CT [13], the question remains open whether in terms of cell biological and physicochemical properties the generated Aβ with the additional residues at its N-terminus is fully identical to the natural Aβ-protein derived from APP. So far, no SPA4CT has been reported that is processed by signal peptidase in such a way that the resulting A4CT has the natural amino acid Asp-1 at its N-terminus.

Here, we describe the generation and proteolytic processing of a mutated SPA4CT protein that is cleaved by signal peptidase in such a way that the resulting A4CT protein has the same N-terminus as A4CT derived from APP, and thus does not contain any additional amino acids at its N-terminus. This mutated SPA4CT protein is a novel precursor for Aβ and a

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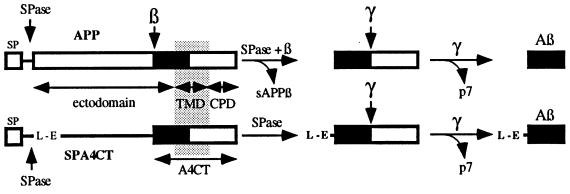

Fig. 1. Proteolytic processing of APP and SPA4CT to Aβ. Schematic representation of APP and SPA4CT. SPA4CT consists of the signal peptide (SP) of APP, the dipeptide leucine-glutamic acid (L-E) and the C-terminal 99 residues of APP (A4CT). Within the sequence of APP, the dipeptide L-E directly follows the signal peptide. The generation of Aβ requires the cleavage of APP by the β- and γ-secretase activities, whereas the β cleavage is obviated in the SPA4CT protein. Aβ derived from SPA4CT starts with the dipeptide L-E at its N-terminus. The cleavage sites of β- and γ-secretases as well as of the signal peptidase (SPase) are indicated. TMD: transmembrane domain; CPD: cytoplasmic domain.

better substrate for the analysis of the enzymatic properties of γ-secretase.

2. Materials and methods

2.1. Cell culture and transfections

COS7 cells were cultured according to the protocol described previously [13] except that DMEM was used instead of a mixture of MEM and F12. Cell culture media were obtained from Sigma. The pCEP4 vector (Invitrogen) with the SPA4CT cDNA inserts was transfected into COS7 cells using Lipofectin (Gibco BRL) as described elsewhere [20]. For each construct two or more independent transfections were analyzed.

2.2. Antibodies

The monoclonal antibodies W02 (for the precipitation of all Aβ peptides regardless of the specific C-terminus), G2-10 (specific for Aβ ending at residue 40) and G2-11 (specific for Aβ ending at residue 42) were raised against synthetic peptides [23]. The polyclonal antibody CT13 was raised against a synthetic peptide comprising the C-terminal 13 residues of A4CT (Eurogentech, Seraing, Belgium).

2.3. Metabolic labeling and immunoprecipitation

In each experiment similar numbers of cells were used. After 45 min preincubation in methionine-free MEM, stably transfected COS7 cells were incubated overnight in methionine-free MEM containing 10% fetal bovine serum and 133 μCi/ml [^{35}S]methionine (Amersham). The conditioned media were centrifuged at 4°C for 1 min at 4000×*g*. Aβ and p3 were immunoprecipitated during 6 h with antibody G2-10 (12.5 μg/ml), G2-11 (17.3 μg/ml) or W02 (5 μg/ml, does not precipitate p3, but only Aβ), and 100 μg protein G-agarose (Boehringer Mannheim), respectively. The immunoprecipitated proteins were separated on 10% Tris-tricine gels [24]. The intensity of the bands was quantified using a Fuji phosphorimager (BAS 1000). The cell lysates were generated as described earlier [13], and A4CT was immunoprecipitated using antibody CT13 and 4 mg of protein A-Sepharose (Pharmacia).

