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Desferrioxamine as an appropriate chelator for ^{90}Nb: Comparison of its complexation properties for M-Df-Octreotide (M = Nb, Fe, Ga, Zr)

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ABSTRACT

The niobium-90 radioisotope (^{90}Nb) holds considerable promise for use in *immuno*-PET, due to its decay parameters ($t_{½}$ = 14.6 h, positron yield = 53%, $E_{\beta^+}^{mean}$ = 0.35 MeV and $E_{\beta^+}^{max}$ = 1.5 MeV). In particular, ^{90}Nb appears well suited to detect *in vivo* the pharmacokinetics of large targeting vectors (50–150 kDa). In order to be useful for *immuno*-PET chelators are required to both stabilize the radionuclide in terms of coordination chemistry and to facilitate the covalent attachment to the targeting vector. Different chelators were evaluated for this purpose in terms of radiolabelling efficiency and stability of the radiolabelled Nb(V) complex and in order to determine the most suitable candidate for conjugation to a biologically relevant targeting vector. For the purpose of studying the complexation properties the niobium radioisotope ^{95}Nb was used as an analogue of ^{90}Nb, by virtue of its longer half-life (35 days) and lower cost (reactor-based production). Acyclic and cyclic chelators were investigated, with desferroxamine [Df: (*N*′-{5-[acetyl(hydroxy)amino]pentyl}-*N*-[5-({4-[(5-aminopentyl)(hydroxy)amino]-4-oxobutanoyl} amino)pentyl]-*N*-hydroxysuccinamide)] emerging as the best candidate. Greater than 99% radiolabelling was achieved at room temperature over a wide pH range. The ^{95}Nb-Df complex is sufficiently stable for *immuno*-PET (<7% degradation over 7 days *in vitro*). As a proof-of-principle, a Df conjugate featuring a well-established targeting vector, (D)-Phe^1-octreotide, was evaluated. The fast labelling kinetics of the unconjugated chelator (Df) were retained for Df-succinyl-(D)Phe^1-octreotide (Df-OC), with > 90% labelling after 1 h at room temperature over the pH range 5–7. Stability studies, performed *in vitro* in serum at physiological temperature (37 °C), revealed that 87 ± 2% of the radiolabelled molecule remained intact after 7 days. Competition studies with relevant metal ions (zirconium$^{(IV)}$, gallium$^{(III)}$ and iron$^{(III)}$) have been performed with Df-OC to gain insight to the relative stability [Nb-Df]-OC complex to transmetallation. At equimolar metal ion concentrations the [Nb-Df]-OC complex showed the greatest overall stability. The favourable radiolabelling characteristics of Df-OC and its stability indicate that Df is a potentially very useful chelator for the development of radiopharmaceuticals for ^{90}Nb-PET.

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1. Introduction

Developments in nuclear medicine, molecular imaging directed towards the diagnosis and therapy of diseases and infections, have called for the investigation of radionuclides to satisfy certain requirements. An attractive new direction in the field is *immuno*-PET, which is viewed as a promising method for the diagnosis of certain cancers [1,2]. *Immuno*-PET employs monoclonal antibodies or antibody fragments as targeting vectors, to enable the visualization of tumors and metastases at the early and later stages of development. These targeting vectors have inherently slow pharmacokinetics, reacting target uptake saturation within a period of several hours or days. Therefore, in the absence of a pre-targeting concept, positron emitting radionuclides with suitable long half-lives are required. The positron emitting radionuclide must be selected according to the biological half-life of the biomolecule [3], and in this respect, commonly used radionuclides such as ^{18}F ($t_{½}$ = 110 min) and ^{11}C ($t_{½}$ = 20.4 min) are practically useless.

Recently several radionuclides with considerably longer half-lives have become available, even commercially, and in some cases have been introduced into clinical application. These include, for example, ^{89}Zr ($t_{½}$ = 78.4 h) [4,5] and ^{124}I ($t_{½}$ = 100.2 h) [6,7]. Typically, engineered targeting vectors have a larger molecular weight and therefore show slower tumor accumulation than those used in *immuno*-PET [8]. The disadvantage of using higher molecular weight targeting vectors, is that there is a corresponding greater radiation dose to the patient associated with the longer-lived radionuclide employed. Therefore it is important that the radionuclide used has a half-life which is as short as possible, taking into account the pharmacokinetics of the targeting vector. In this respect, an intermediate physical half-life, within the range of several hours, but less than one day, would better correlate with the time required for optimal imaging. Accordingly, positron emitters such as ^{64}Cu ($t_{½}$ = 12.7 h) [9,10], ^{86}Y ($t_{½}$ = 17.7 h) [11–13] and ^{76}Br ($t_{½}$ =

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16.2 h) [14,15] have been conjugated with biomolecules, and evaluated for human application.

