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$A\beta^{1-40}$ Peptide Radiopharmaceuticals for Brain Amyloid Imaging: ^{111}In Chelation, Conjugation to Poly(ethylene glycol)-Biotin Linkers, and Autoradiography with Alzheimer's Disease Brain Sections

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The amyloid plaques of Alzheimer's disease (AD) are formed by the neuropeptide $A\beta^{1-42/43}$, and carboxyl terminal truncated forms of this neuropeptide, designated $A\beta^{1-40}$, bind to amyloid plaques of AD autopsy tissue sections. Therefore, $A\beta^{1-40}$ is a potential peptide radiopharmaceutical that could be used for imaging brain amyloid in living subjects with AD, should this neuropeptide be made transportable through the blood-brain barrier (BBB). To accomplish this, the neuropeptide must be modified to enable (i) attachment to a BBB drug targeting system and (ii) labeling with a radionuclide, e.g., 111-indium, suitable for brain imaging by external detection modalities such as single photon emission computed tomography (SPECT). The present studies describe the synthesis of an $A\beta^{1-40}$ analogue that contains a biotin at the amino terminus and a diethylenetriaminepentaacetic acid (DTPA) moiety conjugated to one of the internal lysine residues. The DTPA-[*N*-biotin]-$A\beta^{1-40}$ was purified by gel filtration fast-protein liquid chromatography (FPLC) using two Superose 12HR columns in series, and the structure of the purified peptide was confirmed by matrix-assisted laser desorption ionization (MALDI) mass spectrometry. The binding of the [^{111}In]DTPA-[*N*-biotin]-$A\beta^{1-40}$ to amyloid plaques of AD autopsy tissue sections was demonstrated by film and emulsion autoradiography. A poly(ethylene glycol) (PEG) linker of 3400 Da molecular mass, designated PEG^{3400}, was inserted between the $A\beta^{1-40}$ and the biotin moiety, but this modification diminishes binding of $A\beta^{1-40}$ to the AD amyloid plaques. In summary, these studies describe a novel formulation of biotinylated $A\beta^{1-40}$ that allows radiolabeling with 111-indium. The peptide radiopharmaceutical may be conjugated to an avidin-based BBB drug targeting system to enable transport through the BBB and imaging of brain amyloid in vivo.

INTRODUCTION

Alzheimer's disease (AD) is a chronic neurodegenerative condition that leads to progressive dementia. The dementia of AD correlates with the deposition in brain of amyloid (*1*, *2*), which is formed by a 42−43 amino acid peptide designated $A\beta^{1-42/43}$ (*3*). There are three forms of brain extracellular amyloid in AD, meningeal vascular amyloid (*4*), cortical microvascular amyloid (*5*), and neuritic plaques (*6*). Presently, there is no premortem diagnostic test for AD. In addition, it is not possible to scan the brain of subjects living with AD to determine the level of the $A\beta$ amyloid burden in brain, which can occupy up to 15% of the brain volume in subjects with AD (*7*). The availability of such an amyloid imaging brain scan would not only provide a diagnostic test specific for AD but also allow for assessing the effects in brain of drugs that inhibit the formation of $A\beta$ amyloid.

An amyloid imaging brain scan could be developed with $A\beta^{1-40/42}$ peptide radiopharmaceuticals because radiolabeled forms of these molecules promptly deposit at preexisting amyloid plaques in tissues sections of autopsy AD brain (*8*). The autoradiographic detection of AD amyloid (*8*) using radiolabeled $A\beta$ analogues can be more sensitive than classical immunocytochemical techniques using monoclonal antibodies (MAb) against $A\beta$. Radiolabeled forms of either $A\beta^{1-40}$ (*9*) or anti-$A\beta$ Mabs (*10*) have been administered to aging primates in an attempt to develop amyloid imaging brain scans. In either case, only amyloid at the meninges was imaged because meningeal vessels lack a blood-brain barrier (BBB). Although [^{125}I]$A\beta^{1-40}$ does bind to brain vessels in vivo following systemic administration, there is no measurable transcytosis of this peptide radiopharmaceutical through the BBB in vivo (*11*). The BBB is intact in AD (*12*), and the $A\beta$ amyloid is deposited beyond the BBB in brain (*13*). Therefore, if $A\beta$ peptide radiopharmaceuticals are to be used as in vivo amyloid imaging agents in AD, it may be necessary to conjugate the peptide to BBB drug targeting systems (*14*).

Peptide radiopharmaceuticals such as $A\beta^{1-40/42}$ may be targeted through the BBB by conjugating the peptide to BBB drug-targeting vectors such as peptidomimetic MAbs that undergo receptor-mediated transcytosis through the BBB on endogenous brain capillary endothelial peptide transport systems (*14*). The OX26 murine MAb to the rat transferrin receptor (TfR) undergoes transport through the rat BBB (*15*). However, rats do not develop brain amyloid and the OX26 MAb is specific for rats and cannot be used in other species such as transgenic mouse models (*16*), which develop $A\beta$ amyloid (*17*). However, $A\beta$ amyloid in transgenic mice could be imaged with either the 8D3 or the RI7 rat MAb to the mouse TfR, as these antibodies have been recently shown to cross the mouse BBB via the TfR (*16*). Alternatively, aged (>30 year old) Rhesus monkeys develop $A\beta$ amyloid plaques and represent a potential animal model for imaging brain amyloid (*18*, *19*). The 83-14 MAb to the human insulin receptor (HIR) is an effective BBB drug-

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targeting vector in Old World primates such as rhesus monkeys, but not New World primates such as squirrel monkeys (*20*).

