Optimising conditions for radiolabelling of DOTA-peptides with ^{90}Y, ^{111}In and ^{177}Lu at high specific activities

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Abstract. DOTA-conjugated peptides, such as [DOTA^0,Tyr^3]octreotide (DOTATOC) and [DOTA^0,Tyr^3]octreotate (DOTA-tate), can be labelled with radionuclides such as ^{90}Y, ^{111}In and ^{177}Lu. These radiolabelled somatostatin analogues are used for peptide receptor radionuclide therapy (PRRT). Radioligands for PRRT require high specific activities. However, although these radionuclides are produced without addition of carrier, contaminants are introduced during production and as decay products. In this study, parameters influencing the kinetics of labelling of DOTA-peptides were investigated and conditions were optimised to obtain the highest achievable specific activity. The effects of contaminants were systematically investigated, concentration dependently, in a test model mimicking conditions for labelling with minimal molar excess of DOTA-peptides over radionuclide. Kinetics of labelling of DOTA-peptides were optimal at pH 4–4.5; pH <4 strongly slowed down the kinetics. Above pH 5, reaction kinetics varied owing to the formation of radionuclide hydroxides. Labelling with ^{90}Y and ^{177}Lu was completed after 20 min at 80°C, while labelling with ^{111}In was completed after 30 min at 100°C. The effects of contaminants were systematically categorised, e.g. Cd^{2+} is the target and decay product of ^{111}In, and it was found to be a strong competitor with ^{111}In for incorporation in DOTA. In contrast, Zr^{4+} and Hf^{4+}, decay products of ^{90}Y and ^{177}Lu, respectively, did not interfere with the incorporation of these radionuclides. The following conclusions are drawn: (a) DOTA-peptides can be radiolabelled at high specific activity; (b) reaction kinetics differ for each radionuclide; and (c) reactions can be hampered by contaminants, such as target material and decay products.

Keywords: DOTA – Radiolabelling – Specific activity

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Introduction

DOTA-conjugated peptides, such as the stable somatostatin analogues [DOTA^0,Tyr^3]octreotate (DOTA-tate) and [DOTA^0,Tyr^3]octreotide (DOTATOC), can be readily labelled with radionuclides such as ^{90}Y, ^{111}In and ^{177}Lu. In order for these radiolabelled peptides to be successfully used in peptide receptor radionuclide therapy (PRRT) [1, 2, 3, 4, 5, 6], high specific activities (SAs) are required. A number of biological factors dictate the need for a high SA. First, for in vivo use the amount of (radio)ligand that can be administered is limited by affinity and the amount of receptors. Above the optimal dose a further increase in ligand will increase the competition between unlabelled and labelled ligand for the same receptor and thus lower the uptake of radiolabel into receptor-positive tissue [4]. Second, for peptides that display pharmacological (side)effects, such as DOTA-substance P or DOTA-bombesin, only very small quantities of peptides may be tolerated. For the latter peptide, the amount that can be administered intravenously is limited to 0.1 nmol per minute [7]. Therefore, a high SA will reduce the total peptide amount to be administered. Third, endocytotic mechanisms that affect the cellular internalisation of peptides may become desensitised at high peptide concentrations [8], resulting in lower uptake of radiolabel into target tissue. Additionally, in vitro investigations aimed at measuring receptor-binding affinities require low concentrations of these radioligands (e.g. 10^{-10} *M*) in order to measure receptor–ligand interactions accurately. Unfortunately, the need for high SA is often compromised by conflicting radiochemical parameters that determine reaction rates and yields, i.e. the rate of formation of the metal-DOTA complexes increases with pH [9], but the solubility of In^{3+}, Y^{3+} and Lu^{3+} decreases with pH owing to formation

Table 1. Production methods, target materials, decay products, physical constants and maximal SA for the radionuclides considered

