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Retention of Biologic Activity of Human Epidermal Growth Factor Following Conjugation to a Blood-Brain Barrier Drug Delivery Vector via an Extended Poly(ethylene glycol) Linker

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Human brain gliomas overexpress the receptor for epidermal growth factor (EGF), and radiolabeled EGF is a potential peptide radiopharmaceutical for imaging human brain tumors, should this peptide be made transportable through the blood-brain barrier (BBB) in vivo. Peptide drug delivery to the brain may be facilitated by conjugating peptide radiopharmaceuticals to BBB drug delivery vectors such as the OX26 monoclonal antibody (MAb), which undergoes receptor-mediated transcytosis through the BBB via the brain capillary endothelial transferrin receptor. EGF was biotinylated with NHS-XX-biotin, where NHS = *N*-hydroxysuccinimide and -XX- = bis (aminohexanoyl) spacer arm. The [^{125}I]EGF-XX-biotin rapidly bound to C6 rat glioma cells transfected with the human EGF receptor. However, no binding to the C6 EGF receptor was detected when the [^{125}I]EGF-XX-biotin was bound to a conjugate of streptavidin (SA) and the OX26 MAb. An alternative linker strategy using poly(ethylene glycol) (PEG) of 3400 Da molecular mass (PEG^{3400}) was evaluated, wherein EGF was monobiotinylated with NHS-PEG^{3400}-biotin. Attachment of the [^{125}I]EGF-PEG^{3400}-biotin to the OX26/SA conjugate did not impair binding of the construct to the EGF receptor in C6 glioma cells. The length of the -PEG- spacer arm and the -XX- spacer arm was >200 atoms and 14 atoms, respectively. These studies demonstrate that the use of the extended PEG linker releases steric hindrance of MAb transport vectors on binding of EGF to its cognate receptor on glioma cells. Attachment of EGF peptide radiopharmaceuticals to BBB drug delivery systems such as the OX26 MAb using extended PEG linkers allows for retention of the bifunctionality of the conjugate with binding to both EGF and transferrin receptors.

INTRODUCTION

Human gliomas overexpress the epidermal growth factor (EGF) receptor (*1*). Therefore, radiolabeled EGF is a potential peptide radiopharmaceutical that could be used to image human brain tumors (*2*), using standard external detection modalities such as single photon emission computed tomography (SPECT). However, EGF does not cross the blood-brain barrier (BBB) (*3*). The BBB is intact in the early stages of brain tumor growth and at the margins of the tumor (*4*), especially to larger molecular weight molecules such as peptides or antibodies (*5*). Therefore, the use of EGF as a peptide radiopharmaceutical for imaging human brain tumors will require a BBB drug delivery system.

Peptide drug delivery to the brain is facilitated by conjugation of a nontransportable peptide therapeutic to a BBB delivery vector (*6*). The latter is comprised of a modified protein or receptor-specific monoclonal antibody (MAb) that undergoes receptor-mediated transcytosis through the BBB in vivo. Peptide pharmaceuticals have been delivered through the BBB in rats using an anti-transferrin receptor MAb (*7*) and in Rhesus monkeys using an anti-insulin receptor MAb (*8*). However, it is possible that once a peptide radiopharmaceutical is conjugated to a BBB drug delivery system, the peptide will no longer bind with high affinity to its cognate receptor on brain cells. In this event, prior studies have used a cleavable (disulfide) linker for attachment of the peptide pharmaceutical to the BBB delivery vector (*9*). However, in this case, release of the peptide from the delivery vector requires cleavage of the disulfide to activate the peptide pharmaceutical. Since most disulfide reductase activity may be within the cytosol of cells (*10*), the use of a cleavable disulfide linker requires that the BBB delivery system contain an endosomal release function. A different conjugation strategy, which is examined in the present studies, is to employ an extended, poly(ethylene glycol) (PEG) linker between the peptide radiopharmaceutical and the BBB transport vector, in this case the OX26 MAb which is specific for the rat transferrin receptor. The use of the extended PEG linker increases the length of the linker from 14 atoms, which is the case for the conventional bis (aminohexanoyl) spacer arm, to >200 atoms, in the case of a PEG linker of 3400 Da.

