Systemic Catabolism of Alzheimer’s Aβ40 and Aβ42*

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Jorge Ghiso‡§¶, Marcos Shayo‡, Miguel Calero‡, Douglas Ng‡, Yasushi Tomidokoro‡,
Samuel Gandy**, Agueda Restagno‡, and Blas Frangione¶‡¶

From the Departments of Pathology and Psychiatry, New York University School of Medicine, New York, New York 10016, the Departamento de Microbiologia, Instituto de Salud Carlos III, Madrid 28220, Spain, and the Department of Neurology, Thomas Jefferson University, Philadelphia, Pennsylvania 19107

To better understand the physiologic excretion and/or catabolism of circulating peripheral amyloid β (Aβ), we labeled human Aβ40 (monomeric, with predominant unordered structure) and Aβ42 (mixture of monomers and oligomers in ~50:50 ratio, rich in β-sheet conformation) with either Na125I or 125I-tyramine cellobiose, also known as the cell-trapping ligand procedure, testing their blood clearance and organ uptake in B6SJLF1/J mice. Irrespective of the labeling protocol, the peptide conformation, and the degree of oligomerization, both Aβ40 and Aβ42 showed a short half-life of 2.5–3.0 min. The liver was the major organ responsible for plasma clearance, accounting for >60% of the peptide uptake, followed by the kidney. In vivo, hepatocytes captured >90% of the radiolabeled peptides which, after endocytosis, were preferentially catabolized and excreted into the bile. Biliary excretion of intact as well as partially degraded Aβ species became obviously relevant at doses above 10 μg. The use of biotin-labeled Aβ allowed the visualization of the interaction with HepG2 cells in culture, whereas competitive inhibition experiments with unlabeled Aβ demonstrated the specificity of the binding. The capability of the liver to uptake, catabolize, and excrete large doses of Aβ, several orders of magnitude above its physiologic concentration, may explain not only the femtomolar plasma levels of Aβ but the little fluctuation observed with age and disease stages.

Alzheimer’s disease (AD) is the most frequent type of amyloidosis in humans and the commonest form of clinical dementia. Extracellular Aβ amyloid deposits in the form of amyloid plaques and cerebral amyloid angiopathy as well as intraneuronal neurofibrillary tangles co-exist in the brain parenchyma, being the cognitive areas the most severely affected. Aβ, a 39–42-amino acid-long peptide of unknown biological function, is an internal processing product of a larger type I transmembrane precursor molecule, APP, codified by a single multiexonic gene located on chromosome 21 (reviewed in Ref. 1). A soluble form of Aβ (sAβ) is present in the biological fluids of both normal individuals and AD patients as well as in cytosolic soluble fractions of normal, AD, and Down’s syndrome brain homogenates (2–6). Notably, an increased amount of sAβ has been reported in AD and Down’s syndrome brain tissue in comparison to control individuals (7). Although the primary structures of deposited Aβ and sAβ are indistinguishable, the circulating peptide is predominantly 40 residues long, whereas sAβ42, the major species in parenchymal deposits, is only a minor component of the circulating pool. To the present, it is not clear whether circulating sAβ reflects systemic production, brain clearance, or both. The blood-brain barrier has the capability to modulate sAβ brain uptake and clearance by controlling the uptake of circulating sAβ, either in its free form or bound to its transport apolipoproteins, as well as the elimination of brain-derived Aβ via transport-mediated clearance mechanisms (reviewed in Ref. 8). Experimentally determined transport rates indicate that the receptor for advance glycation end products mediates the influx of free Aβ into the brain (9), whereas low density lipoprotein receptor-related protein 2 (LRP-2, also known as gp330 or megalin) is the receptor involved in the uptake of Aβ-apoE complexes (10). In contrast, brain clearance of Aβ at the blood-brain barrier occurs through different receptors, it is largely mediated by LRP-1 and modulated by the LRP-1 ligands apoE and α2-macroglobulin (11). Different proteases (i.e. nephrilysin, endothelin-converting enzyme, angiotensin-converting enzyme, plasmin, and insulin-degrading enzyme) have been implicated in proteolysis-related clearance of Aβ from the central nervous system (12–15), although their final contribution to the mechanisms of Aβ homeostasis still remains unclear.

The systemic, physiologic sAβ excretion/catabolism mechanisms are poorly understood. Clearance experiments in rats showed that after infusion of radiolabeled Aβ peptides into the lateral ventricle, 40% of the injected radioactivity was present in the blood and urine and taken up by the liver and the kidneys in as little as 3.5 min, indicating not only a fast clearance mechanism but also the involvement of systemic organs in the excretion and/or catabolism (16). The high urinary levels of radioactive tracer compared with those in plasma suggested a key role for the renal clearance. In contrast, immunoprecipitation and mass spectrometry analysis of urine from normal individuals demonstrated the presence of intact sAβ40 at very low levels, accounting for only a minute fraction of the total circulating pool (17), thereby suggesting a different metabolic/excretory pathway.

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† To whom correspondence should be addressed: Dept. of Pathology, New York University School of Medicine, 550 First Ave., TH-432, New York, NY 10016. Tel.: 212-263-7997; Fax: 212-263-6752; E-mail: ghiso01@popmail.med.nyu.edu.

‡ The abbreviations used are: AD, Alzheimer’s disease; Aβ, amyloid β; sAβ, soluble form of Aβ; APP, amyloid precursor protein; TC, tyramine-cellobiose; LRP-1, low density lipoprotein receptor-related protein-1; LRP-2, low density lipoprotein receptor-related protein-2; TTR, transthyretin; SAP, serum amyloid P component; HPLC, high performance liquid chromatography; RP-HPLC, reverse phase-HPLC; MALDI-TOF, matrix-assisted laser desorption ionization-time-of-flight mass spectrometry; CRP, C-reactive protein; SEC-R, serpin-enzyme complex receptor; MeSO2, dimethyl sulfoxide; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; CAPS, 3-(cyclohexylamino)propanesulfonic acid; TES, 2-[2-hydroxy-1-bis(hydroxymethyl)ethyl]aminoethanesulfonic acid; MS, mass spectrometry; PBS, phosphate-buffered saline.