There are several other factors to consider when selecting a radionuclide for application in *immuno*-PET. Radionuclides with a high positron yield (branching) and weak accompanying radiation (β^-, γ) offer high sensitivity PET imaging with a low radiation burden to the patient. Radionuclides with lower β^+-energy are preferable as they facilitate higher spacial resolution and more precise PET-images. Moreover, in terms of wider clinical application, the ability to easily distribute a radionuclide on a commercial scale is of significant benefit.

The half-life of ^{90}Nb ($t_{1/2}$ = 14.6 h) seems to fit well with the pharmacokinetics of antibodies and antibody fragments utilized as targeting vectors. In addition, ^{90}Nb also benefits from a relatively high positron branching (53%) and low positron energy profile ($E_{\beta^+}^{mean}$ = 350 keV, $E_{\beta^+}^{max}$ = 1.5 MeV), which should allow high quality PET images to be obtained. Furthermore, it has been shown that ^{90}Nb can be produced in large batches, and is subsequently isolated from the irradiated zirconium target in sufficient purity for the labelling of biomolecules [16–18]. As a continuation of this work and in order to identify the most adequate bifunctional ligand for pentavalent ^{90}Nb, a range of established ligands have been investigated for the complexation of niobium. The most promising candidate was conjugated to an established peptidic targeting vector, namely octreotide, as a proof-of-principle study.

2. Materials and methods

2.1. Chemicals

Reagents were purchased from Sigma-Aldrich (Germany) and used without further purification unless otherwise stated. Deionized water (18 MΩ cm^{-1}) and ultrapure HCl solution were used. No other special measures were taken regarding working under strict metal-free conditions. Different ligands were applied: Ethylenediaminetetraacetic acid (EDTA) (Fluka, Germany), 1,2-cyclohexylenedinitrilotetraacetic acid (CDTA), diethylene triamine pentaacetic acid (DTPA) (Fluka, Germany), triethylenetetraaminehexaacetic acid (TTHA) (Fluka, Germany), 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) (Strem Chemicals, Germany), 1,4,8,11-tetraazacyclo tetradecane-1,4,8,11-tetraacetic acid (TETA) (Sigma-Aldrich, Germany), desferrioxamine mesylate (Df) (Sigma-Aldrich, Germany), and 4-(2-pyridylazo)resorcinol (PAR) (Merck, Germany) (Fig. 1).

The octapeptide Df-succinyl-(D)Phe^1-Octreotide (SDZ 216-927) (Df-Octreotide) was kindly provided by Novartis AG Basel.

2.2. Production of ^{95}Nb

In research terms, the use of ^{90}Nb is inconvenient as it requires a nearby cyclotron to irradiate the zirconium target. In contrast, the ^{95}Nb radionuclide can be produced by a generator type setup and has a longer half-life ($t_{1/2}$ ~ 35 days). These characteristics, coupled with the fact that its complexation properties are identical to those of ^{90}Nb, render it a cost effective and convenient alternative for studying the complexation properties of ^{90}Nb.

^{95}Nb, ($t_{1/2}$ = 35 days, main photon emission 765.8 keV (100%) was applied as analog of ^{90}Nb. ^{95}Nb was produced via the ^{94}Zr (n, γ) → ^{95}Zr(β^-, $t_{1/2}$ = 64 d) → ^{95}Nb process from natural zirconium granules of 1–3 mm (ChemPur, Germany, 99.8% purity). Neutron capture reactions were performed at the TRIGA reactor at the Institute of Nuclear Chemistry of the Johannes Gutenberg-University Mainz, Germany, and at the research reactor BER II at the Helmholtz Centre Berlin, Germany. At a neutron flux of 2 10^{14} s^{-1} cm^{-2} (BER II) 50 days of irradiation of a 300 mg target generated more than 1.5 GBq of ^{95}Zr. Production of both nuclides, ^{95}Zr and ^{95}Nb, was monitored by gamma spectroscopy, via emission at 724.2 keV (44.2%) and 756.7 keV (54.0%) for ^{95}Zr and via the 765.8 keV (100%) for ^{95}Nb, respectively. The maximum activity of ^{95}Nb as a daughter of the ^{95}Zr/^{95}Nb generator pair appeared at ~67 d after EOB.