In an effort to develop an amyloid brain scan that might be effective in mice, monkeys, or humans, previous studies described the synthesis of Aβ^{1-40} radiolabeled with 125-iodine at the Tyr^{10} residue and monobiotinylated at the amino terminus (*14*). This peptide was bound to a conjugate of streptavidin (SA) and the 83-14 HIRMAb and injected intravenously in young rhesus monkeys (*14*). The Aβ peptide radiopharmaceutical labeled with 125-iodine and conjugated to the HIRMAb drug-targeting system was rapidly transported across the BBB in vivo in primates after intravenous administration, and the brain uptake was visualized by quantitative autoradiography (QAR) of frozen tissue sections of brain (*14*). However, the formulation of Aβ^{1-40} labeled with 125-iodine is not ideal for brain imaging for several reasons. First, peptide radiopharmaceuticals labeled with 125-iodine are rapidly converted by peripheral tissues to radiolabeled small molecules, e.g., iodotryosine, that cross the BBB and yield an artifactually high background of brain uptake (*21*). The high "noise" of the brain-imaging signal is virtually eliminated using peptide radiopharmaceuticals labeled with 111-indium (*21*, *22*). Another reason for developing an Aβ^{1-40} peptide radiopharmaceutical labeled with 111-indium is that ^{125}I is not suitable for external brain imaging with either single photon emission computed tomography (SPECT) or positron emission tomography (PET). The ^{125}I could be substituted with ^{124}I for PET studies, but the availability of the ^{124}I radionuclide is severely limited. Therefore, it would be advantageous to develop Aβ peptide radiopharmaceuticals that are amenable to external brain imaging with SPECT using suitable radionuclides such as 111-indium (In).

The present studies describe a formulation of Aβ^{1-40} that is monobiotinylated at the amino terminus and is radiolabeled with ^{111}In via a chelator moiety, diethylenetriaminenpentaacetic acid (DTPA), which is conjugated to one of the two internal lysine residues, Lys^{16} or Lys^{28}. This form of Aβ is designated [^{111}In]DTPA-[*N*-biotin]-Aβ^{1-40}. The present studies also examine the formulation of an Aβ peptide radiopharmaceutical wherein the biotin moiety is attached to the tip of a single polymeric tail of poly(ethylene glycol) (PEG) of 3400 Da molecular mass, designated PEG^{3400}. In this formulation, the PEG^{3400}-biotin is conjugated to one of the two internal lysine residues. Previous studies have shown that receptor binding of neuropeptides that are conjugated to BBB drug-targeting MAbs is enhanced by placing a PEG linker between the peptide and the BBB drug targeting system (*23*). The insertion of the extended PEG linker releases any mutual steric hindrance between the peptide radiopharmaceutical and the BBB-targeting MAb. This form of Aβ was radiolabeled with ^{125}I and is designated [^{125}I]Aβ^{1-40}−PEG^{3400}-biotin.

EXPERIMENTAL PROCEDURES

Materials. Human Aβ^{1-40} was purchased from Bachem Inc. (Torrance, CA). Biotinylated Aβ ([*N*-biotin]-Aβ^{1-40}), which is monobiotinylated at the amino terminus, was obtained from Quality Controlled Biochemicals Inc. (Hopkinton, MA). [^{125}I]Na was supplied by Amersham (Arlington Heights, IL). [^{111}In]Cl_3 was obtained from NEN Life Science Products Inc. (Boston, MA). NHS-PEG^{3400}-biotin was obtained from Shearwater Polymers (Huntsville, AL), where NHS = N-hydroxysuccinimide and PEG^{3400} = poly(ethylene glycol) of 3400 Da molecular mass. Diethylenetriaminepentaacetic (DTPA) dianhydride was obtained from Aldrich Chemical Co., Inc. (Milwaukee, WI). Chloramine-T was purchased from MCB Reagents (Cincinnati, OH). All other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO). Superose 12 HR 10/30 FPLC columns were obtained from Pharmacia Biotech (Piscataway, NJ). Centricon-3 microconcentrators were obtained from Amicon Corp. (Bevery, MA). Snap-frozen human autopsy Alzheimer's disease brain, used for preparation of frozen sections, was provided by Prof. Harry Vinters (UCLA Department of Pathology/Neuropathology).

Iodination of [*N*-Biotin]-Aβ^{1-40}. [*N*-Biotin]-Aβ^{1-40} (10 μg, 2.1 nmol) was iodinated with ^{125}I (2 mCi, 1.1 nmol) and chloramine T (44 nmol) in 95 μL of 0.05 M phosphate-buffered saline (pH 7.4) at room temperature for 2 min (*14*). After addition of sodium metabisulfite to quench the iodination, 50 μL of 0.05 M phosphate buffer (pH 7.4)/1 mM EDTA was added to the reaction solution. [^{125}I][*N*-biotin]-Aβ^{1-40} was purified by Sephadex G25 size-exclusion chromatography with an elution buffer of 0.01 M phosphate-buffered saline (pH 7.4) containing 0.05% Tween-20. The [^{125}I][*N*-biotin]-Aβ^{1-40} had a trichloroacetic acid (TCA) precipitability of >97% and a specific activity of 159 μCi/μg.