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We investigated the excretion/catabolism of human Aβ40 and Aβ42 species in mice using both iodinated peptides and peptides labeled with an intracellularly trapped ligand procedure based on the use of 125I-t-tyramine-cellobiose (TC), a sugar adduct that is not degraded by mammalian cells and therefore accumulates in the organs involved in the uptake (18). This procedure has been widely used to determine sites of catabolism of many proteins, including some related to amyloid diseases, i.e., transthyretin (TTR) (19) or serum amyloid P component (SAP). Our results indicate that sAβ peptides have a short life in circulation, the liver being the major organ and the hepatocytes the main cell type responsible for their uptake and degradation/excretion. The findings are confirmed in vitro by using hepatocytes in culture and validated in vivo, analyzing Aβ degradation species in the bile of mice injected with human Aβ.

**EXPERIMENTAL PROCEDURES**

**Peptides**

Aβ(1–40) (DAEFRHDSGYEVHHQKLVFFAEDVGSNKGIICILMLWYGVV) and Aβ(1–42) (DAEFRHDSGYEVHHQKLVFFAEDVGSNKGIICILMGLVWYVGILVI) were homogenized to residues 672–711 and 672–713, respectively, of human Aβ precursor protein APP770 as well as a derivative of Aβ(1–40) bearing a single biotin molecule at the N-terminal asparagine residue, were all synthesized at the W. M. Keck Facility at Yale University using N-term-biotinylxycarbonyl chemistry, purified by reverse phase-high performance liquid chromatography (RP-HPLC), their molecular masses corroborated by matrix-assisted laser desorption/ionization mass spectrometry and structurally characterized via size exclusion chromatography using a Superdex 75 10/300 GL column with experimental molecular masses of 4329.1 Da for Aβ40 (expected, 4329.9 Da) and 4513.6 Da for Aβ42 (expected, 4514.1 Da). Peptides KCNTATCQRLAQFLVHSSNGAILSTVQNSTY (37 residues, homologous to human amylin) and KNQCNQNYTPYRKGMQMN (20 amino acids, homologous to the C terminus of APP) were used as unrelated controls for specificity.

Peptides were initially dissolved in either Me2SO or distilled deionized water. The peptides were briefly sonicated, diluted to their final concentration in 50 mM Tris-HCl, 150 mM NaCl, pH 7.4 (TBS), centrifuged at 14,000 × g for 10 min, sterilized using a 0.22-μm pore-size filter (Millex GP; Millipore Corp., Bedford, MA), and either immediately used or stored at −80 °C. If Me2SO was used for solubilization, its final concentration in the sample never represented >1% of the total volume.

**Characterization of Aβ Peptides**

Peptides were analyzed by amino acid sequence and MALDI-TOF mass spectrometry and structurally characterized via size exclusion chromatography and CD spectroscopy. The integrity of their biological activity was assessed through their binding interaction with apolipoprotein J (apoJ).

The degree of oligomerization of the various peptides was measured by size exclusion chromatography using a Superdex 75 10/300 GL column (Amerham Biosciences) equilibrated in TBS, pH 7.4, at a flow rate of 0.5 ml/min. The eluent was monitored by absorbance at 220 nm, and fractions were collected accordingly. Aliquots of 200 ng were immediately lyophilized, re-dissolved in sample buffer, and separated by size exclusion chromatography using a Superdex 75 10/300 GL column with experimental molecular masses of 4329.1 Da for Aβ40 (expected, 4329.9 Da) and 4513.6 Da for Aβ42 (expected, 4514.1 Da). Peptides KCNTATCQRLAQFLVHSSNGAILSTVQNSTY (37 residues, homologous to human amylin) and KNQCNQNYTPYRKGMQMN (20 amino acids, homologous to the C terminus of APP) were used as unrelated controls for specificity.

**Radiolabeling and Characterization of Aβ-labeled Species**

To assess iodine incorporation in Aβ peptides, Michael addition to the terminal Cys residues of nonoxidized, Na[125I] labeled Aβ peptides was used for the uptake experiments. The use of lactoperoxidase consistently rendered lower amounts of oxidizable iodinated peptides while maintaining specific activities at similar levels as IODO-Beads that, in turn, translated in a better yield of nonoxidized, radioiodinated peptides. CD spectroscopy and binding to apoJ were used to demonstrate the lack of structural and functional changes in the Aβ peptides following iodination. The degree of oligomerization of radiolabeled peptides was assessed by Western blot as described above.

Two proteins of different molecular masses and well known half-lives in rodents, human IgG (EMD Biosciences, San Diego, CA) and SAP (Sigma), were also radioiodinated as above and used as controls for the pharmacokinetic parameters described below. Their specific activities were in the range of 50–70 Ci/μg with >95% trichloroacetic acid precipitability in both cases.

Identification of Targeted Amino Acid Residues in the Iodination Procedure—To identify the iodinated amino acid residues, Aβ(1–40) and Aβ(1–42) were labeled with Na[125I] or Na[127I]. The nonoxidized species separated by RP-HPLC identically as above, the resulting peaks subjected to separate proteolytic degradations with three different enzymes (trypsin, endoprotease Gla-N, and endoprotease Glu-C), and the subsequent proteolytic fragments analyzed by MALDI-TOF mass spectrometry to assess iodine incorporation. Each of the HPLC-purified, nonoxidized, Na[127I]-labeled Aβ40 peaks (2–5 μg) was dissolved in 5 ml of distilled water, with equal volumes of either 100 mM Tris-HCl buffer, pH 8, 100 mM phosphate buffer, pH 8, or 100 mM ammonium carbonate buffer, pH 8, and incubated for 3 h at 37 °C with either modified trypsin (Roche Applied Science; 1.25 w/w), endoprotease Asp-N (Roche Applied Science; 1.5 w/w), or endoprotease Glu-C (Roche Applied Science; 1.25 w/w), respectively. Digests were desalted using Zip-Tip C18 micro RP column (Millipore) as recommended by the manufacturer, utilizing 90% acetonitrile, 0.1% trifluoroacetic acid (v/v) for elution prior to MS analysis. To assess iodine incorporation in Aβ42 peptides, identical procedures were followed with the only difference that because RP-HPLC separation of iodinated and nonoxidized species did not result in well-defi-
Tyramine-Cellulose-conjugated Aβ Peptides