EXPERIMENTAL PROCEDURES

Materials. Na[^{125}I] was supplied by Amersham (Arlington Heights, IL). Biotin-XX-NHS was supplied by Cal Biochem (San Diego, CA), where XX = bis (aminohexanoyl) spacer arm and NHS = *N*-hydroxysuccinimide. NHS-PEG^{3400}-biotin was obtained from Shearwater Polymers (Huntsville, AL). Chloramine T was purchased from MCB Reagents (Cincinnati, OH). Recombinant streptavidin (SA), recombinant human epidermal growth factor (EGF), and all other reagents were obtained from Sigma

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Chemical Co. (St. Louis, MO). Vydac C4 (10 × 250 mm) reversed-phase HPLC columns were obtained from Separations Group (Hesperia, CA). TSK-gel G2000 SW_{XL} HPLC columns (7.8 × 300 mm) were obtained from TosoHaas (Montgomeryville, PA). High-Trap copper affinity columns (1 mL) and 1 × 30 cm Superose 12HR columns were obtained from Pharmacia (Piscataway, NJ). Centricon-30 microconcentrators were obtained from Amicon Corp. (Beverly, MA). C6 rat glioma cells transfected with the gene for the human EGF receptor (*2*) were kindly provided by Dr. Robert Fenstermaker of the Roswell Park Cancer Institute (Buffalo, NY), and these cells are designated C6EGFRp.

Preparation of [^{125}I]EGF-XX-biotin. NHS-XX-biotin (65 nmol in 10 μL of 100% dimethyl sulfoxide) was added in an 8:1 molar ratio to 50 μL of EGF (8.3 nmol in 0.05 M $NaHCO_3$, pH 8.3) and this mixture was incubated at room temperature for 60 min. The biotin-XX-EGF was directly iodinated by adding 6 μL (5 μg) to 20 μL of 0.3 M Na_2HPO_4, pH 7.0, followed by the addition of 2 mCi of ^{125}I and 10 μL (0.8 μg) of chloramine T. Following incubation at room temperature for 60 s, an additional 10 μL aliquot (0.8 μg) of chloramine T was added followed 60 s later by the addition of 50 μL (65 nmol) of sodium metabisulfite to terminate the iodination reaction. The mixture was then applied to a Sephadex G-25 gel filtration column followed by elution in 0.1 M Na_2HPO_4/0.15 M NaCl/pH 7.4/0.1% bovine serum albumin (BSA). The [^{125}I]EGF-XX-biotin was labeled to a specific activity of 548 μCi/nmol.

Preparation of [^{125}I]EGF-PEG^{3400}-biotin. NHS-PEG^{3400}-biotin (65 nmol in 100 μL of 0.05 M $NaHCO_3$) was added in an 8:1 molar ratio to 50 μg of EGF (8.0 nmol in 100 μL of 0.05 M $NaHCO_3$, pH 8.3) followed by incubation at room temperature for 60 min. Of this mixture, 20 μL was added to 20 μL of 0.3 M NaH_2PO_4 (pH 7.0) followed by the addition of 2 mCi of ^{125}I and 10 μL (0.8 μg) of chloramine T. After a 60 s incubation, an additional 10 μL (0.8 μg) of chloramine T was added, and 60 s later, the reaction was terminated by the addition of 50 μL (65 nmol) of sodium metabisulfite. Buffer A [0.05 M NaH_2PO_4/0.5 M NaCl/pH 7.0] was added to a volume of 1 mL and the mixture was then applied to a 1 mL High-Trap copper affinity column (*11*). The column was initially washed with 5 mL of water and charged with 0.5 mL of 0.1 M $CuSO_4$ followed by washing with 5 mL of water, followed by application of the sample. The column was washed with 10 mL of buffer A and the [^{125}I]EGF-PEG^{3400}-biotin was eluted with 5 mL of 50 mM imidazole in buffer A; 1 mL of this eluate (250 μCi) was added to a Centricon-3 microconcentrator and the volume was reduced to 200 μL by centrifugation at 7500 rpm (7500 g) for 40 min at room temperature. An aliquot of this fraction was then injected on the gel filtration HPLC column (see below) to determine the mobility of [^{125}I]EGF-PEG^{3400}-biotin. An aliquot was also injected into 2 Superose 12HR gel filtration columns in series and eluted with 0.01 M Na_2HPO_4/0.15 M NaCl/pH 7.4 at 0.5 mL/min, and 0.5 mL fractions were collected and detected at A_{220} by fast protein liquid chromatography (FPLC). The chemical identity of the EGF-[PEG-biotin]$_n$, where n = the number of PEG-biotin moieties per EGF, was determined by matrix assisted-laser desorption ionization mass spectrometry (MALDI). EGF-[PEG-biotin]$_3$ eluted at fractions 54−55 and constituted 7% of the total; EGF-[PEG-biotin]$_2$ and EGF-[PEG-biotin]$_1$ eluted at fractions 58−59 and 63−64 and constituted 35% and 43% of the total, respectively, and unconjugated EGF eluted at fractions 75−76 and comprised 15% of the total.