2.3. Radiochemical separation of ^{95}Nb

The separation protocol was described earlier by Busse et al. [17]. In short, the zirconium metal target (300 mg) was transferred into a 50 mL vial, and water (2 mL) was added. Under ice-cooling, 48% HF (0.63 mL) was added in small portions. After a complete dissolution, 10 M HCl (6 mL) and saturated boric acid (3.4 mL) were added. The ^{95}Nb fraction was extracted with 0.02 M of *N*-benzoyl-*N*-phenylhydroxylamine (BPHA) (Merck, Germany) in $CHCl_3$ (5 mL) by vigorous stirring of the two phases in a 50 mL vial for 20 min. The aqueous phase was additionally washed with $CHCl_3$ (3 mL). The organic phases were combined and washed with a mixture of 9 M HCl/0.001 M HF (2 mL) and with 9 M HCl (2 mL). Re-extraction was carried out with aqua regia (5 mL).

For a final purification of ^{95}Nb from trace amounts of zirconium, an anionic exchange method was employed. After re-extraction, the aqueous phase was evaporated to dryness. The residue was dissolved in a mixture of 0.25 M HCl/0.1 M oxalic acid (0.5 mL) and adsorbed on a small Aminex A27 (Bio-Rad, Germany) 15 ± 2 μm anionic exchange column (20 × 1.5 mm). Elution was performed under slight overpressure of 0.3 bars. After loading, the column was washed with 10 M HCl (100 μL). Residues of Zr were removed by washing with a mixture of 9 M HCl/0.001 M HF (200 μL). ^{95}Nb was eluted by of a mixture of 6 M HCl/0.01 M oxalic acid (200 μL).

2.4. Evaluation of Nb-ligand complexes in aqueous solutions

The following chelating ligands were tested to find an optimal complexation agent for niobium isotopes: EDTA, CDTA, DTPA, TTHA, DOTA, TETA, Df, PAR, cf. Fig. 1.

Complexation of pentavalent niobium with the different chelators was analyzed in terms of distribution of radioactivity of ^{95}Nb between a cation exchange resin (Chelex, Bio-Rad, Germany) and the complex according to Pueschel et al. [19]. Chelex is a chelating resin with iminodiacetic acid groups attached to a styrene-divinylbenzenecopolymer matrix, which was preconditioned with acetate buffer (0.1 M NaOAc, pH 4.7). Portions of 100 mg of resin were placed in eight plastic vials and acetate buffer (1.8 mL) and mixed with 0.01 M chelating solution (200 μL) in each vial. Finally, ^{95}Nb solution in 6 M HCl/0.01 M oxalic acid, 1 μL (50 kBq) was added to each vial. Mixtures were shaken and incubated for 24 hours at room temperature. Aliquots (1 mL) were taken, and radioactivity was measured by gamma spectroscopy. For DOTA and TETA, due to their cyclic structure, kinetic studies of complex formation are generally slower than for the other open chain chelators. For this reason the corresponding solutions were heated for 3 hours at 60 ºC during the 24-hour incubation.

2.5. Influence of the pH on Nb-ligand complex formation

For EDTA, TTHA and Df the influence of different pH values for ^{95}Nb-ligand complex formation was tested. For the precondition of the Chelex resin 0.1 M NaOAc buffer (pH 4.7) or 0.1 M tris(hydroxymethyl)aminomethane (TRIS, pH 7.4) was applied.

Portions of 100 mg of resin were placed in plastic vials and same buffer solutions used for precondition (0.1 M NaOAc and 0.1 M TRIS) were added (1.8 mL) and mixed with 0.01 M chelating solution (200 μL) in each vial. Finally aliquot of ^{95}Nb (1 μL) (10–50 kBq) was added to the mixture. After a 24-hour incubation, aliquots (1 mL) of the solution were taken and analyzed by gamma spectroscopy.