The labeled 125I-TC adduct was prepared as described before (18) by scaling down the reagents 50 times. In brief, 240 μmol each of cellulose, tyramine, and NaBH₄-CN were allowed to react for 6 days at room temperature in 0.2 m sodium phosphate buffer, pH 7.5. Following adjustment of the pH to 5.5, the reaction products were separated on a cation exchange column (0.6 × 18 cm AG-50W, Bio-Rad), and the TC adducts were eluted with 0.5 M NH₄OH. The adduct, further purified in a silicic acid column (0.7 × 26 cm), was eluted with butanol/acetic acid/water (7:1:2) after free tyramine at the end of the column. The purified TC was freeze-dried and stored at −20 °C until used. Approximately 10 nmol (~4.8 μg) were radioiodinated with Na125I (3 mCi) for 30 min at room temperature in a N-chloroformazine sulfonamide-coated tube (10 μg; IODO-GEN; Pierce), and the reaction was stopped by transferring the solution to a tube containing sodium metabisulfite and sodium iodide. After labeling, the 125I-TC adducts were eluted with cyanuric chloride in acetone (1.8 g/liter) and sodium hydroxide (5 μl of 4 mM) for 20 s, and the reaction was quenched by the addition of acetic acid (3 μl of 10% acetic acid). The activated 125I-TC adduct was immediately bound to the corresponding Aβ peptides by addition to 50 μg of each of the peptides (1 mg/ml in 0.1 M phosphate buffer, pH 7.5) and incubated for 1 h at room temperature. In the resulting 125I-labeled peptides were separated by RP-HPLC in a Vydac C4 column using the same linear gradients as above. Specific activities of the labeled TC peptides after purification (typically 20 μCi/μg) were calculated from the integration of the peak areas obtained at 220 nm, and the radioactivity counts were obtained from aliquots of the respective pools.

Plasma Turnover and Organ Distribution

Experiments were performed on 8–12-week-old male B6SLIF1/J mice, (Jackson Laboratories, Bar Harbor, ME) following approval by the Institutional Animal Care and Use Committee at New York University. Typically, groups of 6–8 animals were anesthetized with Isoflurane, and the left jugular vein was isolated. Each group received a bolus intravenous injection of 2 × 10⁷ cpm of either 125I-Aβ40, 125I-Aβ42, 125I-TC Aβ40, or 125I-TC Aβ42 peptides in 200 μl of lactate Ringer’s solution (106 mm NaCl, 1.5 mm CaCl₂, 24 mm sodium lactate, 4 mm potassium lactate, HCl containing 1% ovalbumin). Blood samples were collected from the tail vein at 0.5, 1.5, 5, 15, 30, 60, and 120 min using heparinized capillary tubes (Fisher). To assess the integrity of the peptides, a plasma aliquot at each time point was cold-precipitated with 12% trichloroacetic acid in lactate Ringer’s containing 1% bovine serum albumin. Counts were assessed in a scintillation counter (LS6500; Beckman Instruments, Fullerton, CA), and the total as well as trichloroacetic acid-precipitable counts/min were recorded. Results were expressed as remaining counts/min (percentage of initial counts/min) at each bleed time, with the 0.5-min time point considered as 100%. The half-lives of the radiolabeled peptides were calculated from the clearance curves generated by using a standard exponential decay equation in GraphPad Prism, and pharmacokinetic parameters for the IgG and SAP standard proteins were estimated by using noncompartmental pharmacokinetics data analysis from PK Solutions Software (Montrose, CO) following a bi-exponential equation.

To assess organ distribution, groups of 6–8 animals received an intravenous injection of 2 × 10⁶ cpm of either 125I-Aβ40, 125I-Aβ42, 125I-TC Aβ40, or 125I-TC Aβ42, as above, and were sacrificed by decapitation at 120 min. All organs, carcass, urine, feces, and bile were collected and weighed; the radioactivity was assayed, and the resulting counts/min were normalized per g of wet tissue.

Separation of Hepatic Cell Types

Parenchymal and nonparenchymal liver cells were isolated according to well-established protocols (21, 22). Briefly isoflurane-anesthetized mice were intravenously bolus-injected with 2 × 10⁶ cpm of either 125I-Aβ40, 125I-Aβ42, 125I-TC Aβ40 or 125I-TC Aβ42 in 200 μl of lactate Ringer’s containing 1% ovalbumin for 1 h at room temperature. The labeled liver was removed and an intravenous catheter (Angiocath, BD Biosciences) inserted in the portal vein. The iliumlumbar vein was then sectioned, and the liver was perfused at 37 °C with perfusion buffer (142 mM NaCl, 6.7 mM KCl, 10 mm HEPES, 6 mM NaOH, pH 7.4) at a flow rate of 2 ml/min until the organ was blanched (typically, about 10 min). The liver was subsequently removed from the carcass and further perfused at 37 °C for another 10 min with 0.05% w/v collagenase (type I, Sigma) in 4.8 mM CaCl₂, 68.4 mM NaCl, 6.7 mM KCl, 100 mM HEPES, 66 mM NaOH, pH 7.4. After excision, the liver was placed in 20 ml of ice-cold suspension buffer (68.4 mM NaCl, 5.3 mM KCl, 1.1 mM KH₂PO₄, 0.7 mM NaSO₄, 30.2 mM HEPES, 3.0 mM TES, 36.3 mM Tricine, 0.2 mM NaOH, 1.2 mM CaCl₂, and 0.6 mM MgCl₂, pH 7.4) and gently raked to release the cells. The resulting cell suspension was filtered through a 100-μm nylon mesh to remove connective tissue debris and cell clumps, incubated on a tilting platform at 37 °C for 30 min, cooled in an ice-water bath, and centrifuged at 20 × g (Beckman J-6B) for 2 min at 4 °C. The resulting supernatant was used for further isolation of nonparenchymal cells, which were enriched for hepatocytes, was resuspended in ice-cold wash buffer (perfusion buffer supplemented with 1.2 mM CaCl₂) and further depleted in nonparenchymal cells by four subsequent cycles of low speed centrifugation steps, as above. For the isolation of nonparenchymal cells, all supernatants were pooled, centrifuged at 500 × g for 7 min at 4 °C, resuspended in 2 ml of modified Eagle’s medium (Sigma), overlayed on top of a discontinuous 6, 12, 15% arabinogalactan (Sigma) gradient, and centrifuged at 25 °C for 25 min at 49,000 × g in a Beckman XL ultracentrifuge using a SW-40 rotor (Beckman Coulter). Distribution of the radioactive tracer was assessed in each fraction, and cell morphology was evaluated after cytospin centrifugation (Shandon Cytospin Cytocentrifuge, Thermo Electron Co., Woburn, MA) by Sudan IV and Harris hematoxylin stainings.