Preparation of EGF and OX26 MAb Conjugates. The conjugate of the OX26 MAb and recombinant streptavidin (SA) was prepared with a thiol-ether linkage as described previously (*12*). Briefly, 17 mg of OX26 MAb was thiolated with a 10:1 molar ratio of 2-iminothiolane; 7 mg of recombinant SA was activated with a 20:1 molar ratio of *m*-maleimidobenzoyl *N*-hydroxysuccinimide ester (MBS). The OX26/SA conjugate was labeled with 2.5 μCi of [^{3}H]biotin and was purified on a 2.6 × 92 cm column of Sephacryl S300HR (Pharmacia) followed by elution in 0.01 M Na_2HPO_4/0.15 M NaCl/pH 7.4/0.05% Tween-20 at 30 mL/h, and 3 mL fractions were collected. The conjugate peak was separated from either aggregates, unconjugated OX26 MAb, or unconjugated SA. The number of biotin-binding sites per OX26/SA conjugate was 3.3 ± 0.3, as determined with the [^{3}H]biotin binding assay (*12*).

The conjugate of OX26/SA and [^{125}I]EGF-PEG^{3400}-biotin was prepared by mixing 1 mL of the imidazole eluate from the High-Trap column containing 200 μCi of [^{125}I]EGF-PEG^{3400}-biotin (0.6 nmol) and 400 μg of OX26/SA (2.0 nmol). This volume was reduced to 0.2 mL with a Centricon-30 microconcentrator by centrifugation at 6300 rpm (5000 g) for 20 min at room temperature. The entire mixture was then applied to a TSK-gel G2000 SW_{XL} HPLC gel filtration column, followed by elution in 0.01 M Na_2HPO_4/0.15 M NaCl/pH 7.4/0.05% Tween-20 at a flow rate of 0.5 mL/min for 40 min, and 0.5 mL fractions were collected and counted for ^{125}I radioactivity. The conjugate of [^{125}I]EGF-PEG^{3400}-biotin bound to OX26/SA eluted at 7.0 mL from the column and this conjugate was subsequently used in the radioreceptor assays described below.

The conjugate of OX26/SA and [^{125}I]EGF-XX-biotin was prepared by mixing [^{125}I]EGF-XX-biotin (0.4 nmol) eluted from the Sephadex G-25 column with 60 μg of OX26/SA (0.30 nmol), followed by injection on the TSK G2000 SW_{XL} gel filtration HPLC column and elution as described above. The [^{125}I]EGF-XX-biotin bound to OX26/SA eluted at 7.0 mL from this column and was well separated from nonbiotinylated [^{125}I]EGF, which eluted at 11−12 mL.

