Radiolabeling of Conjugated Antibodies with ^{225}Ac for Targeted Alpha Therapy

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Horizon-Broadening Isotope Production Pipeline Opportunities

1. Abstract

Radiopharmaceuticals have been of interest in the nuclear medicine field because of their ability to diagnose and treat cancer non-invasively and with high efficacy. Radionuclides that decay via alpha emission have been of particular interest due to their high linear-energy transfer (LET). One such alpha emitter, ^{225}Ac, is promising for targeted alpha therapy (TAT) because of its favorable nuclear properties. Targeted alpha therapy consists of conjugating a chelating agent to a targeting vector and labeling it with an alpha-emitting radionuclide. The resulting complex provides a radioactive payload to a specific tumor site, causing double-strand DNA breaks in malignant cells. Our collaborators at Cold Spring Harbor recently developed a novel monoclonal tumor organoid-binding antibody named TOBi-89, which selectively targets pancreatic ductal adenocarcinoma (PDAC) and showed promising results for *in vivo* PET imaging applications.[1] In the present work, a one-step radiolabeling method was investigated for DOTA-antibody conjugates with ^{225}Ac for use in targeted alpha therapy. Significant radiochemical yield was not found with either DOTA-trastuzumab or free DOTA with the reaction conditions tested; further radiolabeling optimization is needed.

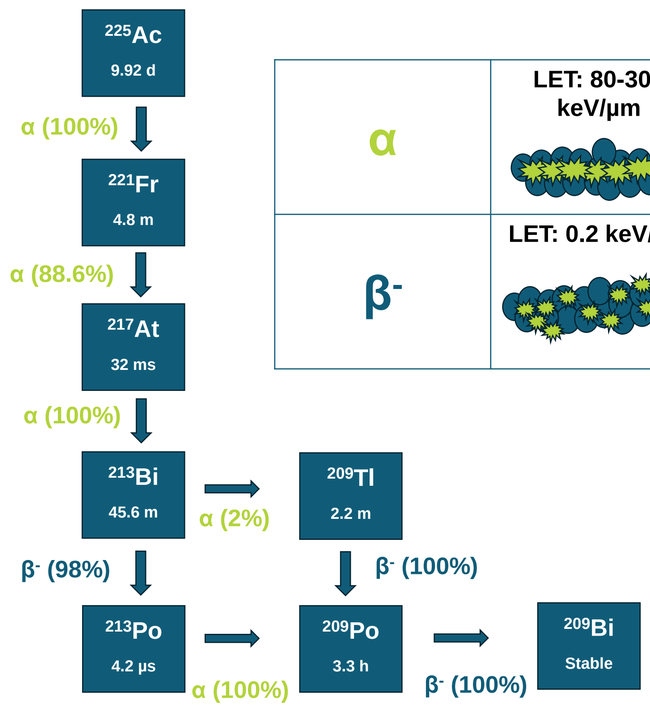
2. Introduction

- Traditionally, radiolabeling of DOTA has required the use of high-temperatures above 60 °C to stably complex ^{225}Ac.[2]
- Most full-length antibodies denature at temperatures above 55 °C.
- Traditional labeling methods cannot be used to label a DOTA-antibody conjugate in one step.
- Two-step labeling methods generally present low yields after purification[2], so a one-step labeling method is desirable.

3. Methods

- Conjugation of DOTA-NCS with trastuzumab was adapted from previously reported procedures.[3] Trastuzumab was used as a surrogate for TOBi-89 due to TOBi-89's limited availability.
- The DOTA-trastuzumab was labeled with ^{225}Ac via two previously reported protocols.[4,5]
- Unconjugated "free" DOTA-NCS was also labeled with ^{225}Ac to assess its capacity for complexation.
- Different variables such as the age/batch of the ^{225}Ac, age/batch of DOTA-NCS, presence of ascorbic acid in the reaction, reaction temperature, and reaction time were tested.
- The radiochemical yield was assessed using radio-iTLC, which were developed in a 10 mM EDTA solution of pH 7, and a 9% NaCl/10 mM NaOH solution.