HepG2 Binding Experiments

Human hepatoma HepG2 cells (ATCC, Manassas, VA) were cultured in growth media (Eagle’s minimum essential medium with 2 mM l-glutamine and Earle’s balanced salt solution adjusted to pH 7.4). All cells were subcultured at a ratio 1:4 with medium replacement every 3 days. For binding experiments, cells were seeded onto Lab-Tek 4-well glass chamber slides (Nalge Nunc International, Naperville, IL) at a concentration of 7.5 × 10⁵ cells in 0.5 ml of growth media per chamber and cultured for 48 h until 60–70% confluence. Cells were washed three times with ice-cold Dulbecco’s PBS and maintained on ice during the subsequent experiment. Biotin-labeled Aβ40 was dissolved in distilled deionized water, briefly sonicated, centrifuged at 14,000 × g for 10 min, sterilized using a 0.22-μm pore filter, diluted to 10 μM in culture medium, and allowed to interact with the HepG2 cells for 2 h at 4 °C. After incubation, cells were washed three times with ice-cold PBS and fixed for 10 min with 4% paraformaldehyde in PBS before incubation with fluorescein-conjugated streptavidin (BioSource; 1:500) for 30 min. Following three PBS washes, nuclear DNA was counterstained with 4′,6-diamidino-2-phenylindole (0.5 μg/ml in Vectashield, Vector Laboratories Inc., Burlingame, CA). Samples were visualized in an Olympus BX51 epifluorescence microscope, and images were acquired and analyzed with the aid of a digital imaging analyzer using Cytovision software version 2.7 (Applied Imaging Co., Santa Clara, CA). The specificity of HepG2-Aβ40 interaction was assessed by competitive inhibition of biotin-labeled Aβ40 binding by 10-fold molar excess of either unlabeled Aβ40 or unrelated peptides amylin and APP (751–770).

Analysis of Aβ in Bile and Urine of Injected Mice and in HepG2 Conditioned Media following Aβ Uptake

Preparation of Anti-Aβ-coated Paramagnetic Beads—Fifty microliters of goat anti-mouse IgG-coated paramagnetic beads (Dynabeads M-280; Dynal Biotech, Lake Success, NY) were allowed to interact with 3 μg each of anti-Aβ mouse monoclonal antibodies 4G8 and 6E10 (recognizing segments 1–16 and 17–24 of Aβ, respectively) for 2 h at room temperature followed by 16 h at 4 °C under constant end-to-end rotation, and subsequently were washed/blocked with PBS containing 0.1% bovine serum albumin.

Aβ Immunoprecipitation in Bile and Urine Samples—Increasing amounts of nonradioabeled human Aβ40 (0, 1, 10, or 100 μg) in 200 μl of lactate Ringer’s solution were bolus-injected intravenously into different sets of mice (n = 2 for 0 μg, n = 8 for 1 μg, n = 7 for 10 μg, and n = 5 for 100 μg injection). The gallbladders and urinary bladders were removed 5, 15, or 30 min after peptide injection. Following washing out of blood, bile and urine were collected (average total volumes: 10 and 490 μl, respectively) and further incubated overnight at 4 °C with anti-Aβ-coated beads resuspended in 1 ml of RIPA buffer (50 mM Tris, pH 8.0, containing 150 mM NaCl, 1% Nonidet P-40 (Sigma), 0.1% sodium deoxycholate (Sigma), 0.1% SDS, 2 mM EDTA, and complete protease inhibitor (Roche Applied Sci-
RESULTS

Size exclusion chromatography immediately after solubilization showed that synthetic Aβ40 was predominantly monomeric. As indicated in Fig. 1A, the bulk of the material (>98%) eluted at 15 min from a Superdex 75 column (fraction 40), 1 min after the elution of cytochrome c (molecular mass 12,384 Da), the lowest molecular mass standard employed in the column calibration. As expected, fraction 40, migrated as a single band of ~4 kDa in SDS-PAGE (Fig. 1A, inset). Aβ40 exhibited a predominantly unordered structure, as indicated by the typical random coil CD profile with a minimum at 198 nm (Fig. 1B). The binding of Aβ40 to apoJ, a useful parameter to evaluate structure stability, displayed a K_d of 1.95 nM (Fig. 1C), well within the values described previously (23).

Freshly solubilized Aβ42, contrary to Aβ40, eluted from the Superdex 75 column in two well defined peaks at 8 and 15 min, with the front peak (fraction 42A) accountable for almost half of the total peptide loaded onto the column. Fraction 42A eluted with the same retention time as its Aβ40 counterpart (Fig. 1A). On SDS-PAGE, fraction 42A resolved as a mixture of monomeric, dimeric, tetrameric, and oligomeric components, whereas fraction 42B, in agreement with its retention time, was composed only of Aβ42 monomers (Fig. 1A, inset). Aβ42 had a predominant β-structure, as indicated by the classical CD profile showing a minimum at 218 nm (Fig. 1B), and interacted with apoJ with high affinity (K_d = 2.44 nM), as reported previously (23).

To estimate half-life in circulation and organ uptake, Aβ40 and Aβ42 were initially labeled with Na[125I]. Because this procedure usually results in concomitant and undesirable peptide oxidati-
Radioiodination of Aβ40 and Aβ42. Ten micrograms of the Aβ peptides were labeled with 2 mCi of Na[125I] (Amersham Biosciences) using lactoperoxidase and standard protocols. A, labeled Aβ40 was separated in a C4 narrowbore column (Vydac) using a 30-min 5–40% linear gradient of acetonitrile in 0.05% trifluoroacetic acid and a flow rate of 200 μL/min. Radioactivity was recovered in the fractions highlighted with a star. The identity of the peaks was assessed via mass spectrometry analysis of identical HPLC peaks obtained in parallel employing Na[127I] instead of Na[125I], under the same experimental conditions. The first three peaks contained predominately located in the tail of the peaks (asterisk); the tail of peak B (arrow) was used for the characterization. Peaks 5 and 6, which typically rendered a 6-peak identical pattern as shown in Fig. 2, were used. The specific activity was estimated based on the area occupied by the radioactive fractions (inset). Continuous line, nonlabeled peptides; broken line, labeled peptides.