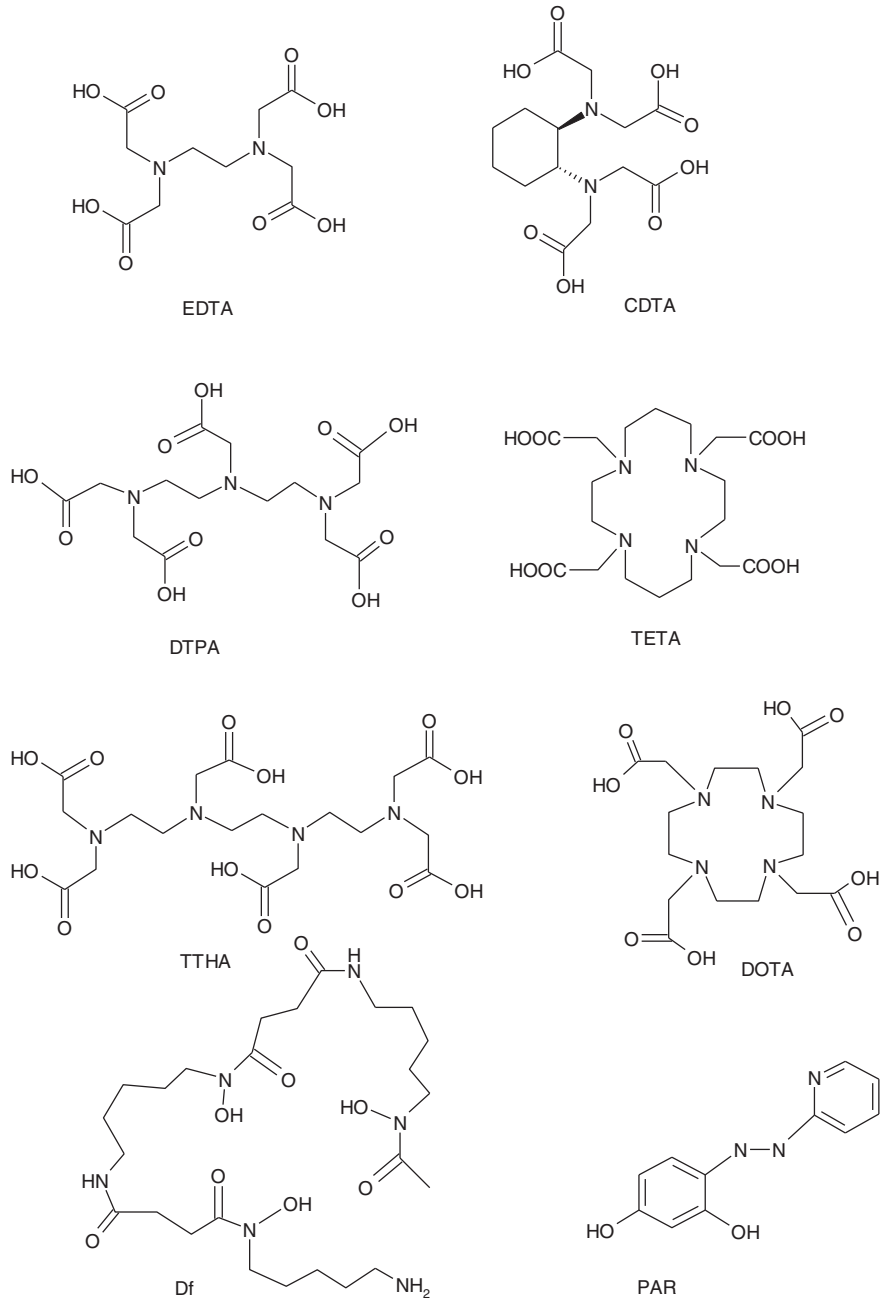

Fig. 1. Different chelators, candidates for a Nb-complexation agent.

2.6. Kinetics of the formation of Nb-Df complexes

To investigate the formation kinetics of ^{95}Nb-Df complexes, the following procedure was applied. In 1 mL reaction vials, to a solution of 0.1 μM Df (200 μL) in 0.1 M acetate buffer (pH 4.7) and 6 M NaOH (1 μL), the ^{95}Nb solution (1 μL) (10–50 kBq) was added while stirring. Samples were taken for TLC analysis at appropriate time points (1, 5, 10, 20 and 30 min). Two different systems of TLC analysis were employed. As solid phase for both systems, C-18 modified silica gel on aluminum support (Merck, Germany) were used, and as mobile phases, a 0.25 M oxalic acid solution and a mixture of 0.07 M KH_2PO_4/MeCN (40/60), respectively.

2.7. Stoichiometry of the Nb-Df complexes

Different stoichiometric proportions of Nb/Df were examined using a ^{95}Nb + ^{nat}Nb$^{(V)}$ mixture. Aliquots of 10, 20 ,40 and 200 μL of 10^{-3} M Nb$^{(V)}$ standard solution (Alfa Aesar, Germany) spiked with ^{95}Nb in 0.1 M HCl/0.1 M oxalic acid were mixed with 0.02 μM Df in 0.1 M NaOAc (pH 4.7) in a 3 mL reaction vial. As a result, Nb$^{(V)}$ concentrations were $5 \cdot 10^{-6}$, 10^{-5}, $2 \cdot 10^{-5}$ and 10^{-4} M which equaled the Nb/Df proportions 1/1, 1/2, 2/1, 10/1, respectively. Reaction mixtures were filled-up to 2 mL with acetate buffer (pH 4.7) and were incubated at room temperature for 24 hours.