Synthesis and Iodination of Aβ^{1-40}-PEG^{3400}-Biotin. NHS-PEG^{3400}-biotin (230 nmol in 50 μL of 0.1 M $NaHCO_3$) was added in a 2:1 molar ratio to 500 μg of Aβ^{1-40} (115 nmol) in 0.033 M $NaHCO_3$, pH 8.3, followed by incubation at room temperature for 60 min. The entire mixture was then applied to two Superose 12 HR 10/30 fast-protein liquid chromatography (FPLC) columns in series, followed by elution in 0.01 M phosphate-buffered saline (pH 7.4) at a flow rate of 0.5 mL/min for 90 min (*21*). For detection of conjugates, UV absorption of 205 nm was used. Aβ^{1-40} containing a single PEG^{3400}-biotin moiety and designated Aβ^{1-40}-PEG^{3400}-biotin, eluted at 58 min from the column, as demonstrated by mass spectrometry analysis (below). Aβ^{1-40}-PEG^{3400}-biotin (1.3 mL from FPLC) was applied to a Centricon-3 microconcentrator and the volume was reduced to 0.3 mL by centrifugation at 7500*g* for 60 min at room temperature. Aβ^{1-40}-PEG^{3400}-biotin (2.3 nmol) was iodinated with ^{125}I (2.6 mCi, 1.4 nmol) and chloramine T (44 nmol) in 95 μL of 0.05 M phosphate-buffered saline (pH 7.4) at room temperature for 2 min (*1*). After addition of sodium metabisulfite to quench the iodination, 50 μL of 0.05 M phosphate buffer (pH 7.4)/1 mM EDTA was added to the reaction solution. [^{125}I]Aβ^{1-40}-PEG^{3400}-biotin was purified by Sephadex G25 size-exclusion chromatography with a elution buffer of 0.01 M phosphate-buffered saline (pH 7.4) containing 0.05% Tween-20. [^{125}I]Aβ^{1-40}-PEG^{3400}-biotin had a TCA precipitation of >98% and a specific activity of 135 μCi/μg.

Synthesis and ^{111}In-Labeling of DTPA-[*N*-Biotin]-Aβ^{1-40}. DTPA dianhydride [2.7 μmol in 20 μL of dimethyl sulfoxide (DMSO)] was added in a 50:1 molar ratio to 250 μg of [*N*-biotin]-Aβ^{1-40} (54 nmol) in 200 μL of 0.05 M $NaHCO_3$, pH 8, followed by incubation at room temperature for 30 min. The entire mixture was then applied to two Superose 12 HR 10/30 FPLC columns in series, followed by elution in 0.01 M phosphate-buffered saline (pH 7.4) at a flow rate of 0.5 mL/min for 90 min. For detection of conjugates, UV absorption of 205 nm was used. [*N*-Biotin]-Aβ^{1-40} containing a single DTPA moiety conjugated to one of two internal lysine residues on Aβ^{1-40} (Lys^{16},Lys^{28}) and designated DTPA-[*N*-biotin]-Aβ^{1-40}, eluted at 64 min from the columns, as demonstrated by mass

spectrometry analysis (below). Two millicurie of ^{111}In (0.4 nmol) in 38 μL of 0.05 N HCl was added to 1.0 nmol of DTPA-[*N*-biotin]-Aβ$^{1-40}$ in 0.1 mL of phosphate-buffered saline (pH 7.4). After 30 min at room temperature, [^{111}In]-DTPA-[*N*-biotin]-Aβ$^{1-40}$ was separated from unreacted free ^{111}In by Sephadex G25 size-exclusion chromatography with an elution buffer of 0.01 M phosphate-buffered saline (pH 7.4) containing 0.05% Tween-20. The specific activity of the [^{111}In]DTPA-[*N*-biotin]-Aβ$^{1-40}$ was 388 μCi/μg.

Matrix-Assisted Laser Desorption Ionization (MALDI) Mass Spectrometry. Mass spectra of Aβ$^{1-40}$-PEG^{3400}-biotin and DTPA-[*N*-biotin]-Aβ$^{1-40}$ eluted from the two Superose 12 HR 10/30 columns in series were recorded with a reflector time-of-flight instrument (PerSeptive Biosystems Voyager RP) used in the linear mode with stainless steel targets (*21*). An aliquot of sample solution (0.5 μL) was overlaid on top of matrix (α-cyano-4-hydroxycinnamic acid), and after drying, the target was inserted into the mass spectrometer, and time/intensity data from 50 to 100 scans was averaged and converted to mass/intensity data.