For Aβ42, the separation of the different species was not as defined as for Aβ40. Although the two main groups (oxidized and nonoxidized) were well discriminated (Fig. 2, insets), the tail of peak B (arrow) was used for the in vivo experiments. A and B, insets, Western blot analysis of nonoxidized [127I]-Aβ40 and [127I]-Aβ42 using a combination of 6E10 and 4G8 antibodies as described under “Experimental Procedures” and Fig. 1. Circular dichroism spectroscopy (C) and binding interaction with apoJ (D) were used to demonstrate the lack of structural and functional changes in the Aβ peptides following iodination. Continuous line, nonlabeled peptides; broken line, labeled peptides.
Aβ Turnover and Tissue Uptake

Fig. 3. Identification of targeted amino acid residues in the iodination procedure. Aβ40 was labeled with Na[127I]; nonoxidized species were separated by RP-HPLC, and the resulting peaks (denoted as peaks 4–6 in Fig. 2A) were subjected to separate proteolytic degradations with modified trypsin, endoproteinase Asp-N, and endoproteinase Glu-C. The subsequent proteolytic fragments were analyzed by MALDI-TOF-mass spectrometry to assess iodine incorporation, as described under “Experimental Procedures.” Top panel shows the amino acid sequence of Aβ40 and the cleavage sites of the different enzymes employed. The four residues amenable for iodination (His-6, Tyr-10, His-13, and His-14) are indicated in boldface. The table illustrates the peptides containing the potential iodination sites originated by proteolysis of the nonoxidized peaks 4–6 with the different enzymes. Theoretical and experimental molecular masses are shown. Deviations from the theoretical m/z values are shown in parentheses.

| Enzyme | Proteolytic peptide | m/z (Theoretical) | Peak 4 | Peak 5 | Peak 6 |
|--------|---------------------|------------------|--------|--------|--------|
| Trypsin | HDSGVEVHQQK | 1337.4 | 1337.5 | 1463.8 (+126.4) | 1589.9 (+252.5) |
| Asp-N | DAEFRH | 775.8 | 775.3 | 775.1 | 775.9 |
|         | DSGYEVHQQKLVFAE | 1917.1 | 1917.3 | 2043.4 (+126.1) | 2169.9 (+252.8) |
| Glu-C  | FrHDSGYE | 1011.0 | 1011.5 | 1137.3 (+126.3) | 1264.0 (+253.0) |
|         | VHHQKLVFAE | 1355.6 | 1355.7 | 1355.4 | 1355.5 |

The subsequent CD spectra were observed for Aβ40, 125I-Aβ40, and 127I-Aβ42. The four residues amenable for iodination (His-6, Tyr-10, His-13, and His-14) are indicated in boldface. The table illustrates the peptides containing the potential iodination sites originated by proteolysis of the nonoxidized peaks 4–6 with the different enzymes. Theoretical and experimental molecular masses are shown. Deviations from the theoretical m/z values are shown in parentheses.

Radioiodinated Aβ40 and Aβ42 were bolus-injected into the bloodstream of the mice, and blood samples were collected at various time points used to calculate the half-life of the peptides, whereas harvest of the various organs at the end of each experiment and assessment of the incorporated radioactivity provided information about organ uptake. Fig. 4A illustrates the rate of blood removal for 125I-Aβ40 and 125I-Aβ42 corrected for trichloroacetic acid precipitation at each time point. The corresponding half-lives, calculated via standard exponential decay equations, were 2.4 min for 125I-Aβ40 and 2.8 min for 125I-Aβ42. As controls, parallel experiments were carried out under identical conditions using molecules with known half-life, i.e. monomeric SAP and IgG with half-lives in mice of 7.0–8.25 (24) and 17–28 h (25), respectively. The experimental values, 7.2 h for SAP and 19 h for IgG were well within the range of published values, thereby validating the results obtained for the Aβ peptides.

Organ uptake of 125I-labeled peptides was evaluated 120 min after the intravenous bolus injection. As indicated in Fig. 4B, ~30% of the injected radioactivity was distributed mostly in the liver, kidney, stomach, and intestine, whereas the rest was located mainly in the head and carcass. The values were similar for 125I-Aβ40 and for 125I-Aβ42; the stomach was the only organ where the capture of 125I-Aβ42 seemed to be larger than 125I-Aβ40. Most interestingly, trichloroacetic acid experiments with plasma samples obtained at the time of organ harvest (2 h after the injection of the peptides) indicated that only 23 ± 3% of the total radioactivity was trichloroacetic acid-precipitable. This decrease did not originate from enzymatic degradation of Aβ peptides within the blood compartment because 125I-Aβ species incubated in vitro with fresh mouse plasma for up to...
125I-Aβ peptides. Groups of 8–12-week-old B6SJLF1/Mice were anesthetized with isofluorane and intravenous bolus-injected with 2 × 10^7 cpm of the radiolabeled Aβ40 and Aβ42 peptides. A, at different time point intervals (0.5, 1.5, 5, 15, 30, 60, and 120 min), blood samples were drawn from the tail by using heparinized capillary tubes, and plasma radioactivity was assessed. Acid-soluble radioactivity, associated with the presence of Aβ degradation fragments, was assessed by trichloroacetic acid precipitation, as described under “Experimental Procedures.” The half-lives for the 125I-Aβ40 and 125I-Aβ42 peptides were calculated with GraphPad Prism based on the clearance curves generated by using standard exponential decay equations. Results represent percentage of trichloroacetic acid-precipitable counts/min compared with the 0.5-min value considered as 100%. Each point represents the mean ± S.D. of 6–8 animals. B, at the end of the experiment (120 min), animals were euthanized, and the different organs were collected and weighed, and radioactivity was determined as above. Values are expressed in % of total radioactivity.

120 min at 37 °C resulted in no significant changes in the amount of acid-soluble material, suggesting that the radiolabeled material detected in blood 2 h after injection most likely reflected the presence of both intact protein and labeled breakdown products that had been released from the site(s) of uptake and catabolism and had not yet been excreted.

To corroborate the organ uptake data and circumvent the problem of peptide processing and degradation, we performed parallel experiments using Aβ peptides coupled to 125I-TC. The TC adduct, synthesized as described under “Experimental Procedures,” was labeled with Na[125I] before being reacted with the Aβ primary amines. Although no oxidation derivatives were produced by this procedure, the final labeled peptide eluted from the HPLC as a single broad peak (not shown), indicating that the final Aβ 125I-TC product was a heterogeneous mixture of labeled peptides, with TC moieties likely attached to one or more of the available primary amines at positions 1, 17, and 28. Despite this heterogeneity, blood clearance curves obtained for 125I-TC Aβ40 and 125I-TC Aβ42 (Fig. 4) were almost identical to those shown in Fig. 4A for 125I-Aβ species, and the calculated half-life values of 2.6 min for 125I-TC Aβ40 and 3.0 min for 125I-TC Aβ42 were in excellent agreement with those values obtained for 125I-Aβ40 and 125I-Aβ42. The similarity of the curves as well as the calculated half-life values indicated that both Aβ40 and Aβ42 were cleared from blood with similar kinetics, and the coupling of 125I-TC to the peptides did not significantly alter their plasma clearance.