2.8. Synthesis and in vitro stability of Nb-Df octreotide

A solution of 1 μM of Df-octreotide (Fig. 2.) in 0.1 M NaOAc (pH 6) with an addition of 0.1 μM NaN_3 was used. This solution can be stored for days at −20ºC.

7 μl (7 nmol) of the Df-Octreotide solution was mixed with 6 M NaOH (1 μL), a ^{95}Nb solution (1 μL) (10–50 kBq) and filled-up with 500 μL of ammonium acetate (pH 5.0), KH_2PO_4/Na_2HPO_4 (pH 7.4) or TRIS (pH 8.6) buffer solutions. Aliquots were taken at different time

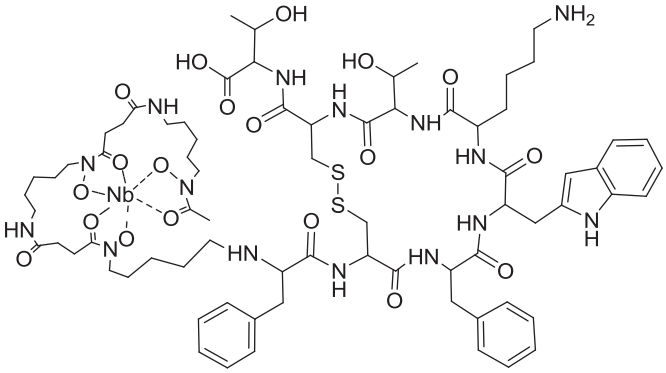

Fig. 2. Structure of ^{95}Nb-Df-Octreotide complex.

points, and the kinetics of ^{95}Nb-Df-Octreotide formation was monitored via TLC. As solid phase cellulose and as mobile phase a mixture of NaOAc/MeCN (55/45) were applied.

2.9. Competitive stability studies of Nb-Df

Competitive complexation experiment was carried out for Nb complexes with EDTA and Df. A solution of 1 μM EDTA spiked with ^{95}Nb in acetate buffer (pH 4.7) was mixed with 0.01 μM Df. The ^{95}Nb-Df complex formation was measured by TLC. As a mobile phase 0.25 M oxalate solution (pH 8.5), solid phase silica gel on aluminum plates RP-18 (Merck, Germany) were used.

2.10. Stability studies of Nb-Df and Nb-Df-octreotide in serum

To examine the applicability of Nb-Df and Nb-Df-octreotide for medical purposes, stability tests in serum were performed. 120 mg of lyophilized human serum albumin (Sigma Aldrich, Germany) were dissolved in water (2 mL). 290 μL of 0.1 M NaOAc (pH 4.7) were mixed with of 1 μM Df solution (5 μL), 6 M NaOH (3 μL) and ^{95}Nb solution (3 μL) (30–150 kBq), and incubated at room temperature for 1 hour under gentle stirring.

In case of Df-Octreotide, a 0.1 M NaOAc solution (290 μL), pH 4.7, was mixed with 5 nM Df-Octreotide (5 μL), 6 M NaOH (3 μL), and finally a ^{95}Nb solution (3 μL) (30–150 kBq) was added. The mixture was incubated for 1 hour at room temperature under intensive stirring. For both samples, TLC analysis was performed after 1 hour. In another test, the ^{95}Nb-Df (7 μL) and the ^{95}Nb-Df-Octreotide (6 μL) solutions were combined with serum (200 μL) and incubated under gentle stirring at 37ºC. At different time points, aliquots were taken and analyzed by TLC. As solid phase, cellulose was used and as mobile phase NaOAc.

2.11. Stability studies of Nb-Df-octreotide using competing metals such as Zr, Fe and Ga

Standard solutions (1 μM) for inductively coupled plasma mass spectrometry (ICP-MS) of $Zr^{(IV)}$, $Fe^{(III)}$, $Ga^{(III)}$ and $Nb^{(V)}$ in 0.09 M oxalic acid solution were applied (Alfa Aeasr, Germany). In the first experimental setup, in four 3 mL reaction vials solutions of 0.1 M NaOAc (pH 4.7) (1.84 mL), Df-Octreotide (20 μL) and 0.1 M oxalic acid (100 μL) (pH 8.5), ^{95}Nb solution (1 μL) (10–50 kBq) and a 1 μM stable $Nb^{(V)}$ standard solution (20 μL) were added. The mixtures were incubated under gentle stirring. After 24 hours, 1 μM standard solutions (20 μL) of $Zr^{(IV)}$, $Fe^{(III)}$, $Ga^{(III)}$ or $Nb^{(V)}$ were added to one of the prepared vials with $^{95+\ nat}$Nb-Df-octreotide (Fig. 3). At appropriate time points, aliquots (3 μL) were taken and analyzed by TLC. A mobile phase of 0.25 M oxalate solution (pH 8.5) and a solid phase RP-18 silica gel on aluminum plates were used.