Film and Emulsion Autoradiography. Snap-frozen Alzheimer's disease cortex was provided by the UCLA Department of Pathology/Neuropathology, and 15 μm sections were cut on a Bright cryostat and thaw-mounted to glass slides for autoradiography of tissue sections, where the BBB is nonexistent (*11*). The slides were warmed to room temperature, air-dried, and incubated for 30 min with TBM buffer (0.05 M Tris-HCl, 0.1% BSA, 10 mM $MnCl_2$, pH 7.4) (*8*). Then the slides were incubated with 250 μL of TBM buffer containing 0.5 μCi/mL of either [^{125}I][*N*-biotin]-Aβ$^{1-40}$ or [^{125}I]Aβ$^{1-40}$-PEG^{3400}-biotin. After 2 h at room temperature, the slides were washed four times with 2 min wash in Tris/BSA buffer (0.05 M Tris-HCl, 0.1% BSA, pH 7.4) at 4 °C followed by two 5 s washes in H_2O at 4 °C.

For autoradiography with the ^{111}In, it was necessary to omit the $MnCl_2$ from the mixture, as this divalent cation competed with the ^{111}In for chelation to DTPA; these slides were preincubated in Tris/BSA buffer for 30 min at room temperature, then incubated for 2 h in Tris/BSA buffer containing 1.0 μCi/mL of [^{111}In]DTPA-[*N*-biotin]-Aβ$^{1-40}$, and washed as for [^{125}I]labeled conjugates.

After drying, half of the slides were dipped with emulsion (NTB3; Eastman Kodak Co., Rochester, NY) in the dark room for 5 s followed by air-drying at room temperature for 30 min, and then exposed at −20 °C in the dark for up to 2 weeks (*11*). Only the ^{125}I reacted with the emulsion, as the ^{111}In provided no reaction in the emulsion autoradiography, perhaps due to dissociation of the ^{111}In by components of the emulsion. The other half of slides were placed in apposition to Kodak Biomax MS X-ray film and exposed for up to 2 weeks at room temperature. The film was scanned with a Hewlett-Packard ScanJet IIcx/T flatbed scanner and transferred to Adobe Photoshop on a Power Macintosh 7100/66 microcomputer, and prints were generated with a Kodak printer.

RESULTS

Three different forms of Aβ$^{1-40}$ peptide radiopharmaceutical were synthesized (Figure 1). All three had the dual modifications of (i) attachment of a radionuclide and (ii) attachment of a biotin group. The monobiotinylation enables conjugation to a BBB drug targeting vector comprised of a transporting MAb and streptavidin or avidin. In the first formulation (Figure 1A), the Aβ$^{1-40}$ is radiolabeled by attachment of 125-iodine to the single Tyr residue at position 10, and the biotin is attached at the amino terminus with a short (<15 atom) linker between the biotin and the amino terminus. In the second formulation (Figure 1B), the Aβ$^{1-40}$ is radiolabeled at Tyr^{10} with ^{125}I, and is biotinylated at one of the two internal lysine residues via an extended PEG^{3400} linker. In the third formulation (Figure 1C), the Aβ$^{1-40}$ is biotinylated at the amino terminus with a short linker and is radiolabeled with ^{111}In via a DTPA chelator moiety conjugated to one of the two internal lysine residues (Figure 1C).

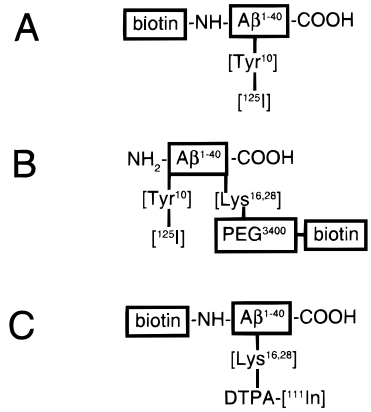

Figure 1. Structures of [^{125}I][*N*-biotin]-Aβ$^{1-40}$ (A), [^{125}I]Aβ$^{1-40}$-PEG^{3400}-biotin (B), and [^{111}In]DTPA-[*N*-biotin]-Aβ$^{1-40}$ (C). The ^{125}I was attached to Tyr^{10}, and the -PEG^{3400}-biotin or the DTPA was conjugated to the epsilon amino group of one of the two internal lysine residues at position 16 or 28.