2.4. Plasmid construction

Wild-type and mutated plasmids pBS/SPA4CT-DIR were generated by cloning the *Eco*RI/*Spe*I fragments of the corresponding pUC18/A4CT plasmids [13] into the vector pBS/SPC99 [25] that was digested with *Eco*RI/*Spe*I. The *Sma*I/*Spe*I fragments of these constructs were cloned into the pCEP4 vector that was digested with *Nhe*I/*Pvu*II, thus generating the constructs pCEP4/SPA4CT-DIR. pBS/SPA4CT-DA was generated using the Quik Change Site Directed Mutagenesis Kit (Stratagene), suitable oligonucleotides and pBS/SPA4CTrev [13] as DNA template. The *Kpn*I/*Spe*I fragment of this construct was cloned into the pCEP4 vector that was digested with *Kpn*I/*Nhe*I. The identity of the construct obtained by PCR was confirmed by DNA sequencing. The generation of plasmids pCEP/SPA4CT-LE and pCEP/SPA4CT-LE V46F was described previously [13].

2.5. Radiosequencing

After SDS-PAGE, [^{3}H]phenylalanine-labeled proteins were radiosequenced as described before [26].

3. Results

The first SPA4CT protein described in the literature possessed the signal peptide of APP fused to the C-terminal 99 residues of APP (A4CT, C99) via the dipeptide leucine-glutamic acid (LE) (Fig. 2, [21]). Therefore, we here refer to this protein as SPA4CT-LE in order to distinguish it from the two mutant SPA4CT proteins termed SPA4CT-DIR and SPA4CT-DA (Fig. 2). Both mutants were generated in order to create a novel SPA4CT protein, which can serve as the precursor for Aβ with the authentic N-terminus of Aβ at amino acid Asp-1. For both mutated SPA4CT proteins the signal peptidase cleavage site was determined by radiosequencing. Furthermore, the processing of both proteins to $Aβ_{42}$ and $Aβ_{40}$ was analyzed and compared with SPA4CT-LE.

According to the rules for the substrate specificity of the eukaryotic signal peptidase (see Section 4), we modified the residues leucine and glutamic acid between the signal peptide of SPA4CT-LE and the A4CT sequence. The new construct SPA4CT-DIR (*dir*ect fusion of the signal peptide to the A4CT sequence) lacks the two amino acids leucine and glutamic acid, whereas SPA4CT-DA carries Asp and Ala instead of leucine and glutamic acid (Fig. 2).

The three constructs encoding SPA4CT-LE, DIR and DA were stably transfected into COS7 cells. Upon expression and membrane insertion the SPA4CT proteins are cleaved by signal peptidase and converted to A4CT. Immunoprecipitation of A4CT from the cell lysate of metabolically labeled COS7 cells shows that A4CT-DA has a slightly lower apparent molecular weight than A4CT-LE (Fig. 3A). In the lysate of vector transfected control cells, A4CT could not be detected (data not shown). For A4CT-DIR two bands representing A4CT were detected with apparent molecular weights of 11 and 11.5 kDa. The minor band (11.5 kDa) had the same molecular weight as A4CT-DA, whereas the major band (11

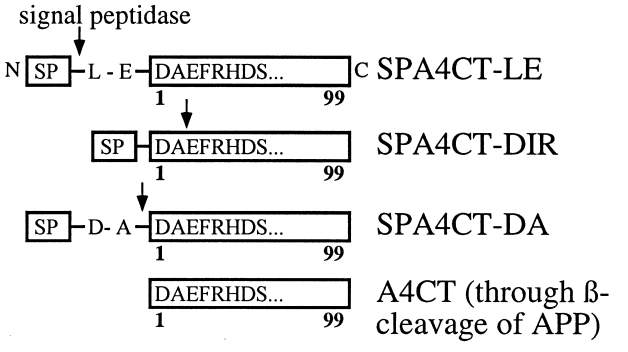

Fig. 2. Schematic representation of the SPA4CT proteins. SPA4CT-DIR consists of the signal peptide (SP) of APP directly fused to the C-terminal 99 residues of APP (A4CT). SPA4CT-LE contains a leucine (L) and a glutamic acid (E) between the signal peptide and the A4CT sequence, and SPA4CT-DA an aspartic acid (D) and an alanine (A). The amino acid sequences are shown in the usual one-letter code. N- and C-termini (N, C) of the proteins are indicated as well as the cleavage sites (arrow) of signal peptidase as determined by radiosequencing. The numbers indicate the position of the residues within the A4CT sequence.