	^{57}Co	^{67}Ga	^{90}Y	^{111}In	^{177}Lu
Production method	Reactor (n, pn)	Cyclotron (p, 2n)	Generator	Cyclotron (p, 2n)	Reactor (n)
Target	^{58}Ni	^{68}Zn	^{90}Sr	^{112}Cd	^{176}Lu
Decay product	^{57}Fe	^{67}Zn	^{90}Zr	^{111}Cd	^{177}Hf
Physical constants					
$t_{1/2}$ (days)	270.9	3.26	2.67	2.83	6.71
pmoles per mCi	2080	25	20.5	21.5	51.4
Maximal SA (mCi per nmol)					
Theory[a]	0.5	40	49	46	19
In practice[b]	n.d.	10	11	22	3

Labelling conditions with ^{57}Co and ^{67}Ga were not optimised, but taken to be identical to those of ^{111}In: 30 min at 100°C
SA, Specific activity, expressed as mCi per nmol; n.d., not determined
[a] Since 1 nmol DOTA can incorporate 1 nmol (radio)nuclide, this number indicates the maximal theoretical SA of the radiolabelled DOTA-peptides
[b] Highest value achieved; this implies a ratio of DOTA over radionuclide [practice (b) over theory(a)] of 4, 4 1/2 and 2 1/4 for ^{67}Ga, ^{90}Y and ^{111}In, respectively

of hydroxides [10]. Although recently labelling of DOTA analogues with ^{90}Y and ^{177}Lu was reported at pH 7–8 [11, 12], we encountered the above-mentioned solubility problems when high concentrations of these radionuclides were used at pH 7–8. Therefore we decided to perform this study at pH 5 or lower.

The studies presented here were undertaken to determine the optimal conditions for radiolabelling DOTA-peptides, using the radionuclides ^{90}Y, ^{111}In and ^{177}Lu and DOTATOC and DOTA-tate as model reactants. We investigated parameters that influence reaction kinetics and radiochemical yields in order to define conditions that result in maximal achievable SAs. This report also summarises the effects of ever-present contaminants, such as nuclides formed by decay of the radionuclides.

Materials and methods

Ligands and radionuclides. DOTA-peptides were dissolved in 0.01–0.05 *M* acetic acid in Milli-Q water. As the buffer, 25 m*M* sodium ascorbate (quencher) in 50 m*M* sodium acetate was used. ^{90}Y was from Pacific Northwest National Laboratory (Richland, Wash., USA) and from MDS Nordion (Fleurus, Belgium). ^{177}Lu was from Missouri University Reactor Research (MURR, St. Louis, Mo., USA) and from NRG (Petten, The Netherlands). $^{57}CoCl_2$ in 0.1 *M* HCl was from MDS Nordion (Vancouver, BC, Canada). $^{111}InCl_3$ was from Mallinckrodt Medical (Petten, The Netherlands). All these radionuclides were delivered in 0.01–0.2 *N* HCl. $^{67}GaCl_3$ in 0.7–0.8 *M* HCl was from Mallinckrodt Medical (Petten, The Netherlands) and from MDS Nordion (Vancouver, BC, Canada) in 0.05 *M* HCl.

Reaction conditions: the effects of pH and temperature. The experiments were performed in small volumes (typically 40–75 µl) using double-sealed plastic reaction tubes (PCR thermocycler tubes, max volume 125 µl, MoBiTec, ITK Diagnostics, Uithoorn, The Netherlands). Heating was performed in a temperature-controlled heating block (Grant, Fisher Scientific, Zoeterwoude, The Netherlands). The pH of the reaction mixture was measured after the reaction. All experiments were carried at least in duplicate, using at least three different production batches of the radionuclides from the suppliers mentioned above. All chemicals were purchased from Aldrich Chemicals (Zwijndrecht, The Netherlands), and were of the highest analytical grade available. The radiochemical purity of the radiolabelled DOTATOC and DOTA-tate was studied at room temperature in the presence of 4 m*M* DTPA pH 5. High-performance liquid chromatography and instant thin-layer chromatography were performed as described previously (see [13] and [14] respectively)..

Maximal achievable SA, and effects of contaminants. In order to determine the maximal achievable SA (≥ 98% incorporation of ^{67}Ga, ^{90}Y, ^{111}In or ^{177}Lu), radiolabelling was performed using a constant amount of the radionuclides and increasing amounts of ligand. To investigate the effects of the contaminants present, experiments were performed adding known amounts of contaminants to the reaction vial at the start of the radiolabelling. For all radionuclides considered, the target material and the decay products are summarised in Table 1, and all were tested concentration dependently. The starting conditions in the absence of added contaminants were chosen to be critical, using a low mol/mol ratio of DOTA over radionuclide.