In an additional experimental setup the test was conducted in an opposite direction. To each of the four polyethylene 3 mL reaction vials, 0.1 M NaOAc (1.84 mL), Df-octreotide (20 μL) (pH 4.7) and 0.1 M oxalic acid (100 μL) (pH 8.5) were added. 1 μM standard solutions (20 μL) of the metals Zr, Fe, Ga and Nb were added. The mixtures were then incubated under gentle stirring. After 24 hours a 1 μM standard solution (20 μL) of $Nb^{(V)}$ and the ^{95}Nb solution (1 μL) were added. At appropriate time points, aliquots (3 μL) were taken and analyzed by TLC, as mobile phase 0.25 M oxalate solution (pH 8.5) and as solid phase RP-18 silica gel on aluminum plate were used.

3. Results

3.1. Evaluation of ^{95}Nb-ligand-complexes in aqueous solutions

The individual ligands showed different capability for ^{95}Nb complex formation (Fig. 4). Desferrioxamine yielded the best results with 82 ± 3% of complex formation at room temperature, pH 4.7, at 24 hour time point in comparison with all other tested chelators. TTHA, DTPA and EDTA showed also good complexation properties with niobium (73 ± 2%, 54 ± 4% and 53 ± 3% respectively). For the cyclic chelators DOTA and TETA, in spite of 3 hours heating at 60ºC during the reaction process, less than 20% of ^{95}Nb-ligand complex formation were observed.

3.2. Influence of the pH on the ^{95}Nb-ligand -complex formation

For EDTA and TTHA, increasing the pH to 7.4 caused a very strong decreased in complex formation with ^{95}Nb. In case of EDTA, difference in complexation yield between pH 4.7 and 7.4 was 32 ± 2%, for TTHA the difference was even higher (64 ± 3%). In case of Df, no significant difference was found between the two pH values.

3.3. Kinetics of the formation of ^{95}Nb-Df complexes

The kinetics of the formation of ^{95}Nb-Df complexes was monitored at various time points during incubation of mixture for half an hour at room temperature in acetate buffer (pH 5). Already after the first minute of reaction, more than 70% of complex formation was observed. At 5 and 10 min of incubation high yield of 86 ± 5% and 95 ± 3%, respectively, can be achieved. At later time points between 20 and 30 min the labeling yields approach ≥ 98% of product formation which is much faster than in presence of Chelex resin as in the previous experiments. Results allow us to conclude that for appropriate labeling 15 to 20 minutes of reaction period is sufficient.

3.4. Stoichiometry of Nb-Df complexes

Results of the stoichiometry tests showed for a stoichiometric ratio of Df/$^{95+\ nat}$Nb = 2/1 and also 1/1 an almost quantitative $^{95+\ nat}$Nb-Df complex formation (Table 1). An increasing content of $Nb^{(V)}$ of a 1/2 and 1/10 ratio led to an Nb-Df complex formation of 49 ± 2% and 13 ± 3%, respectively. Both values correlate very well with the results of a ratio of 1/1. These results show that for an efficient and high yield complexation of niobium with desferrioxamine a 1/1 ratio already gives excellent results and a 2-fold excess of Df over $Nb^{(V)}$ allows quantitative Nb-Df complexation.

3.5. Synthesis of ^{95}Nb-Df octreotide

For the first testing of the application of ^{90}Nb as a potential candidate for PET imaging, labeling of ^{95}Nb with Df-octreotide was performed. Here, the simple chelate Df turns into a bifunctional derivate, yielding Df-succinyl-(D)Phe^{1}-octreotide. However, this conjugation creates a different structure of the chelate (one amino group replaced on succinyl linker to attach octreotide) compared to initial Df. Therefore it is