C6EGFRp Radioreceptor Assays. C6EGFRp cells were grown in Dulbecco's modified Eagle medium (DMEM) with 4.5 g/L D-glucose, 4 mM glutamine, 10% fetal bovine serum, and 250 μg/mL G418, used as a selection agent, in 24-well cluster dishes. At 24 h prior to the radioreceptor assays, the medium was changed to DMEM with 4.5 g/L D-glucose, 1% fetal bovine serum, and 1 μM dexamethasone to induce expression of the EGF receptor (*2*). The assay was terminated by aspiration of medium, and washing wells with 0.01 M HEPES/0.15 M NaCl/pH 7.4 (HBS buffer). Radioreceptor assays were performed in the 24-well cluster dishes at 23 °C with 200 μL of final volume in the HBS buffer with 0.1% BSA. The wells contained 0.1 μCi/mL (0.18 nM) of [^{125}I]EGF conjugate and varying concentrations of unlabeled recombinant EGF ranging from 0.1 nM to 1 μM. Experimental incubations were performed from 15 to 120 min. The cell monolayer was solubilized by the addition of 0.5 mL of 1 N NaOH and incubated at 55 °C for 30 min or overnight at room temperature followed by counting for ^{125}I radioactivity and measurement of cell protein with the bicinchoninic acid (BCA) protein assay (Pierce Chemical Co.). Binding was expressed as percent of medium counts bound per milligram of cell protein and represents both surface bound and internalized ligand. Binding of the OX26/SA conjugate to C6 cells was confirmed by adding [^{3}H]biotin complexed to OX26/SA to cultured C6 cells; binding increased linearly with incubation time and was 4−5%/mgp at 60 min of incubation.

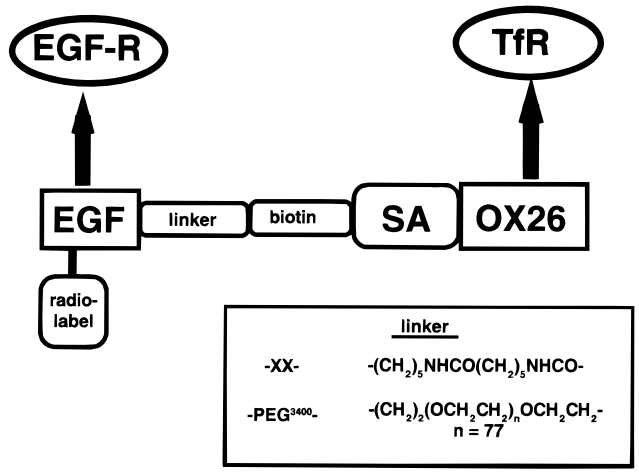

Figure 1. Structure of conjugate of EGF attached to the OX26 MAb using either a bis (aminohexanoyl) spacer arm (-XX-) or a 3400 Da poly(ethylene glycol) (PEG) spacer arm. The stable thioether linkage between OX26 and streptavidin (SA) is prepared in parallel with monobiotinylation of EGF using either NHS-XX-biotin or NHS-PEG^{3400}-biotin. The construct is prepared to ensure retention of the bifunctionality of the conjugate with binding to both the EGF receptor (EGF-R) via the EGF moiety or to the transferrin receptor (TfR) via the OX26 MAb moiety. The EGF is radiolabeled by iodination of EGF tyrosine residues. The length of the -XX- linker is 14 atoms and the length of the -PEG^{3400}- linker is >200 atoms.

RESULTS

The structure of the EGF/OX26 conjugate using a biotin/streptavidin linker is shown in Figure 1 for either the -XX- linker or the -PEG^{3400}- linker. The -XX- linker is 14 atoms long and the -PEG^{3400}- linker is >200 atoms long (Figure 1).

The binding of [^{125}I]EGF-XX-biotin to the C6EGFRp cells was avid and reached equilibrium by 60 min of incubation (Figure 2A) and was completely saturated by unconjugated EGF (Figure 2B). In contrast, conjugation of [^{125}I]EGF-XX-biotin to OX26/SA resulted in a more than 5-fold inhibition of binding of the EGF to the C6 cells (Figure 2A) and this binding was independent of unlabeled EGF added to the medium (Figure 2B). These results indicated that biotinylation, per se, of EGF did not impair binding to the EGF receptor, but attachment of EGF-XX-biotin to the OX26/SA drug delivery system essentially eliminated binding of the conjugate to the EGF receptor. Therefore, binding of the conjugate to the EGF receptor was assessed following the use of a PEG linker coupling EGF to OX26/SA.

