Cy5.5-Aminohexanoic acid-RPLALWRS-aminohexanoic acid-C-G4-PAMAM-PEG-AF750

PB-M7NIR

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Chemical name:	Cy5.5-Aminohexanoic acid-RPLALWRS-aminohexanoic acid-C-G4-PAMAM-PEG-AF750	
Abbreviated name:	PB-M7NIR	
Synonym:		
Agent category:	Peptide	
Target:	Matrix metalloproteinase-7 (MMP-7)	
Target category:	Enzyme	
Method of detection:	Optical, near-infrared (NIR) fluorescence	
Source of signal/contrast:	Cy5.5	
Activation:	Yes	
Studies:	• *In vitro* • Rodents	No structure is currently available in PubChem.

Background

[PubMed]

Optical fluorescence imaging is increasingly used to obtain biological functions of specific targets (1, 2). However, the intrinsic fluorescence of biomolecules poses a problem when fluorophores that absorb visible light (350–700 nm) are used. Near-infrared (NIR) fluorescence (700–1,000 nm) detection avoids the background fluorescence interference of natural biomolecules, providing a high contrast between target and background tissues. NIR fluorophores have wider dynamic range and minimal background as a result of

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reduced scattering compared with visible fluorescence detection. They also have high sensitivity, resulting from low infrared background, and high extinction coefficients, which provide high quantum yields. The NIR region is also compatible with solid-state optical components, such as diode lasers and silicon detectors. NIR fluorescence imaging is becoming a non-invasive alternative to radionuclide imaging.

Extracellular matrix (ECM) adhesion molecules consist of a complex network of fibronectins, collagens, chondroitins, laminins, glycoproteins, heparin sulfate, tenascins, and proteoglycans that surround connective tissue cells, and they are mainly secreted by fibroblasts, chondroblasts, and osteoblasts (3). Cell substrate adhesion molecules are considered essential regulators of cell migration, differentiation, and tissue integrity and remodeling. These molecules play a role in inflammation and atherogenesis, but they also participate in the process of invasion and metastasis of malignant cells in the host tissue (4). Invasive tumor cells adhere to the ECM, which provides a matrix environment for permeation of tumor cells through the basal lamina and underlying interstitial stroma of the connective tissue. Overexpression of matrix metalloproteinases (MMPs) and other proteases by tumor cells allows intravasation of tumor cells into the circulatory system after degrading the basement membrane and ECM (5).

Several families of MMPs are involved in atherogenesis, myocardial infarction, angiogenesis, and tumor invasion and metastases (6-9). MMP expression in normal cells, such as trophoblasts, osteoclasts, neutrophils, and macrophages, is highly regulated. Elevated levels of MMPs have been found in tumors associated with a poor prognosis for cancer patients (10). The peptide aminohexanoic acid(AXH)-RPLALWRS-(AXH)-C (M7) was found to be a MMP-7 substrate that is cleaved between the L and W residues. Scherer et al. (11) used this sequence with a Cy5.5 NIR dye molecule to attach to a Generation 4–polyamidoamine-polyethylene glycol (PEG)-AF750 dendrimer to form fluorescence-quenched dendrimer, Cy5.5-(AXH)-RPLALWRS-(AXH)-C-G4-PAMAM-PEG-AF750 (PB-M7NIR). The Cy5.5 molecules are in close proximity, which results in fluorescence quenching because of the close proximity of the Cy5.5 molecules. AF750 molecules are conjugated to the dendrimer and act as an internal fluorescence reference. The NIR fluorescence signal will increase when the L-W bond is cleaved by MMP-7, releasing Cy5.5-containing fragments. Cy5.5 is a NIR fluorescent dye with an absorbance maximum at 675 nm and an emission maximum at 694 nm with a high extinction coefficient of 250,000 $M^{-1}cm^{-1}$. PB-M7NIR is being developed for NIR fluorescence imaging of MMP-7 expression in tumors, atherosclerosis, myocardial infarction, and other diseases.

Synthesis

[PubMed]

Scherer et al. (11) prepared PB-M7NIR by conjugation of Cy5.5-M7 to G4-PAMAM-PEG-AF750. A methanolic solution (5 mM) of the M7 peptide was reacted with 0.8 molar equivalent of Cy5.5-NHS in dimethyl sulfoxide (DMSO) (7 mM), and triethylamine was

added to 1% (v/v) to fluorescently label the peptide at the N-terminal amine. Cy5.5-M7 was coupled to G4-PAMAM-PEG. To synthesize the thioether-bonded conjugate in Cy5.5-M7-PAMAM-PEG, the PAMAM-PEG conjugate was first activated by treatment with *N*-succinimidyl iodoacetate (SIA) (8 mg/ml methanol, 20 molar equivalents/PAMAM). After reaction for 20 minutes at room temperature, the SIA-activated PAMAM-PEG was incubated with the reduced Cy5.5-M7 peptides in methanolic solution (8 peptides/PAMAM) for 24 hours at room temperature. To label the PAMAM dendrimer scaffold with AF750, the Cy5.5-M7-PAMAM-PEG was dissolved in 50 mM in Na_2CO_3 (pH 9) and reacted for 18 h with up to 8 molar equivalents of AF750-NHS (7 mM in DMSO). PB-M7NIR was purified with ultrafiltration. There were approximately eight molecules of Cy5.5-M7 and approximately six molecules of AF750 per PB-M7NIR.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Scherer et al. (11) showed that PB-M7NIR exhibited five-fold increases in Cy5.5 NIR fluorescence signal with MMP-7 incubation with no change in the AF750 reference fluorescence signal. McIntyre et al. (12) showed that FITC-M7-PAMAM-TMR (PB-M7VIS) was cleaved by MMP-2, MMP-3, and MMP-7. However, PB-M7VIS was 56-fold more active with MMP-7 than with MMP-2 and 13-fold more active with MMP-7 than with MMP-3. PB-M7VIS was not a substrate for cathepsin B or L.

Animal Studies

Rodents

[PubMed]

Scherer et al. (11) performed biodistribution studies of PB-M7NIR (1 nmol/mouse) injected intravenously to mice (n = 13) bearing SW480neo human colon cancer cells (MMP-7–negative) on one flank and SW480mat human colon cancer cells (MMP-7–positive) on the opposite flank. Optical imaging was performed in the Cy5.5 and AF750 channels for 4 h after injection. Cy5.5 NIR fluorescence signal above the background was visualized at 1 h and increased by approximately one-fold at 3 h as compared with the signal at 1 h. On the other hand, the AF750 reference signal was lower than the Cy5.5 signal with little increase over time. The Cy5.5/AF750 ratios at 4 h after injection were 70 for the MMP-7–negative tumors and 275 for the MMP-7–positive tumors. Histological imaging of tumor sections revealed a strong Cy5.5 signal at the tumor-stroma interface and in the ECM of MMP-7–positive tumor sections, but not in the MMP-7–negative tumor sections with Cy5.5/AF750 ratios of 692 and 2, respectively. No blocking or MMP-7 inhibition experiments were performed.

Other Non-Primate Mammals

[PubMed]

No publication is currently available.

Non-Human Primates

[PubMed]

No publication is currently available.

Human Studies

[PubMed]

No publication is currently available.

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