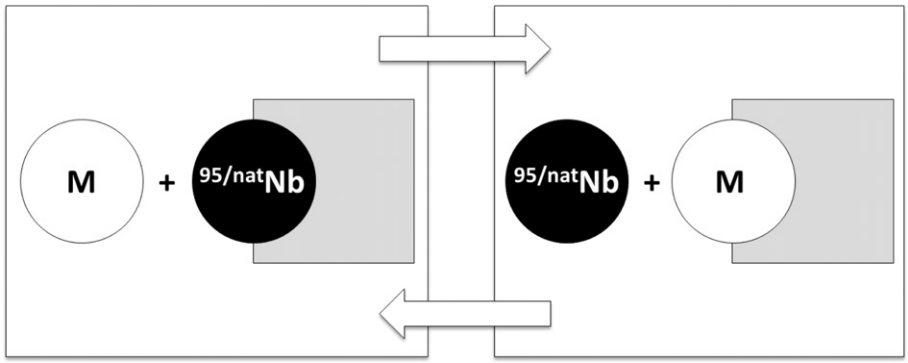

Fig. 3. Scetch of the studies on the stability $^{95+\ nat}$Nb-Df-octreotide using competing metals (Zr^{IV}, Fe^{III}, Nb^{V} and Ga^{III}).

important to prove that the stability of the derivate forms complexes with Nb not significantly weaker than with the initial desferrioxamine. However, labeling kinetics showed that already after 30 min at room temperature, for all tested pH values, labeling yields are more than 90% and for pH 5.0 are >99% (Fig. 5). After 1 hour at different pH values the labeling yields varied between 97% to 100%. These results show that one hour incubation is efficient to reach an almost quantitative labeling yield and an optimal pH for labeling is 5. However, pH 7.4 and 8.6 also showed good kinetics of complex formation and might be preferred in case of high pH sensitive molecules as targeting vectors different to octreotide. Thus, even after bifunctionalization and the loss of one NH_2-functionality, the bifunctional Df-derivative retains excellent complex formation properties for $Nb^{(V)}$.

3.6. Comparative studies of the in vitro stability of Nb-Df with EDTA and Nb-Df-octreotide versus analogue M-Df-octreotide (M = Zr, Fe and Ga) complexes

A competitive experiment of ^{95}Nb-EDTA versus desferrioxamine showed a relatively fast transchelation of ^{95}Nb from EDTA to ^{95}Nb-Df. At 30 min of incubation, the ratio of ^{95}Nb-EDTA/^{95}Nb-Df was 1/1. After 120 min less than 10% of the ^{95}Nb-EDTA was left, and at 180 min more than 95% of ^{95}Nb-Df was found.

Competitive studies with other metals that can be present in the labeling mixture (Fe, Zr and Ga) and can act as a competitor of Nb during the labeling procedure or *in vivo* (Fe) showed that the complex formation of Nb-Df is more preferred than all other tested metals (Fig. 6).

For a first experimental setup, the ^{95}Nb solution was added to M-Df-octreotide (M = Zr, Fe, Ga, Nb) to test if $Nb^{(V)}$ will displace the other metals in the complex.

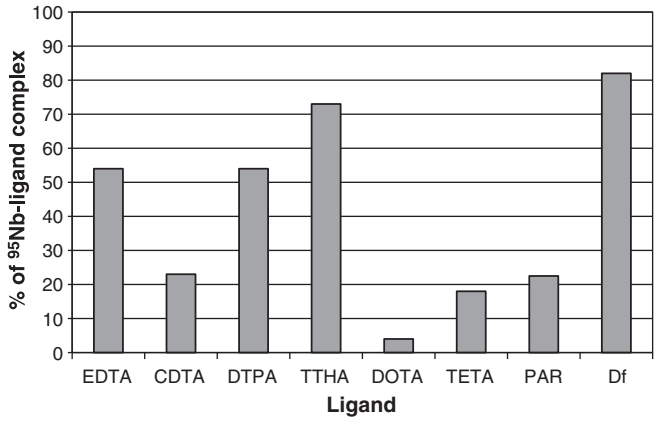

Fig. 4. Percentage of the ^{95}Nb-ligand-complex formation using different chelators at pH 5 (24 hours, RT, 0.01 M ligand concentration).

As a result, the capacity of Df-Octreotide complex formation for pentavalent niobium is higher than for all the other metals evaluated. After addition of the ^{95}Nb solution, more than 50% of $^{95+\ nat}$Nb-Df-octreotide complex was formed for Zr, Fe and Ga already after 30 min. After three days, the Zr sample contained already 80 ± 2% of $^{95+\ nat}$Nb-Df-octreotide. In case of Fe and Ga, the percentages of $Nb^{(V)}$ displacing the M-Df-octreotide complexes were 86 ± 2% and 89 ± 3%, respectively. In the case of ^{95}Nb versus ^{nat}Nb-Df-octreotide complexation, a 50 ± 4% value was approached as expected.