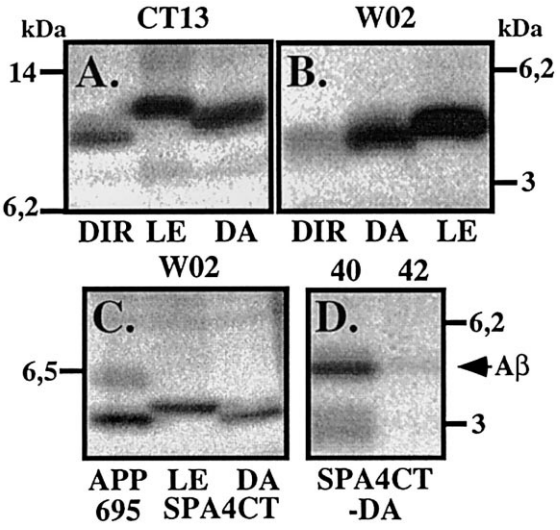

Fig. 3. Proteolytic processing of SPA4CT. A4CT and Aβ were immunoprecipitated from the cell lysate or the conditioned medium of COS7 cells stably expressing the indicated SPA4CT protein. The antibody used for the immunoprecipitation is given above each panel. A: A4CT in the cell lysate. B–D: Aβ in the conditioned medium. CT13: polyclonal antibody against the C-terminus of A4CT; W02, 40, 42: monoclonal antibodies specific for Aβ in general (W02), for the C-terminus of $Aβ_{40}$ (40) and the C-terminus of $Aβ_{42}$ (42). APP695: full-length APP with 695 residues. DIR, LE, DA: SPA4CT-DIR, LE, DA.

kDa) had a lower molecular weight than both A4CT-LE and A4CT-DA (Fig. 3A).

To determine the cleavage site of the signal peptidase, the different A4CT proteins were immunoprecipitated from the cell lysate of metabolically [^{3}H]phenylalanine-labeled cells and subsequently radiosequenced (Fig. 4). A4CT-DA has the radioactive phenylalanine in reaction cycle 4, showing that the signal peptide is cleaved together with the two additional residues Asp and Ala. Thus, A4CT-DA starts at the N-terminus with Asp-1 and, hence, is identical to A4CT derived from APP by β-secretase cleavage ([4] and references therein). In reaction cycle 6 of A4CT-DA, the radioactivity was still slightly above the background level, suggesting that a minor fraction of A4CT-DA starts at the N-terminus with the two additional residues Asp-2/Ala-1. SPA4CT-LE starts at the N-terminus with the two amino acids leucine and glutamic acid as reported previously [21]. For A4CT-DIR, the 11 kDa band was radiosequenced. The radioactive phenylalanine was detected in reaction cycle 2, revealing that the signal peptidase cleaved between residues Ala-2 and Glu-3 (Fig. 4). In our initial experiments with SPA4CT-DIR, the protein contained additionally the V46F mutation, which is linked to a familial form of Alzheimer's disease (for a review, see [5]). The radiosequencing described here was also carried out using the SPA4CT-DIR with the V46F mutation. Since this mutation is located in the transmembrane domain distant from the cleavage site of the signal peptidase, we assume that the products of SPA4CT-DIR and SPA4CT-DIR-V46F processing do not differ in their N-termini. In addition, both proteins have the same apparent molecular weight on an electrophoresis gel (data not shown).

The 11.5 kDa band of SPA4CT-DIR with the very low intensity was not radiosequenced, but might start at the N-terminus with Asp-1, as its apparent molecular weight is the same as that of A4CT-DA. This shows that in the case of SPA4CT-DIR two cleavage sites are possible for signal peptidase but that the one after Ala-2 is the predominant one.