Results

Reaction kinetics

Reaction kinetics with ^{90}Y, ^{111}In and ^{177}Lu were found to be optimal at pH 4–4.5, with a steep decrease at lower

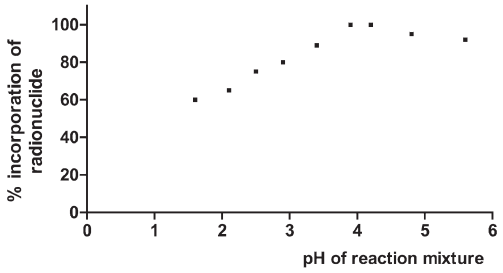

Fig. 1. Formation of ^{177}Lu-DOTATOC as a function of pH after 20 min at 80°C, as measured by the % incorporation of the radionuclide. Similar results were found with ^{90}Y and with DOTA-tate as ligand

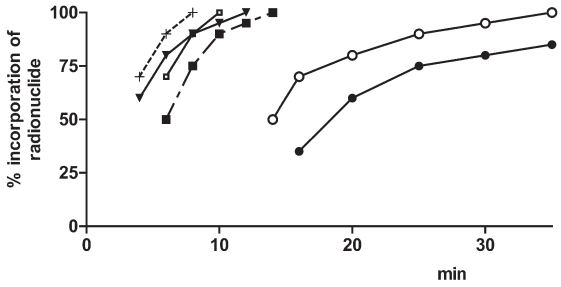

Fig. 2. Formation of radiolabelled DOTATOC at pH 4 as a function of time of incubation, as measured by the % incorporation of the radionuclide. Similar results were found with DOTA-tate as ligand. +, ^{177}Lu at 80°C; ▼, ^{177}Lu at 100°C; ■, ^{90}Y at 80°C; □, ^{90}Y at 100°C; ●, ^{111}In at 80°C; ❍, ^{111}In at 100°C

pH and a slow decrease at higher pH (Fig. 1). In addition, % incorporation of ^{111}In and ^{177}Lu at pH ≥5 became non-reproducible: after centrifugation of these reaction vials, precipitation was found. The reaction kinetics were also found to be time- and temperature-dependent: reactions were complete with ^{90}Y and ^{177}Lu after 20 min at 80°C, and with ^{111}In after 30 min at 100°C (Fig. 2). Table 1 shows the highest achieved SAs of ^{67}Ga, ^{90}Y and ^{111}In, implying a mol/mol ratio of DOTA over radionuclide of 4, 4 1/2 and 2 1/4, respectively. For ^{177}Lu the mol/mol ratio was even 1.2 (see also Discussion). We were still able to label at high SAs 2 weeks after the production of ^{177}Lu (data not shown).

Effects of contaminants of maximal achievable SA

To validate the test model the effect of the addition of unlabelled Y, In and Lu to the corresponding radionuclide was investigated. As expected, the dilution of the SA of the radionuclide decreased its incorporation in the DOTA-chelator in a concentration-dependent manner (Fig. 3, Table 2). The addition of nuclides such as Hf, Zr and Sr had no effect on the % incorporation of the radionuclides, indicating that these nuclides are not competitors under these reaction conditions. In contrast, the effect of addition of nuclides such as Fe and Cd clearly showed that they are strong competitors for the incorporation of radionuclide in the DOTA-chelator, as shown in Fig. 3 and Table 2. Table 1 also contains data on labelling experiments with ^{57}Co and ^{67}Ga using the same conditions as those for ^{111}In, 30 min at 100°C. With the Mallinckrodt-produced ^{67}Ga, pH was difficult to control since the radionuclide is delivered in 0.7–0.8 *M* HCl. More importantly, the [Zn] (Zn is the target and decay product of ^{67}Ga, see Table 1) was measured by ICP (data not shown) and found to exceed [Ga] frequently by >100-fold, with a dramatic effect on achieving high SAs.