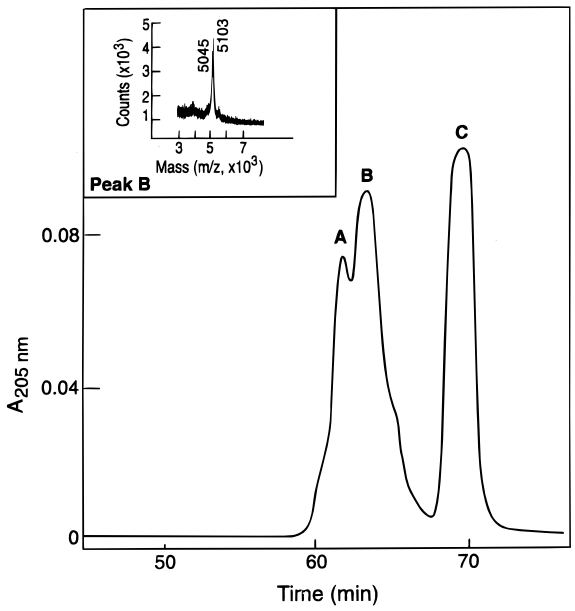

Figure 2. Purification of DTPA-[*N*-biotin]-Aβ$^{1-40}$ by FPLC. Reaction mixture of [*N*-biotin]-Aβ$^{1-40}$ and DTPA dianhydride was applied to two Superose 12 HR 10/30 columns in series, followed by elution in 0.01 M phosphate-buffered saline (pH 7.4) at a flow rate of 0.5 mL/min for 90 min. Peak A, $(DTPA)_2$-[*N*-biotin]-Aβ$^{1-40}$; peak B, DTPA-[*N*-biotin]-Aβ$^{1-40}$; peak C, DTPA. (Insert) MALDI mass spectra shows molecular mass of 5045 Da for peak B, the DTPA-[*N*-biotin]-Aβ$^{1-40}$ (theoretical M_r, 5045 Da). Peak A, $(DTPA)_2$-[*N*-biotin]-Aβ$^{1-40}$ showed molecular mass of 5420 Da (theoretical M_r, 5420) with the MALDI analysis.

The DTPA-[*N*-biotin]-Aβ$^{1-40}$ (peak B, Figure 2) was separated from unreacted *N*-biotin-Aβ$^{1-40}$ (peak C, Figure 2) and $DTPA_2$-[*N*-biotin]-Aβ$^{1-40}$ (peak A, Figure 2) by gel filtration FPLC using two Superose 12HR columns in

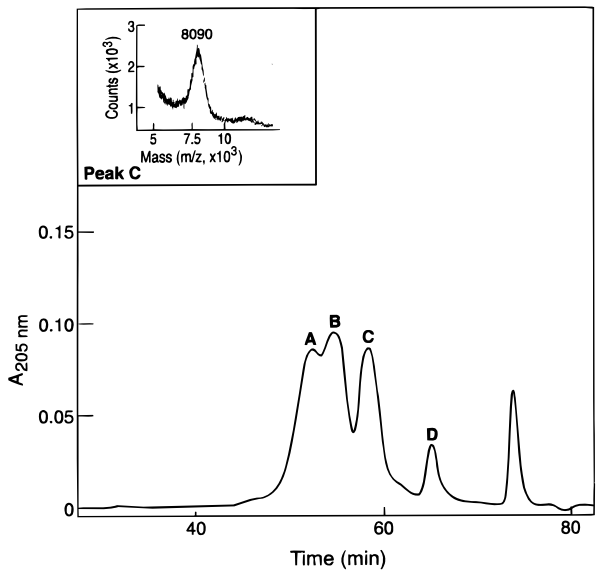

Figure 3. Purification of Aβ^{1-40}-PEG^{3400}-biotin by FPLC. Reaction mixture of Aβ^{1-40} and NHS-PEG^{3400}-biotin was applied to two Superose 12 HR 10/30 columns in series, followed by elution in 0.01 M phosphate-buffered saline (pH 7.4) at a flow rate of 0.5 mL/min for 90 min. Peak A, Aβ^{1-40}-(PEG^{3400}-biotin)$_3$; peak B, Aβ^{1-40}-(PEG^{3400}-biotin)$_2$; peak C, Aβ^{1-40}-PEG^{3400}-biotin; peak D, Aβ^{1-40}. (Insert) MALDI mass spectra shows mean molecular mass of 8090 Da for peak C, Aβ^{1-40}-PEG^{3400}-biotin (theoretical M_r, 7981 Da). Peak A, Aβ^{1-40}-(PEG^{3400}-biotin)$_3$ and peak B, Aβ^{1-40}-(PEG^{3400}-biotin)$_2$ showed mean molecular masses of 15 356 Da (theoretical M_r, 15283 Da) and 11 676 Da (theoretical M_r, 11 632), respectively, with the MALDI analysis. The (PEG^{3400}-biotin)$_n$ nomenclature, where $n = 1$, 2, or 3, refers to the number of PEG^{3400} strands conjugated to the Aβ^{1-40}, which can attach to Lys^{16}, Lys^{28}, or the amino terminus. The theroretical M_rs are approximations owing to the uncertainty of the exact M_r of the PEG^{3400}.

series. MALDI mass spectrometry analysis (inset, Figure 2) demonstrated the observed molecular mass of the DTPA-[*N*-biotin]-Aβ^{1-40} was 5103−5045 Da, with a theoretical molecular mass (M_r) of 5045 Da. MALDI mass spectrometry showed the observed molecular mass of the Aβ conjugate in peak A was 5420 Da, which correlates with the theoretical molecular mass (5420 Daltons) for the [*N*-biotin]-Aβ^{1-40} that contains DTPA moieties conjugated to both internal lysine residues, designated (DTPA)$_2$-[*N*-biotin]-Aβ^{1-40}. The purity of the peptide following gel filtration FPLC through two columns in series was demonstrated by the absence of peaks of contaminating peptides in the MALDI mass spectrometry analysis.

The Aβ^{1-40}-PEG^{3400} biotin was purified by gel filtration FPLC using two Superose 12HR columns in series, which allowed for separation of unconjugated Aβ^{1-40} (peak D, Figure 3) from Aβ conjugates containing 3, 2, or 1 PEG^{3400}-biotin moieties (peaks A, B, and C of Figure 3, respectively). The MALDI mass spectrometry analysis of peak C revealed a molecular mass of 8090 Da (inset, Figure 3), which correlates with the predicted theoretical M_r of 7981 Da. The precise theoretical M_r is an approximation owing to uncertainties regarding the length and M_r of the PEG^{3400}. The M_r of peak A was 15 356 Da as opposed to the theoretical M_r of 15 283 Da for the Aβ conjugate with three PEG^{3400}-biotin residues attached to both internal Lys residues and to the free amino terminus. The MALDI mass spectrometry analysis of peak B indicated the mass of this peptide was 11 676 Da, which correlates with the theoretical M_r of 11 632 Da for the Aβ^{1-40} containing two PEG^{3400}-biotin resides (peak B, Figure 4).