A4CT-DA was processed to Aβ as shown in Fig. 3B. As expected, Aβ derived from A4CT-DA had the same electrophoretic mobility as Aβ derived from APP (Fig. 3C) and a slightly lower apparent molecular weight than the Aβ derived from A4CT-LE (Fig. 3B,C). This result is in good agreement with the lack of the two residues at the N-terminus of A4CT-DA compared with A4CT-LE. Equal amounts of A4CT-DA and A4CT-LE were processed to similar amounts of Aβ (data not shown). Moreover, as reported previously for Aβ derived from A4CT-LE [13], the Aβ derived from A4CT-DA consists of the C-terminally ragged peptides $Aβ_{42}$ and $Aβ_{40}$ as determined by immunoprecipitation using the $Aβ_{42}$ and $Aβ_{40}$ monoclonal antibodies G2-11 and G2-10, respectively (Fig. 3D). Antibody G2-10 also precipitated peptides with an apparent molecular weight of about 3 kDa, corresponding to the $p3_{40}$ and $p3.5_{40}$ peptides (Fig. 3D) which are N-terminally shortened compared with $Aβ_{40}$ and which are generated by the subsequent action of first α- and then γ-secretase [27]. The same p3 and p3.5 peptides are also generated from A4CT-LE as shown previously [13,14]. In several independent experiments, the relative $Aβ_{42}/Aβ_{40}$ ratio for A4CT-DA was found to be 5.0 ± 1.7%, which is in agreement with the corresponding ratios found for Aβ derived from A4CT-LE (4.7 ± 1.3%) and APP695 (4.7 ± 1.2%) [13]. These results show that A4CT-DA is processed by γ-secretase in the same manner as A4CT-LE and as A4CT derived from APP. But compared with A4CT-LE, A4CT-DA has the advantage of having the same N-terminus as A4CT derived from APP.

In contrast to A4CT-DA and A4CT-LE, only low amounts of Aβ were detected in the conditioned medium of COS7 cells expressing A4CT-DIR (Fig. 3B), clearly showing that A4CT-DIR was poorly processed by γ-secretase. Intracellular Aβ was not detected in the cell lysate (data not shown), ruling out the possibility that Aβ was generated but not secreted.

It is unclear why A4CT-DIR is only poorly processed to Aβ. To analyze whether A4CT-DIR was rapidly intracellularly degraded, e.g. due to misfolding of the protein, the half-life for the intracellular degradation of A4CT-DIR was determined in several pulse-chase experiments and compared with the half-life of A4CT-LE (experiment not shown). The

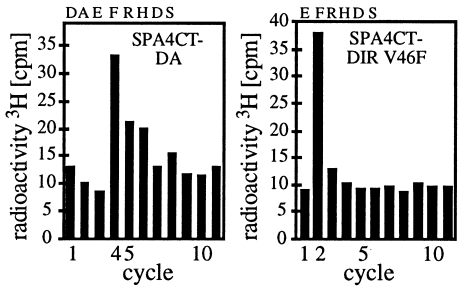

Fig. 4. Radiosequencing of the N-terminus of A4CT-DIR V46F and A4CT-DA. A4CT was immunoprecipitated from the cell lysate of [^{3}H]phenylalanine-labeled COS7 cells and radiosequenced. The radioactivity in each degradation step was determined and is indicated as counts per minute (cpm). The amino acids that correspond to each degradation step are shown in the usual one-letter code above the diagrams. A radioactive phenylalanine was found in degradation cycle 2 for SPA4CT-DIR V46F and cycle 4 for SPA4CT-DA.

half-life for both proteins was found to be 20 min (data not shown) showing that A4CT-DIR was not degraded more rapidly than A4CT-LE and that the different N-termini of both proteins did not affect their half-life. Therefore, the reduced processing of A4CT-DIR by γ-secretase was not due to a rapid intracellular degradation of A4CT-DIR. In the pulse-chase experiments described here, SPA4CT-DIR and SPA4CT-LE carried the V46F mutation, as for the radiosequencing described above. For the same reasons as mentioned above, we assume that the half-life of both proteins was not affected by this mutation.