The [^{125}I]EGF-PEG^{3400}-biotin eluted from TSK gel filtration HPLC column at a volume of 13.5 mL (Figure 3). However, after conjugation to OX26/SA, the [^{125}I]EGF-PEG^{3400}-biotin eluted at 7.0−7.5 mL from the column (Figure 3). Conjugation of EGF-PEG^{3400}-biotin to OX26/SA increased the molecular mass of the EGF construct from approximately 10 000 Da (Experimental Procedures) to 210 000 Da, since the OX26/SA has a size of 200 000 Da. The binding of [^{125}I]EGF-PEG^{3400}-biotin to the C6EGFRp cells was high (Figure 4A) and was comparable to the binding of the [^{125}I]EGF-XX-biotin (Figure 2A). The binding of [^{125}I]EGF-PEG^{3400}-biotin to the C6 cells was nearly completely displaced by the addition of unlabeled EGF to the medium, and the ED_{50} of this inhibition was approximately 1 nM (Figure 4B). This ED_{50} of EGF inhibition of the binding of [^{125}I]EGF-PEG^{3400}-biotin to the C6 cells approximates the ED_{50} of EGF inhibition of the binding [^{125}I]EGF-XX-biotin to these cells (Figure 2B). The conjugation of [^{125}I]EGF-PEG^{3400}-biotin to OX26/SA did not impair the binding of the EGF construct to the C6 cells (Figure 4A), and approximately 50% of the binding was inhibited by unlabeled EGF added to the medium (Figure 4B). The [^{125}I]EGF-XX-biotin or the [^{125}I]EGF-PEG^{3400}-biotin was metabolically stable during all radioreceptor assay incubations as the trichloroacetic acid (TCA) precipitability of medium ^{125}I radioactivity was >98% at all time points.

DISCUSSION

Human brain tumors frequently overexpress the EGF receptor (*13*, *14*). Radiolabeled monoclonal antibodies (MAb) to the EGF receptor have been used to image human gliomas in vivo (*15*). However, MAbs do not cross the BBB (*6*) and can image human brain tumors only when there is preexisting BBB disruption. For example, radiolabeled nonspecific MAbs image advanced human gliomas just as well as EGF receptor-specific MAbs, especially in patients with prior radiation therapy to the brain tumor (*15*), which causes BBB disruption (*16*). In the early stage of growth of a human brain tumor in an untreated patient, even metastatic tumors to brain (*17*),