Film autoradiography examined the binding of the three different Aβ^{1-40} peptide radiopharmaceuticals to amyloid plaques in frozen sections of autopsy AD brain (Figure 3). All three formulations of the Aβ peptide radiopharmaceutical bound to the amyloid plaques with a predilection for gray matter and sparing of the central white matter track. The binding of the [^{111}In]DTPA-[*N*-biotin]-Aβ^{1-40} to the amyloid plaques (Figure 4B) was comparable to the binding of [^{125}I][*N*-biotin]-Aβ^{1-40} (Figure 4A). Although there was still extensive binding of the [^{125}I]Aβ^{1-40}−PEG^{3400}-biotin to the amyloid plaques, this was diminished compared to the other two formulations (Figure 4C).

Emulsion autoradiography studies were also performed and these showed that the ^{111}In formulation did not react with the emulsion. The two Aβ peptide radiopharmaceutical formulations containing the ^{125}I radionuclide did react with the emulsion, and these studies are shown in Figure 5. There was extensive binding to amyloid plaques at both the meningeal surface and internally in the brain

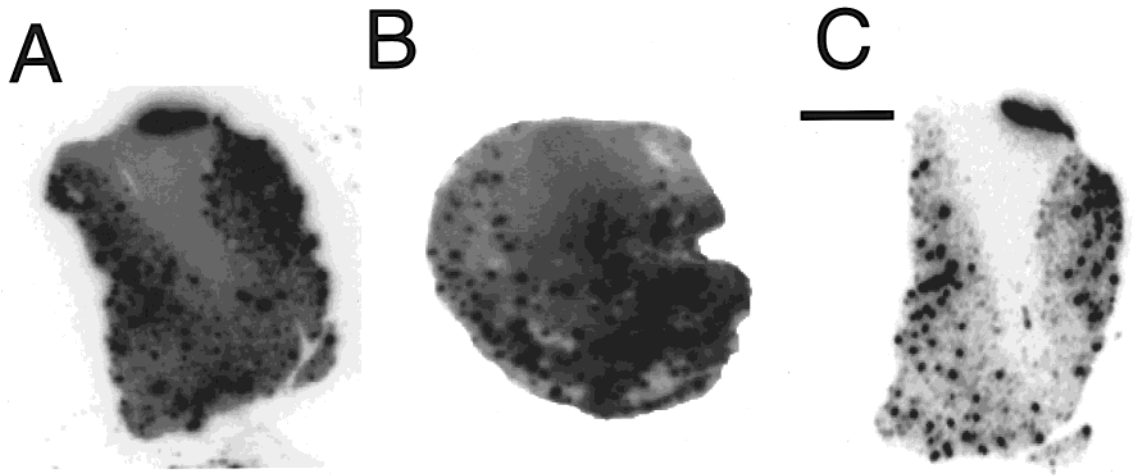

Figure 4. Film autoradiography showing binding of [^{125}I][*N*-biotin]-Aβ^{1-40} (A), [^{111}In]DTPA-[*N*-biotin]-Aβ^{1-40} (B), and [^{125}I]Aβ^{1-40}-PEG^{3400}-biotin (C), and to amyloid plaques of frozen sections of human Alzheimer's disease brain. Magnification bar = 4 mm. The central white matter track is spared of amyloid. The amyloid plaques are small structures with a diameter less than 100 μm, as viewed by emulsion autoradiography (*11*). However, when the amyloid plaques are viewed with film autoradiography, such as that shown in Figures 4 and 5, there is a coalescence of the plaque signals (*11*) to yield the large plaque structures of a diameter >1 mm, as shown in the figure.

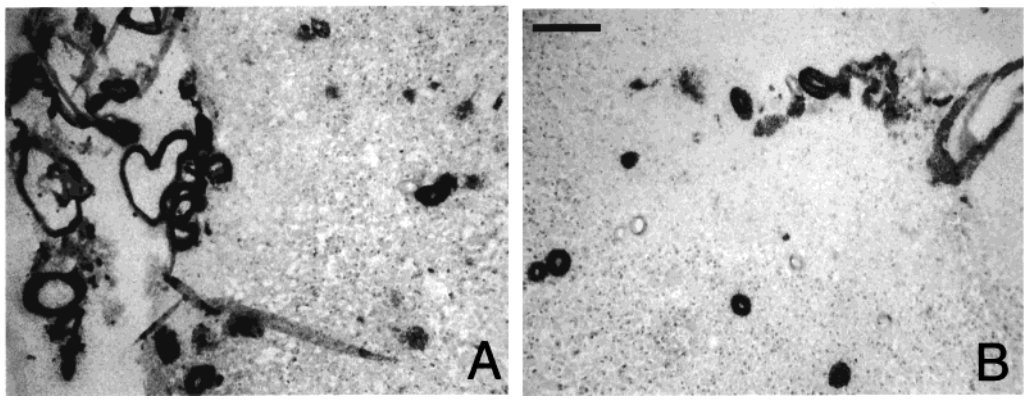

Figure 5. Emulsion autoradiography showing binding of [^{125}I][*N*-biotin]-Aβ^{1-40} (A) and [^{125}I]Aβ^{1-40}-PEG^{3400}-biotin (B) to amyloid plaques of frozen sections of human Alzheimer's disease brain, as viewed under bright field microscopy. Magnification bar = 200 μm.