4. Discussion

The proteolytic processing of APP by β-secretase occurs within the ectodomain of APP at a short distance from the transmembrane domain. This cleavage leads to the secretion of the large N-terminal ectodomain of APP (β-APPs) and to the generation of the C-terminal, membrane-bound fragment A4CT (C99), which is further cleaved by the protease activity called γ-secretase. γ-Secretase has not yet been identified, but recent studies have provided evidence that presenilin 1 is γ-secretase [15,16]. Presenilin 1 is a polytopic membrane protein that is required for γ-cleavage [28]. Furthermore, a mutational analysis suggested that presenilin 1 might be an aspartyl protease. However, the question remains open whether presenilin 1 directly cleaves A4CT or whether it contributes to γ-cleavage through a different mechanism, e.g. by being a cofactor of γ-secretase.

The γ cleavage is necessary to generate the C-terminus of the Aβ peptide. Both A4CT and Aβ have Asp-1 as the N-terminal amino acid when derived from APP.

In order to study γ-secretase directly and without any influence of the β-secretase activity, A4CT may be directly expressed in eukaryotic cells. To ensure the correct membrane insertion, the N-terminal signal peptide of APP with 17 amino acids was added to the A4CT cDNA construct, which was then cleaved by signal peptidase [10,13,20–22,25]. To simplify the cloning strategy, additional amino acid residues were used to link the signal peptide and A4CT: either the dipeptide LE (leucine and glutamic acid) (SPA4CT-LE [21,22]), which directly follows the signal peptide within the APP sequence, or only the single amino acid leucine (L) [10].

In each case the signal peptidase removes the 17 residue signal peptide, generating A4CT proteins with a modified N-terminus carrying the additional dipeptide LE or only the single amino acid L at their N-terminus. Although all these proteins were processed by γ-secretase to Aβ, the Aβ had also the N-terminal additional residues compared with the APP-derived Aβ.

We here describe a novel SPA4CT construct (SPA4CT-DA), the translation product of which is cleaved by signal peptidase at the authentic peptide bond (N-terminal to Asp-1 of A4CT), leading to the generation of an A4CT protein having the same N-terminus as A4CT derived from APP. According to the substrate specificity of the eukaryotic signal peptidase [29,30], we generated two new SPA4CT constructs with mutations at the signal peptidase cleavage site. In one construct (SPA4CT-DIR) the two residues leucine and glutamic acid between the signal peptide and A4CT were deleted (Fig. 2). In the other construct (SPA4CT-DA), both residues were replaced on the DNA level by Asp and Ala (Fig. 2).

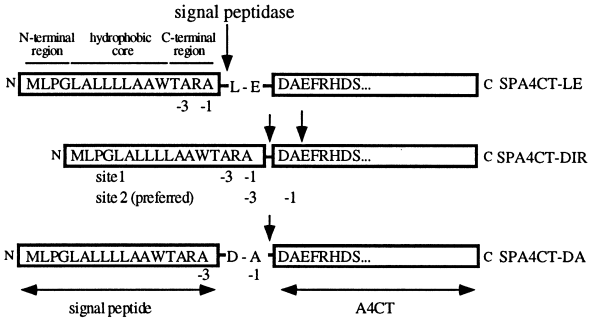

Fig. 5. Cleavage of SPA4CT-LE, DIR and DA by signal peptidase. The amino acid sequences are shown in the usual one-letter code. N- and C-termini (N, C) of the proteins are indicated. The amino acid positions −1 and −3 that determine the cleavage site (arrow) of signal peptidase are shown below the sequences. For details see the text.

Two rules have been established for the substrate specificity of the signal peptidase (for reviews see [29,30]). The −1/−3 rule requires small hydrophobic residues like alanine at positions −1 and −3 with respect to the cleavage site. The amino acid at position −2 is often a large or charged residue. Therefore, SPA4CT-LE may only be cleaved at a single peptide bond, leading to an A4CT protein that starts with the dipeptide Leu-Glu at the N-terminus (Fig. 5). SPA4CT-DIR may be cleaved at two different peptide bonds (Ala-1/Asp-1 and Ala-2/Glu-3) and SPA4CT-DA at three different peptide bonds (Ala-3/Asp-2, Ala-1/Asp-1, Ala-2/Glu-3). The second empirical rule predicts which of the different possible peptide bonds will mainly be cleaved. This is most often the peptide bond that is located at a distance of six residues C-terminal to the end of the hydrophobic core of the signal peptide. A typical signal peptide may be subdivided into three subdomains (Fig. 5): the N-terminal charged region, the central hydrophobic core and the C-terminal region [29–31]. Using this second rule, the preferred cleavage site within SPA4CT-DA is the peptide bond Ala-1/Asp-1, which leads to the generation of A4CT that has Asp-1 as the N-terminus in the same way as A4CT derived from APP. In fact, this is the most favored cleavage site used by signal peptidase as shown by radiosequencing. Only a minor amount of the protein has been cleaved at the peptide bond Ala-3/Asp-2. The preferred cleavage site for SPA4CT-DIR should be the peptide bond Ala-2/Glu-3, which we confirmed by radiosequencing this protein. Taken together, the cleavage sites that we found for the different SPA4CT proteins are in good agreement with the empirical rules known for the substrate specificity of the signal peptidase.