As in the previous case of the 125I-Aβ species, organ uptake of 125I-TC-labeled peptides was evaluated 120 min after the intravenous bolus injection. As indicated in Fig. 5B, most of the remaining radioactivity (~65% of the injected values) was located in the liver. The kidney and small intestine each accounted for less than 10%, and only traces of radioactivity were located in other viscera. Most interestingly, plasma samples tested for the trichloroacetic acid precipitability of the peptides bearing the TC moiety after the 2-h experiments showed trichloroacetic acid-precipitable values in the range of 76 ± 6% indicating that, in contrast to the case of 125I-Aβ, the 125I-TC-labeled peptides remaining in circulation had not been significantly degraded. Normalization of the TC data according to
the organ weight (expressed as counts/min/g of tissue) revealed significant amounts of labeling also captured in the gallbladder, spleen, and small intestine as well as excreted to the urine, whereas the A\textsubscript{40}/H9252 retention in the carcass lost significance (not shown). Taken together, the data indicate a preponderant hepatic uptake and excretion to the bowel, most likely via the biliary ducts.

The identity of the main cells responsible for the liver uptake was assessed by isolation of hepatic parenchymal and nonparenchymal cells 10 min after intravenous bolus injection of either \textsuperscript{125}I-TC-A\textsubscript{40} or \textsuperscript{125}I-TC-A\textsubscript{42}, as described under “Experimental Procedures.” Hepatocytes were purified from nonparenchymal cells by a series of centrifugations at low speed, whereas the resulting supernatants were pooled, pelleted, and loaded onto a 6–15% arabinogalactan discontinuous gradient to further separate nonparenchymal cells. In this type of gradient, pure lipocytes are recovered at the interface between aqueous medium and 6% arabinogalactan, sinusoidal endothelial cells with a small portion of Kupffer cells, and some remaining lipocytes in the 6–8% interface, sinusoidal endothelial cells together with Kupffer cells (50:50 ratio) in the 8–12% interface, and remnant endothelial and Kupffer cells, together with erythrocytes, leukocytes, and bile duct epithelial cells, at the bottom of the tube (21, 22). Radioactivity values, assessed in the different gradient layers as well as in the pooled hepatocyte fractions purified by low speed centrifugations, indicated that hepatocytes were the cells involved in the uptake of the labeled A\textsubscript{40} (>90% radioactivity), whereas Kupffer cells were only accountable for <2% of the total uptake.

For further evaluation of the capability of the liver to catabolize A\textsubscript{40}, different concentrations of intact nonlabeled human A\textsubscript{40} were intravenous bolus-injected into the bloodstream of a group of mice, their gallbladders and urinary bladders removed shortly after injection, the bile and urine A\textsubscript{40} species immunoprecipitated with a mixture of 6E10 and 4G8 anti-A\textsubscript{40} antibodies coupled to paramagnetic beads and further identified by mass spectrometry analysis. Fig. 6 illustrates a representative MALDI-TOF analysis of the A\textsubscript{40} species immunoprecipitated from the bile of mice injected with 100 \textmu g of peptide. In addition to intact A\textsubscript{40}, C-terminally as well as N-terminally degraded peptides were clearly identified 5 min after the bolus injection in agreement with the short plasma turnover. In many cases, peptides bearing the oxidized Met-35 residue, indicative of \textit{in vivo} oxidation as part of the catabolic processing mechanisms, were also identified (Fig. 6). Bile immunoprecipitation experiments did not retrieve any A\textsubscript{40} species from the groups injected with either 1 or 10 \textmu g and analyzed under identical conditions, suggesting that intact A\textsubscript{40} and large degradation fragments are only secreted into the bile after a certain threshold had been surpassed. These results correlated with similar immunoprecipitation experiments performed with urine samples from mice injected with either 1 or 10 \textmu g of \textsuperscript{125}I-A\textsubscript{40} and collected 30 min after injection. In these samples, neither intact nor partially degraded A\textsubscript{40} peptides were re-
retrieved despite the appreciable radioactivity (data not shown), suggesting that the labeling excreted into the urine represents either free iodo-tyrosine, short Aβ/H9252 fragments, or both.

The role of hepatocytes in Aβ/H9252 capture was confirmed by binding experiments performed using human HepG2 cells and biotin-labeled Aβ40. The peptide, custom-synthesized with a single biotin moiety at its N terminus, was synthesized with a single biotin moiety at its N terminus. Superdex 75 elution profile and corresponding SDS-PAGE pattern were assessed by Western blot analysis performed as described under “Experimental Procedures,” and Fig. 1 indicates that biotinylated Aβ40 was >97% monomeric. Secondary structure of biotin-labeled Aβ40, evaluated by CD spectroscopy. Far-UV light (195–260 nm) spectra were recorded with a Jasco J-720 spectropolarimeter, and the results are expressed in terms of mean residue ellipticity (degree-cm$^2$-dmol$^{-1}$). C, HepG2 (7.5 × 10$^6$ cells/well) were grown to 60–70% confluence on 4-well glass chamber slides and incubated with 10 μM of biotin-labeled Aβ40 for 2 h at 4 °C in the absence (panel 1) or presence (panel 2) of 10-fold molar excess of nonbiotinylated Aβ40. Surface-bound biotinylated Aβ was assessed via fluorescence microscopy after staining with fluorescein isothiocyanate-conjugated streptavidin for 30 min. Nuclear DNA was counterstained with 4′,6-diamidino-2-phenylindole. Magnification, ×400. D, mass spectrometry analysis of Aβ species in HepG2 conditioned media. Following biotinylated Aβ40 binding at 4 °C, cells were incubated at 37 °C to allow peptide internalization; media were replaced at various time points (5–60 min) and subjected to immunoprecipitation and MS analysis to assess processing and degradation. The figure illustrates the MS data obtained with conditioned media at 5 (top panel) and 60 min (bottom panel) after immunoprecipitation with 6E10/4G8-coated paramagnetic beads. Mass spectrometry analysis was performed as described in Fig. 6 for bile samples. B, biotin; M$_{SO}$, methionine sulfoxide; M$_{SOS}$, methionine sulfone.
oxidized Aβ40 species were already evident, indicating the presence of rapid in vivo oxidative mechanisms, in agreement with the bile data. At this time point, heterogeneous C-terminal fragments Aβ(1–39), Aβ(1–38), and Aβ(1–37) were minor components. One hour after internalization, the majority of the intact Aβ40 species were Met-35 sulfoxide and sulfone derivatives, and the C-terminally truncated species had slightly increased. The Aβ immunoprecipitation data shown in Fig. 7, carried out employing anti-Aβ-coated beads, were confirmed in parallel experiments using streptavidin-coated beads (not shown).