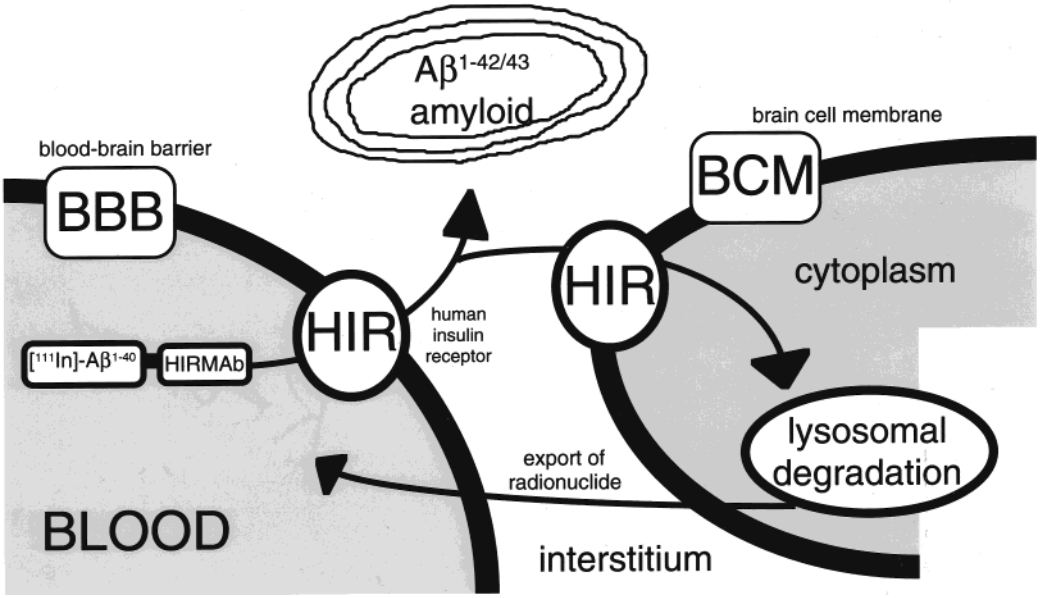

Figure 6. Pathways of metabolism of Aβ^{1-40} conjugates in brain. The mono-biotinylated Aβ^{1-40} (Figure 1) is bound to a conjugate of streptavidin or avidin and a peptidomimetic monoclonal antibody (MAb) that undergoes receptor mediated transcytosis through the blood-brain barrier (BBB). In the case of humans or rhesus monkeys, the MAb is directed against the human insulin receptor (HIR), which is also localized to brain cell membranes (BCM) (*24*) and mediates the endocytosis of the conjugate into brain cells. Once inside brain cells, the conjugate is degraded in the lysosome with export of the radionuclide to blood, which occurs with a $t_{1/2}$ of 16 h in the young rhesus monkey lacking brain A$\beta^{1-42/43}$ amyloid (*14*). In subjects with A$\beta^{1-42/43}$ brain amyloid, the plaques in the brain interstitial space enable deposition of the Aβ^{1-40} peptide radiopharmaceutical on to the surface of the extracellular amyloid plaque. This deposition is hypothesized to delay the metabolic degradation and prolong the residence time in brain of the Aβ^{1-40} peptide radiopharmaceutical, such that at delayed imaging times (e.g., 48−72 h after isotope administration) the brain radioactivity persists at the regions of high plaque content. Conversely, in subjects without brain amyloid, the brain radioactivity returns to background levels at the 48−72 h images (*14*).

parenchyma using the [^{125}I, *N*-biotin] Aβ^{1-40} (Figure 5). Similar to the results with the film autoradiography (Figure 4), there was a diminished binding to the amyloid plaques in the emulsion autoradiography using the [^{125}I]Aβ^{1-40}−PEG^{3400}-biotin formulation as shown in Figure 5B.

DISCUSSION

The results of the present studies are consistent with the following conclusions. First, it is possible to prepare a formulation of Aβ (Figure 1C) that has the dual modifications of (i) attachment to a radionuclide, ^{111}In, suitable for SPECT external imaging of brain, and (ii) attachment of a biotin moiety to enable conjugation to a BBB drug targeting system. This formulation of the Aβ peptide radiopharmaceutical still avidly binds to the amyloid plaques of AD brain (Figure 4). Second, the insertion of a PEG^{3400} linker to spatially separate the Aβ peptide radiopharmaceutical from the biotin linker does not confer any enhanced binding of the Aβ peptide radiopharmaceutical at the amyloid plaques and may inhibit binding (Figures 4 and 5).