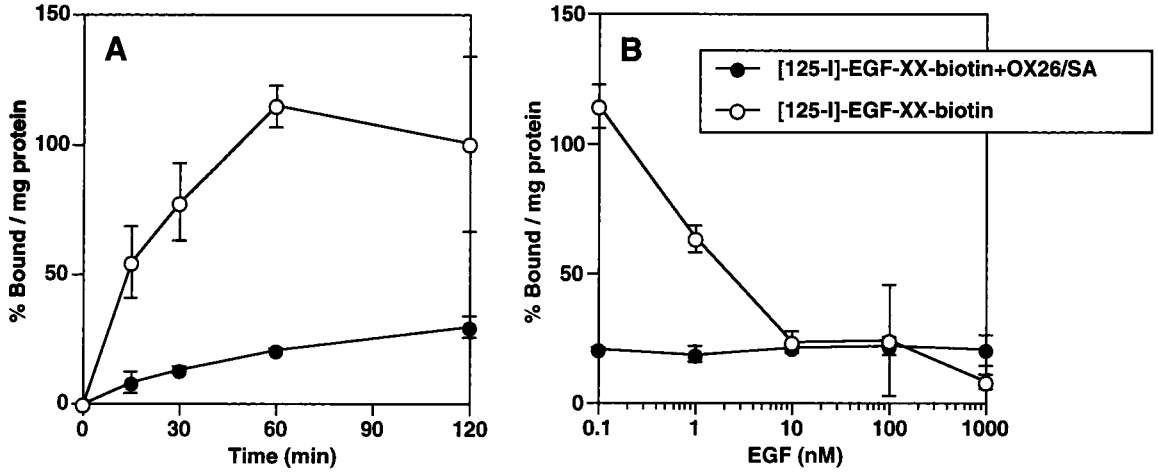

Figure 2. Radioreceptor assay showing binding of either [^{125}I]EGF-XX-biotin conjugated to OX26/SA (closed circles) or binding of [^{125}I]EGF-XX-biotin (open circles) to C6 rat glioma cells transfected with the human EGF receptor. Data are mean ± SE (n = 3 replicas at each point). (A) Time course of binding of either construct to the cells. (B) Saturation analysis was performed at 60 min incubations by adding varying concentrations of unlabeled recombinant EGF to the medium.

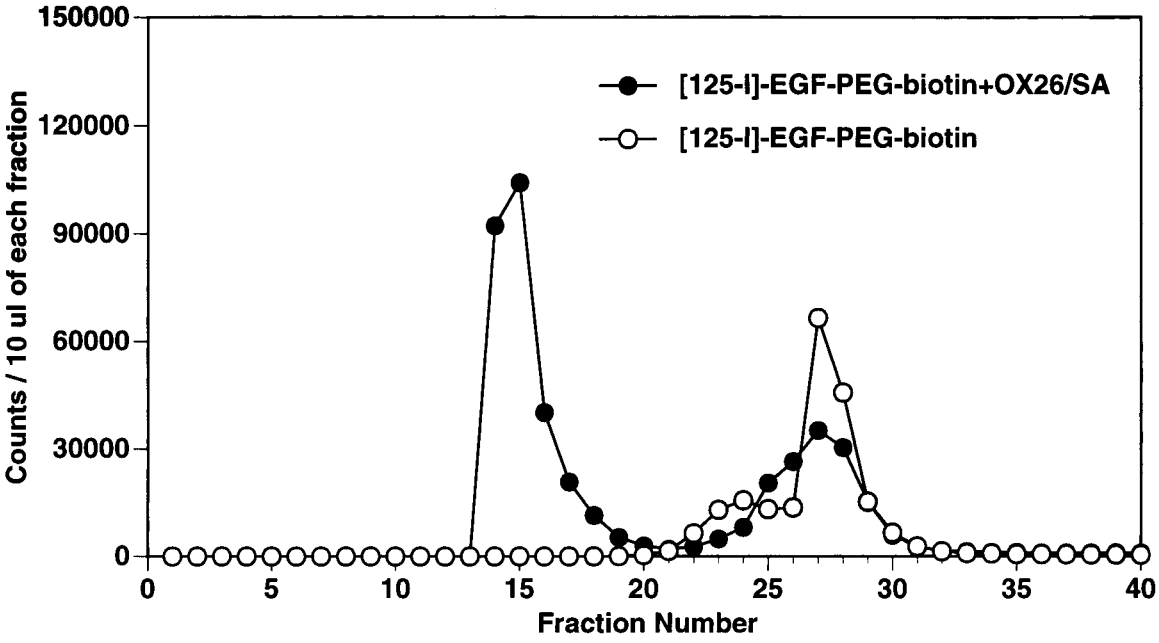

Figure 3. Elution of either [^{125}I]EGF-PEG^{3400}-biotin (open circles) or [^{125}I]EGF-PEG^{3400}-biotin conjugated to OX26/SA (closed circles) from a TSK-gel G2000 SW_{XL} gel filtration HPLC column. Each fraction was 0.5 mL and was accounted for ^{125}I radioactivity.