**DISCUSSION**

Understanding the catabolism of sAβ species has both physiological and pathological implications. Soluble Aβ is present in biological fluids in femtomolar concentrations, with little variation between health and disease states. Urine levels account only for a minute portion of the circulating sAβ (17) ruling out free excretion through the kidney, a common pathway for low molecular mass proteins/metabolites, as the mechanism accounting for the low plasma sAβ levels. In addition, Aβ peptides show high tendency to aggregate/polymerize and adopt a variety of assembly states, e.g., monomers, dimers, oligomers, protofibrils, and fibrils. Increasing data appear to indicate that soluble oligomers are the active species related to synaptic loss and dementia associated with AD (26–29). Stable oligomers of Aβ42 have been identified in vivo and isolated from brain, plasma, and cerebral spinal fluid (30–32). Because they correlate better with the severity of neurodegeneration (33, 34) the role of fibrillar species in the disease pathogenesis has been questioned. Furthermore, experiments with nonfibrillar structures including oligomers, amyloid-derived diffusible ligands (35–39), and protofibrils (40, 41) demonstrated that these peptide assemblies are also neurotoxic in culture. Therefore, it is highly relevant to understand how both monomeric and oligomeric peptide loads are uptaken/catabolized in vivo. The Aβ peptides used in our experiments were either exclusively monomeric, as in the case of Aβ40, or a combination of monomeric and oligomeric species in ~50:50 ratio, as in Aβ42. The labeling of choice for catabolism assessment was radioiodination because of its high sensitivity and versatile possibilities for quantitative assessments. Under our experimental conditions, either one or two iodine atoms were incorporated to the tyrosine residue located at position 10 of Aβ, whereas the three available histidine residues (amino acids 6, 12, and 13), also amenable for iodination, were not modified. Undesirable oxidation products artificially created by the iodination procedure and primarily consisting of Met-35 sulfoxide derivatives, minimized by selecting a mild oxidative protocol for the iodination procedure, were separated from the relevant nonoxodized iodinated species by a simple HPLC purification, a step that also allowed the separation of unlabeled peptide as well as free unreactive iodine. Under these conditions, it was found that the “native” structural characteristics of Aβ40 and Aβ42, namely the degree of aggregation assessed by Western blot, secondary structure estimated by changes in the corresponding CD spectra, and biological activity measured by the binding affinity to apoE, were almost identical to those of the unlabeled peptides allowing us to conclude that, under the present conditions, the radioiodination procedure did not induce major conformational changes in the Aβ peptides. Both Aβ40 and Aβ42 cleared from the blood very rapidly (<3 min) and localized in various organs, particularly liver, spleen, kidney, and stomach. No significant differences between Aβ40 and Aβ42 were observed in clearance kinetics and organ uptake, suggesting that both peptides may follow similar major metabolic pathway(s), and that the degree of oligomerization does not significantly alter these parameters in view of the similar handling of monomeric Aβ40 and oligomeric Aβ42 species. However, an increase of acid-soluble radioactivity in the plasma samples as a function of time was a constant finding throughout our experiments, a clear warning sign that the plasma levels of radiotracer were not indicative of the sole presence of intact peptide but of a mixture of Aβ plus degradation products and free iodine. As indicated by the lack of Aβ degradation by plasma in vitro, these nontrichloroacetic acid-precipitable fragments were not generated in situ by proteolytic enzymes present in the blood, strongly suggesting that proteolysis occurred in systemic organ(s) and the resulting fragments were released into the circulation to be excreted in the urine. These may explain why, despite the presence of detectable radioactivity in urine after infusion of 125I-labeled Aβ40 and Aβ42, immunoprecipitation with 4G8 and 6E10 in combination with mass spectrometry failed to detect measurable amounts of intact Aβ. A similar behavior with respect to trichloroacetic acid precipitation has been observed previously in clearance experiments in rats in which 30 min after injection only around 34% of Aβ remaining in the circulation was tri-chloroacetic acid-precipitable (42).

To gather better indication about the cells involved in the process of Aβ catabolism and to circumvent the problem of the peptide degradation, we used compounds that can accumulate within the cells responsible for the uptake. This so-called intracellularly trapped ligand methodology has been extensively used to identify tissue sites of degradation and intracellular transport pathways of many proteins (18, 19, 43–46). The tracer in this case is a TC adduct with hydrophilic properties that preclude its solubility in lipid bilayers. This compound is poorly degraded by mammalian cells, is susceptible to oxidative iodination, and does not modify the metabolic behavior of the labeled protein (18). In our experiments using TC labeling, the plasma decay kinetics of 125I-TC Aβ40 compared with 125I-Aβ40 as well as 125I-TC Aβ42 versus 125I-Aβ42 were almost identical, indicating that coupling to the TC adduct did not significantly alter the metabolic characteristics of the Aβ peptides. As before, no significant differences in the kinetics between Aβ40 and Aβ42 were observed, and the plasma half-life for both Aβ40 and Aβ42 seemed to be in the range of 3 min, in close agreement with previously published data in rats (47). Examples of molecules with short half-lives (<5 min) are many hormones and peptide hormones, among them somatostatin, thyrotropin-releasing hormone, oxytocin, and insulin (48, 49). Noteworthy, insulin is degraded by the highly conserved thiil metalloprotease insulin-degrading enzyme (50), also known to break down several peptides capable of forming amyloid fibrils in vitro and in vivo, including glucagon, amylin, atrial natriuretic peptide, calcitonin, and Aβ (51).