The pathways involved in imaging the Aβ amyloid of AD brain in vivo are outlined in Figure 6. The Aβ peptide radiopharmaceutical must be formulated with dual modifications that enable (a) attachment of a radionuclide, e.g., 111-indium, suitable for SPECT imaging, or 124-iodine, suitable for PET imaging, and (b) conjugation to a BBB drug targeting system. The latter is a peptidomimetic MAb that undergoes receptor-mediated transcytosis through the BBB (*15*) and may be the 83-14 MAb that targets the BBB insulin receptor in rhesus monkeys (*18*), or the 8D3 or RI7 MAb that targets the BBB TfR in mice (*16*). The insulin receptor or the TfR are widely expressed on brain cells (*24*, *26*), and binding of the peptidomimetic MAb to the brain cell insulin receptor or TfR will enable

receptor-mediated endocytosis into brain cells behind the BBB (Figure 6). Following entry into the lysosomal system of brain cells, the amyloid imaging agent is degraded, and the radionuclide is exported to the systemic circulation. This has been demonstrated previously in young primates lacking brain amyloid and the half time of conjugate degradation and ^{125}I-radionuclide clearance from brain is 16 h in the living rhesus monkey (*14*). Consequently, >90% of the peptide radiopharmaceutical initially taken up by the primate brain lacking amyloid is cleared by 48 h after single intravenous administration of the conjugate (*14*). In subjects with significant quantities of amyloid plaque in brain, and in patients with AD, this can form up to 15% of the brain volume (*7*), it is hypothesized that the Aβ peptide radiopharmaceutical will be selectively sequestered by the extracellular amyloid in brain (*14*), as depicted in Figure 6. Although Aβ^{1-40} is rapidly degraded, its degradation is markedly delayed upon binding to preexisting amyloid plaques (*26*). Therefore, the brain scan at 48 h after intravenous administration of the Aβ peptide radiopharmaceutical conjugated to a BBB drug targeting system may show persistent radioactivity at regions containing extensive amyloid compared to the minimal radioactivity in regions with no amyloid. The hypothesis that the brain ^{111}In radioactivity will be selectively exported from brain in nonamyloid-bearing regions cannot be verified until these studies are actually executed in mouse or primate animal models of Aβ brain amyloid. However, the ability of the brain to rapidly export ^{111}In following brain uptake of a ^{111}In-peptide radiopharmaceutical conjugated to a BBB drug targeting system has been recently demonstrated in an experimental brain tumor model (*22*). The export of
^{111}In from the brain in vivo contrasts with cultured cells, where ^{111}In is sequestered within the cell (*27*, *28*).

The administration of Aβ amyloid imaging agents to humans for brain scanning by SPECT will require the radiolabeling of Aβ analogues with a SPECT-compatible radionuclide such as 111-indium. The present studies show that one of the internal lysine residues may be conjugated with DTPA to enable chelation of 111-indium. The conjugation of the DTPA chelator moiety to one of the internal lysine residues at Lys^{16} or Lys^{28} does not inhibit the binding to amyloid plaques (Figure 4B). The Lys^{16} is contiguous with the sequence of Aβ that runs from Leu^{17} to Asp^{22}, and which plays an important role in binding of Aβ analogues to amyloid plaques (*29*−*31*). Other active sites in the Aβ molecule that are critical to binding to amyloid plaques must also be in the carboxyl terminus of the peptide, because Aβ^{1-28} does not bind to amyloid plaques, whereas Aβ^{10-35} does bind to plaques (*32*). The conjugation of PEG^{3400}-biotin to one of the two internal lysine residues results in a decreased binding of the peptide to amyloid plaques as shown by the film and emulsion autoradiography studies (Figures 4 and 5). Conjugation of DTPA to one of the internal lysine residues does not affect amyloid binding (Figure 4C). Therefore, the conjugation of an internal lysine residue, per se, would not be expected to inhibit amyloid binding. In previous studies with an epidermal growth factor (EGF) peptide radiopharmaceutical developed for imaging brain tumors expressing the EGF receptor, the insertion of a PEG^{3400} linker between the neuropeptide and the biotin actually enhanced binding of the EGF to its cognate receptor (*23*). The PEG^{3400} linker eliminates steric hindrance of peptide binding to the EGF receptor caused by conjugation of the peptide to the BBB drug targeting MAb. However, in the case of the Aβ^{1-40} peptide analogues, the conjugation of the >200 atom long PEG^{3400} linker may interfere with Aβ^{1-40} binding to amyloid plaques, although this is only a partial inhibition (Figures 4 and 5).

In summary, these studies describe a novel formulation of Aβ^{1-40} that involves conjugation of DTPA to an internal lysine residue of Aβ^{1-40} that contains a biotin residue at the amino terminus. This allows for the dual conjugation of the amyloid imaging peptide to (a) 111-indium, and (b) a BBB drug targeting system, which is comprised of a peptidomimetic MAb and an avidin or streptavidin moiety. Genetically engineered MAb/avidin genes and fusion proteins have been prepared (*33*, *34*), and a chimeric form of the 83-14 HIRMAb has been genetically engineered (*35*). These advances, in conjunction with progress in the synthesis of multifunctional amyloid-imaging agents (Figure 1), may enable the administration of these conjugates in humans for brain imaging of amyloid in subjects living with AD.

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