The γ-secretase cleavage sites of A4CT-DA are identical to the cleavage sites of A4CT-LE and A4CT derived from APP [13]. Neither the amount of Aβ derived from A4CT nor the 42/40 ratio of Aβ shows a difference between A4CT-LE and A4CT-DA. From this we conclude that the N-terminus of A4CT does not influence the γ cleavage. Similar results have been observed for the A4CT-derived protein p3CT (Fig. 1) that is still processed by γ-secretase, although lacking the N-terminal 16 amino acids of Aβ (for an overview see [5]).

In contrast to SPA4CT-LE and SPA4CT-DA, SPA4CT-DIR, which has Glu-3 as the N-terminal amino acid after signal peptidase cleavage, was only poorly processed by γ-secretase to Aβ. The reason for this unexpected finding is so

far not known. We considered whether these N-terminal residues (Asp-1 and Ala-2) are required for the binding of the A4CT protein to γ-secretase. However, this assumption seems very unlikely, as the N-terminal residues of A4CT are obviously not required for γ cleavage as described above for p3CT.

In COS7 cells, A4CT-DIR is as stable as A4CT-LE as shown by pulse-chase analysis. Thus, we can also exclude that A4CT-DIR is rapidly degraded before coming into contact with γ-secretase.

Another explanation why A4CT-DIR is processed to only low amounts of Aβ is that A4CT-DIR is mistransported. A4CT-DIR could be transported differently compared with A4CT-LE and A4CT-DA so that A4CT-DIR might not reach the compartment in which the γ-secretase activity is located. This would imply that residues Asp-1 and Ala-2 that are lacking in the A4CT-DIR protein are important for the correct transport of newly synthesized A4CT. So far we have no experimental evidence for this, but it would be in agreement with a recent study [25] which analyzed the transport of wild-type and mutated APP proteins in primary rat hippocampal neurons. That study showed that APP was mainly sorted to axons, whereas A4CT-DIR was mainly sorted to the somato-dendritic compartments. Moreover, C111, which is 12 residues longer at the N-terminus than A4CT, was transported to axons as well as to the somato-dendritic compartments [25]. Thus, at least in primary rat hippocampal neurons, the N-terminus of A4CT has an important influence on the intracellular transport of A4CT.

In summary, we have created a novel and preferred substrate for the analysis of Alzheimer's disease γ-secretase. This SPA4CT-DA construct is the only one among several existing SPA4CT constructs, which is cleaved by signal peptidase at the correct position, so that the generated A4CT has the same N-terminus as A4CT, which is produced by β-secretase cleavage of APP. Not only the A4CT -protein, but also the Aβ derived from SPA4CT-DA is N-terminally truncated by two residues compared with the Aβ derived from SPA4CT-LE. Previously, it has been shown in vitro that Aβ peptides which are shorter at their N-terminus aggregate more rapidly [32]. Therefore, we expect that Aβ lacking the N-terminal dipeptide Leu-Glu aggregates faster than Aβ with the additional dipeptide at its N-terminus. Since the rapid aggregation of the Aβ peptides seems to be a determining factor for the successful generation of transgenic animals mimicking the pathologic lesions of Alzheimer's disease [33], SPA4CT-DA might be more valuable than SPA4CT-LE for generating such animals which may assist in the development of therapeutic strategies for Alzheimer's disease.

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