By using the TC approach, the data clearly highlighted the prominent role of the liver in the catabolism of Aβ peptides, accounting for more than 60% uptake of the peptide. By using a different methodology, other investigators (52) have also recently indicated the importance of the liver in Aβ uptake. The liver is an important site for the catabolism of many plasma proteins, among them TTR (19), apoA-I (45), retinol-binding protein (53, 54), orosomucoid (53), tissue plasminogen activator (55, 56), ß2-macroglobulin (57), C-reactive protein (CRP) (43), and SAP (24, 43, 43). Although it is common to find more than one organ involved in the clearance of plasma proteins, in fewer cases, e.g. CRP and SAP, the liver is the sole organ responsible for their catabolism (43). Within the liver, a similar diversity is observed with respect to the specific cells involved in the uptake; sometimes both parenchymal and nonparenchymal liver cells take up the protein, whereas in other cases only the hepatocytes are involved in the clearance and degradation, as is the case for TTR, apoA-I, CRP, and SAP (19, 43, 45). In our
in vivo experiments, the hepatocytes were the main cells involved in the Aβ uptake (>90%) and catabolism. These results correlate well with our in vitro data using HepG2 cells in culture. The patchy membrane pattern observed at 4 °C following biotin-labeled Aβ40 binding together with the specific inhibition with unlabeled Aβ40 (and not by unrelated peptides) suggest that the uptake mechanism may be receptor-mediated. The likely candidates are the integrins, a group of heterodimeric receptors involved in cell adhesion and cell-cell interactions. Of particular interest for the uptake and internalization of Aβ is the α2β1 integrin, a fibronectin receptor that mediates cell attachment to nonfibrillar Aβ and is present in the surface of parenchymal liver cells. The amino acid sequence RHDS (positions 5–8 of Aβ) resembles the general integrin recognition sequence RGDS present in many extracellular matrix proteins, among them fibronectin. Indeed, the RHDS sequence has been identified as the α2β1 integrin recognition site in binding and cell-adhesion experiments (58) and is demonstrated to be biologically relevant for Aβ endocytosis and degradation in α2β1-transfected cells (59). Because this receptor-ligand interaction promoted the clearance of Aβ by cultured cells reducing the formation of fibrillar matrix and its associated toxic effects, it was proposed that α2β1 may regulate brain cell survival by in vivo binding and sequestering Aβ.

Another likely candidate for Aβ uptake is the serpin-enzyme complex receptor (SEC-R), a cell-surface protein identified in a variety of cells, e.g. hepatocytes, mononuclear cells, neutrophils, and neurons (60). SEC-R has the ability to mediate the endocytosis and lysosomal degradation of several serine proteases complexed to various serine-protease inhibitors (serpins), among them thrombin-heparin cofactor II, elastase-α1-antitrypsin, and thrombin-antithrombin, whereas noncomplexed serpins or free proteases do not interact with the receptor. Competition experiments have indicated that a hydrophobic stretch of five amino acids located within the C terminus of various serpins (FFVFLM in α1-antitrypsin, FMLII in α1-antichymotrypsin, FLVFI in antithrombin, and FLFLI in heparin cofactor II) is likely to be responsible for their interaction with the enzymatically active ligands. It has been postulated that this region is located in a hidden area of the serpin molecule and becomes accessible to interact with the SEC-R only after this region is located in a hidden area of the serpin molecule the enzymatically active ligands. It has been postulated that α2β1 may regulate brain cell survival by in vivo binding and sequestering Aβ.


diffuse AD progresses to florid dementia and eventually culminates in death within a few years of symptom onset (65). The present findings indicate that the liver has the capability to uptake, degrade, and excrete large doses of Aβ, several orders of magnitude above the physiological levels of circulating Aβ, and may explain its low plasma concentration, usually in the femtomolar range and with very little fluctuation.

Oxidative stress resulting from the unregulated production of reactive oxygen species has been lately implicated in the progression of AD (65). Amyloid plaques in affected brains contain remarkably high concentrations of the redox-active metals copper, zinc, and iron, and increasing evidence seems to indicate that homeostasis of these metals and their respective binding proteins are significantly altered in AD. More recently, it was demonstrated that reactions of copper with Aβ resulted in the production of Met-35 sulfoxide (66), an oxidation product capable of exerting detrimental neurotoxic effects (67). Note-worthy, our immunoprecipitation studies in mouse bile as well as in HepG2 conditioned media demonstrated the presence of Aβ species oxidized at Met-35, thereby raising the possibility that they originated by copper-mediated redox processes. It is known that the copper concentration included in the culture media is capable of generating Met-35 sulfoxide species in Aβ peptides (68), whereas in vivo, under normal physiological conditions, about 98% of copper excretion occurs via the bile, being hepatocytes actively involved in the process (69). The clearance and uptake experiments described herein were performed with nonoxidized Aβ species. Whether oxidized Aβ peptides originated by any of these redox mechanisms are catabolized/uptaken differently remains to be determined.

A direct implication of our data may be reflected in some of the current therapeutic strategies being explored for AD treatment. Synthetic homologues of Aβ, in particular Aβ42, are being used in active immunization protocols for therapeutic purposes. Studies in animal models have shown that Aβ immunization may provide protection against, and sometimes reverse, the AD-like pathology in transgenic animals (reviewed in Ref. 70). The application of active immunization protocols in human clinical trials raised safety concerns based on the capability of Aβ42 to cross the blood-brain barrier, seed fibril formation, and even attach to existing amyloid lesions, promoting plaque formation and an increase in toxicity (71). In view of these issues and the reports of encephalitis in some patients immunized with intact Aβ42 peptide in a phase II clinical trial (72), alternative approaches using the same concept are being actively pursued, like the use of Aβ fragments and nontoxic Aβ derivatives as immunogens or in passive immunization protocols (73). Immunization procedures generally involve the subcutaneous or intradermal injection of the immunogen emulsified with a suitable adjuvant. Under these conditions, the immunogen might be released from the site of application, reaching the circulation where it is systemically distributed. Safety concerns regarding the release of Aβ42 into the blood and its potential contribution to the deposited Aβ as a negative side effect of the vaccination strategy should be counterbalanced with the knowledge of the capability of the liver to uptake, catabolize, and excrete large doses of the peptide. Even in the event that a large dose of free Aβ42 suddenly gains access to the circulatory system, the liver would reduce its plasma concentration by 50% in a short period of time (~ 3 min in mice), physiologically minimizing its potentially harmful effects. Because no data are yet available regarding the Aβ catabolic parameters in cases in which the peptide is either bound to a carrier molecule (it being either one of the multiple plasma protein carriers described, e.g. apoJ, apoE, albumin, etc.), to a specific antibody generated by active or passive immunization, or to a component of the adjuvant used in vaccination procedures, further studies are needed to assess the impact that these interactions may have on the homeostasis of the Aβ peptides. Although it would be predictable to assume that the binding of Aβ to large carrier molecules will extend the half-life of the peptide in circulation toward the half-life of the carrier (usually much longer than that of the Aβ), the affinity/avidity of the protein-peptide interactions will certainly influence the final outcome.