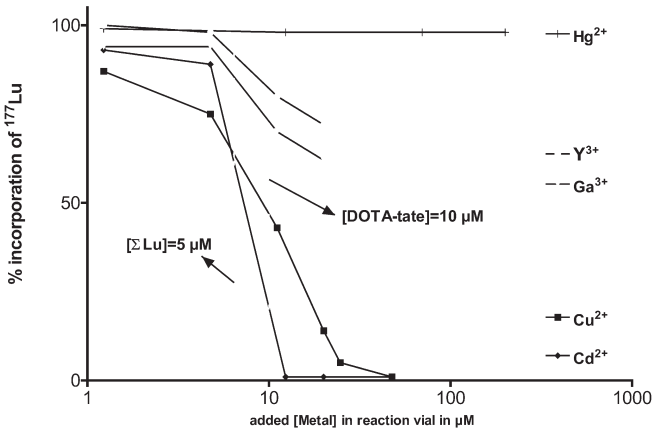

Fig. 3. Effects of contaminants on the incorporation of ^{177}Lu in DOTA-tate by the controlled addition of non-radioactive nuclides

Table 2. Effects of different concentrations of metal ions, as contaminants in the reaction vial, on incorporation of radionuclides in DPTA-ligand

0	+	++
Ag^{+}	Ga^{3+}	Cd^{2+}
Hf^{4+}	Y^{3+}	Co^{2+}
Hg^{2+}		Cu^{2+}
Sr^{2+}		In^{3+}
Zr^{4+}		Fe^{2+}
		Lu^{3+}
		Ni^{2+}
		Zn^{2+}

0, ≤10% at 10 µ*M*; +, ≥10% at 1–10 µ*M*; ++, ≥10% at 1 µ*M*

Discussion

We recently reported that Cd is a strong competitor for ^{111}In incorporation in the DOTA-chelator, and since ^{111}Cd is formed from decaying ^{111}In, the highest SA is achieved immediately after the production of ^{111}In [15]. Analogously, achieving a high SA with ^{67}Ga will be very difficult owing to the [$^{67/68}$Zn], present from target (^{68}Zn)

and formed during decay. Even if the [^{68}Zn] is low or zero at the end of production of ^{67}Ga, after one half-life of ^{67}Ga the [^{67}Ga]=[^{67}Zn].

This study provides insights into the effects of contaminants in daily radiolabelling practice when high SAs are required. It also improves the interpretation of the concentrations of contaminants as mentioned in the data sheet provided by the manufacturer of the radionuclides. For instance, the data sheet of ^{90}Y (MDS Nordion) states that the maximal concentration of Zn in ^{90}Y will not exceed the level of 30 μg per Ci of ^{90}Y. If this is so, however, it implies that the mol/mol ratio for Zn will be more than 20 times that for Y; achieving a high SA would then not be possible.

In Table 1 the highest achieved SAs of ^{67}Ga, ^{90}Y, ^{111}In and ^{177}Lu are presented, and imply a mol/mol ratio of DOTA over radionuclide of 4, 4 1/2, 2 1/4 and 6, respectively. This reflects the reaction conditions, including pH, temperature and the use of very pure reactants. From Table 2 it can be seen that Hf is not a competitor for ^{177}Lu under our reaction conditions. This implies that the ingrowth of Hf has no consequences for the maximal achievable SA. The rate of incorporation is >99%, even at a mol/mol ratio (DOTA-peptide over $^{176+177}$Lu) of 1.2. In addition, a high SA can still be achieved 2 weeks after the production of ^{177}Lu, confirming that Hf is not a competitor for Lu in the incorporation in DOTA. Although stability constants of DOTA with many nuclides are available in the NIST database [16], data on reaction kinetics are scarce. However, from the data presented here it can be concluded that the reaction kinetics of Lu, Y and In with DOTA are in the order: Lu>Y>>In.

In conclusion, DOTA-peptides can be radiolabelled at high SA. Reaction kinetics differ for each radionuclide: with ^{90}Y and ^{177}Lu, conditions were optimal at pH 4–4.5 and the reactions were complete after 20 min at 80°C, while labelling with ^{111}In was completed after 30 min at 100°C. Reactions can be hampered by contaminants, such as target material and decay products.

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