Figure 1: ^{225}Ac Decay Chain

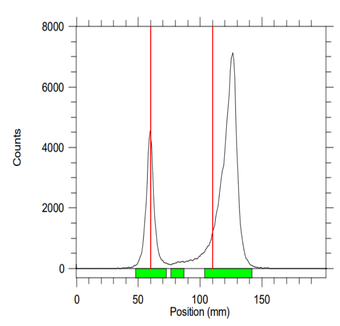


Labeling Methods

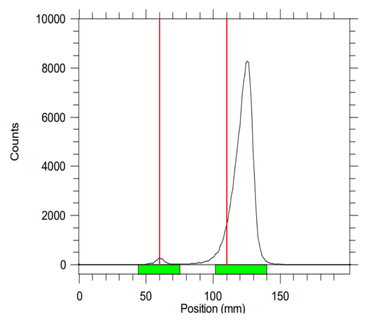
Method 1	Method 2
• 0.1 mg DOTA-trastuzumab • 20 µL of 150 mg/mL ascorbic acid • 25 µL 2M TMAA buffer • 15 µCi ^{225}Ac React at 37 °C Timepoints: 15, 30, 60, 120 min	• 0.1 mg DOTA-trastuzumab • 100 µL 2M tris buffer • 50 µL 20% ascorbic acid • 15 µCi ^{225}Ac React at 45 °C Timepoints: 15, 30, 60, 120 min

Table 1: [^{225}Ac]Ac-DOTA-Trastuzumab Labeling Studies

Variable	Labeling Method	RCY (t = 2 h) n = 2
Fresh ^{225}Ac	Method 2	6.21%
Fresh ^{225}Ac	Method 1	29.96%
Old ^{225}Ac	Method 2	11.96%



Radio-iTLC strip from July 2nd, using labeling method 1 at the 2-hour time point. RCY = 42.22%, solvent system was 10 mM EDTA, pH 7. Complexed ^{225}Ac will stay at the solvent front, while "free" ^{225}Ac will travel.



Rerun of the same iTLC strip 24 hours later. RCY = 1.90%. It is hypothesized that ^{213}Bi was being complexed instead of ^{225}Ac. This necessitated the need to optimize labeling of free DOTA.

Table 2: [^{225}Ac]Ac-DOTA Labeling Studies (conducted standardly at 60 °C)

Variable	Labeling Method	RCY (t = 1 h) n = 2
Freshly prepared reagents	Method 1	2.17%
Freshly prepared reagents	Method 2	2.47%
Presence of ascorbic acid (+)	Method 2	0.85%
Presence of ascorbic acid (-)	Method 2	6.33%
90 °C	Method 2	4.42%
Old DOTA-NCS	Method 2	5.39%

5. Discussion

- Labeling yields remained low throughout a variety of reaction conditions.
- Above-average labeling yields were observed with method 1 when labeling DOTA-trastuzumab, but it appears that ^{225}Ac daughters, such as ^{213}Bi, were competitively complexing with DOTA.
- Complexation of DOTA with ^{225}Ac seemed to be influenced minimally by the reaction conditions tested, however the age/batch of the DOTA-NCS and presence of ascorbic acid seemed to affect the radiochemical yield the most.
- iTLC data showed that EDTA was competitively binding with ^{225}Ac, demonstrating that the DOTA is not completely and stably complexing.

6. Future Directions

- Continue to determine the effect of the batch and age of ^{225}Ac on the labeling.
- Investigate the optimal pH for deprotonation and complete complexation with the DOTA chelator.
- Test different chelators to fit the Ac^{3+} ionic radius
- Develop efficient iTLC solvent systems and update reagents.

7. References

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8. Acknowledgements

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