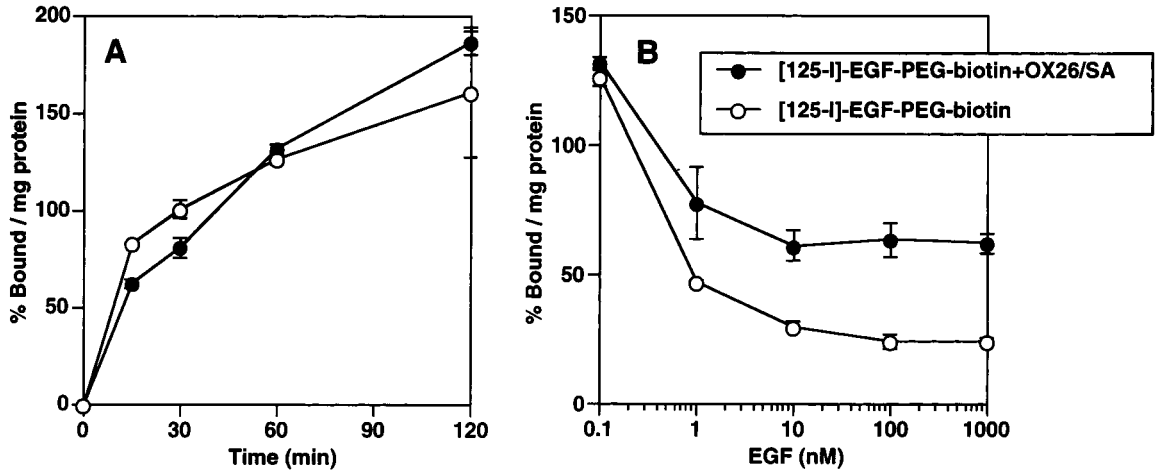

Figure 4. Radioreceptor assay showing binding of either [^{125}I]EGF-PEG^{3400}-biotin conjugated to OX26/SA (closed circles) or binding of [^{125}I]EGF-PEG^{3400}-biotin (open circles) to C6 rat glioma cells transfected with the human EGF receptor. Data are mean ± SE (n = 3 replicas at each point). (A) Time course of binding of either construct to the cells. (B) Saturation analysis was performed at 60 min incubations by adding varying concentrations of unlabeled recombinant EGF to the medium.

the BBB is intact, and it is at this stage that imaging and diagnosis of nascent brain tumors is most needed. Imaging of early brain tumors where the BBB is intact with either MAbs or peptide radiopharmaceuticals such as EGF requires the attachment of the tumor imaging agent to a BBB drug delivery system. The present studies make an initial evaluation of the molecular formulation required to attach EGF to a BBB transport vector in such a way that binding of the peptide to the EGF receptor is retained following conjugation to the delivery vector.

The results of these studies are consistent with the following conclusions. First, human EGF can be biotinylated with succinimide esters of biotin to target the ϵ-amino groups of lysine residues, with retention of ligand affinity for the EGF receptor. Second, when EGF-XX-biotin is conjugated to OX26/SA, the EGF no longer binds to the EGF receptor (Figure 2). Third, substitution of the 14 atom bis(aminohexanoyl) spacer arm between the biotin moiety and the EGF with a >200 atom extended poly(ethylene glycol) linker restores affinity of EGF for its cognate receptor despite conjugation of the peptide to the BBB delivery vector, OX26/SA (Figure 4).

Biotinylation of EGF with NHS-XX-biotin targets the ϵ-amino moiety of surface lysine residues. Murine EGF lacks internal lysine residues, but human EGF has 2 lysine residues at positions 28 and 48 (*18*, *19*). Although the amino terminus is also a target for biotinylation with succinimide esters, the biotinylation was performed at pH 8.3, which preferentially exposes the ϵ-amino moieties of lysine residues to the succinimide ester (*20*). The EGF-XX-biotin still binds to the human EGF receptor in transfected C6 rat glioma cells as EGF inhibits this binding with an ED_{50} of approximately 1 nM (Figure 2). The total binding of either [^{125}I]EGF-XX-biotin or [^{125}I]EGF-PEG^{3400}-biotin, expressed as percent bound per $(\text{milligram})_p$, is nearly identical to the total binding of unconjugated [^{125}I]EGF to the C6-$EGFR_p$ cells (*2*), indicating that neither biotinylation nor pegylation, per se, impairs ligand binding to the human EGF receptor. These results are consistent with earlier immunocytochemistry experi-

ments showing that biotinylated EGF binds to the EGF receptor in frozen sections of human glioma cells in vitro (*1*). Moreover, EGF-biotin detects the EGF receptor on human glioma cells immunocytochemically with a greater sensitivity than that achieved with monoclonal antibodies to the EGF receptor (*1*). The retention of ligand affinity for its cognate receptor following biotinylation on lysine residues, and the fact that mouse EGF lacks internal lysine residues (*18*, *19*), suggests that the lysine residues at positions 28 or 48 of human EGF do not participate in binding to the EGF receptor. However, when the OX26 MAb is conjugated to streptavidin via a stable thioether linkage and this complex is then bound to EGF-XX-biotin, the EGF no longer binds to the EGF receptor (Figure 2). These observations suggest that binding of EGF-XX-biotin to OX26/SA causes significant steric hindrance of binding of EGF to its cognate receptor. Although a vasoactive intestinal peptide (VIP) analogue has been successfully attached to OX26/SA with the biotin-XX-linker, and with retention of peptide affinity for the cognate receptor (*12*), the attachment of opioid peptides to OX26/SA with this linker eliminates peptide affinity for the opioid receptor (*9*). In the event that the non-cleavable (amide) -XX- linker eliminates ligand and receptor binding following attachment of the peptide to the delivery vector, previous studies have employed a cleavable (disulfide) -SS- linker between the biotin moiety and the therapeutic peptide. In this case, the peptide becomes biologically active with restoration of affinity for its cognate receptor following cleavage of the disulfide bond and release of the peptide from the OX26/SA delivery system (*9*). However, if the majority of the disulfide reductase resides in the cytosol of brain cells (*10*), it is necessary for the vector-SS-peptide conjugate to undergo release from the intracellular endosomal compartment following receptor-mediated endocytosis into the brain cell. In the absence of an obligatory endosomal release function built into the delivery system, the exposure of the conjugate to the cytoplasmic disulfide reductase may be minimal.

Given the potential limitations of either the noncleavable (amide) -XX- linker or the cleavable (disulfide) -SS- linker, there is a need for a third approach for conjugating peptide therapeutics to MAb-based BBB delivery systems that allows for retention of receptor affinity of the peptide therapeutic but does not require a disulfide cleavage. These requirements are satisfied with the use of an extended PEG linker between the biotin moiety and the peptide therapeutic. The radioreceptor assays in Figure 4 show that EGF-PEG^{3400}-biotin binds with high affinity to the EGF receptor, and the ED_{50} of EGF inhibition of this binding is 1 nM, which is identical to ED_{50} of EGF inhibition of EGF-XX-biotin binding to the EGF receptor (Figure 2). Therefore, the placement of the 3400 Da PEG moiety on a surface lysine residue of EGF does not impair peptide binding to the EGF receptor. When the EGF-PEG^{3400}-biotin is conjugated to OX26/SA, there is no impairment in the binding of the EGF to its cognate receptor on C6 glioma cells (Figure 4). The time course of EGF-PEG^{3400}-biotin binding to C6 glioma cells is identical with or without attachment to OX26/SA (Figure 4A). In addition, more than 50% of the binding of [^{125}I]-EGF-PEG^{3400}-biotin conjugated to OX26/SA is displaced by native EGF in the medium (Figure 4B). In the presence of saturating concentrations of EGF (>10 nM) there is still significant binding of [^{125}I]EGF-PEG^{3400}-biotin conjugated to OX26/SA (Figure 4B), and this residual binding is a result of conjugate binding to the transferrin receptor on the C6 glioma cells.

In summary, these studies describe the molecular formulation of an EGF peptide radiopharmaceutical that is conjugated to the OX26 MAb BBB drug delivery system, using an extended poly(ethylene glycol) linker between EGF and the biotin moiety. In contrast to a short bis(aminohexanoyl) -XX- linker, the PEG linker allows for retention of EGF binding to its cognate receptor on rat glioma cells. Many human brain gliomas overexpress the EGF receptor (*1*, *13*, *14*), and EGF peptide radiopharmaceuticals are potential imaging agents for detection of these tumors (*2*). However, EGF does not cross the BBB (*3*), which is consistent with the absence of EGF receptors on endothelial cells (*21*). An optimal use of EGF peptide radiopharmaceuticals to image human brain tumors may require a molecular reformulation of the peptide that involves conjugation to a BBB drug delivery vector.

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