To prove the results, the experimental setup was performed the other way around. $^{95}Nb^{(V)}$ was complexed with Df-octreotide first, and then all other metals (Zr, Fe, Ga and Nb) were added to check if they can displace $^{95+\ nat}$Nb from the ^{95}Nb-labeled octreotide (Fig. 7). After three days of incubation of the mixture with Zr and $^{95+\ nat}$Nb-Df-octreotide, the solution showed only 20 ± 1% of Df-octreotide displaced by Zr. For Fe and Ga, the percentages of Df-octreotide complex formation after three days of incubation were even less, only 14 ± 2% and 11 ± 1%, respectively. The results clearly demonstrate that $Nb^{(V)}$ presents the best capacity for complexation with desferrioxamine in comparison with all other evaluated metals.

3.7. Stability studies of Nb-Df and Nb-Df-octreotide in serum

The ^{95}Nb-Df complex incubated in serum at 37ºC showed excellent *in vitro* stability. Over two days, less than 4% and after seven days less than 6% of free ^{95}Nb was found. Slightly increasing degradation of the complex with 11 ± 1% free ^{95}Nb was observed after ten days.

A slightly decreased *in vitro* complex stability was detected after two days of incubation for the ^{95}Nb-Df-octreotide complex in comparison to the ^{95}Nb-Df complex. After seven days 9 ± 2% of free ^{95}Nb was found, and at day ten this amount increased to 13 ± 2%.

4. Discussion

It was shown that desferrioxamine is the most appropriate ligand for the fast and quantitative formation of stable complexes with $Nb^{(V)}$ and radioniobium isotopes among the other common chelators tested. In addition, $Nb^{(V)}$ desferrioxamine complexes are more stable

Table 1
Experimental and theoretically calculated $^{95+\ nat}$Nb-Df complex formation yields at different Df/Nb ratios (24 hours, RT, pH 4.7 NaOAc).

Ratio of Df/Nb	Nb-Df complex formation yields (%)	Theoretically calculated yields for Df/Nb at 1/1 (%)
2/1	≥99	100
1/1	96 ± 5	100
1/2	49 ± 3	50
1/10	13 ± 4	10

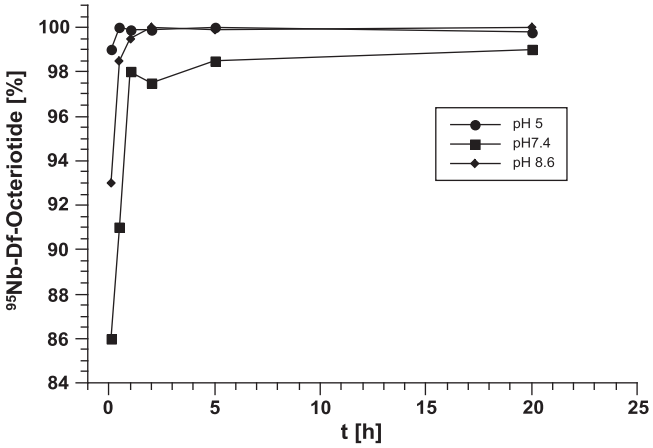

Fig. 5. Kinetics of the formation of ^{95}Nb-Df-octreotide at various pH values (RT, 7 nmol Df-Octreotide).

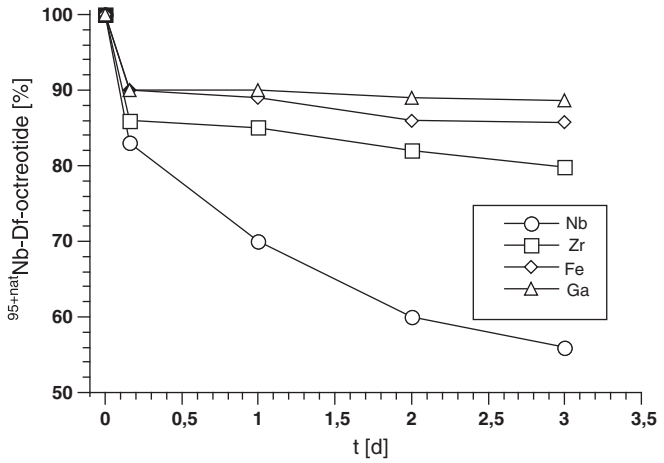

Fig. 7. Stability of the $^{95+\ nat}$Nb-Df-octreotide after addition of Zr^{IV}, Fe^{III}, Nb^{V} and Ga^{III}.

when compared with other metals involved (Zr, Fe and Ga). This in particular is relevant, because the increasing application of the positron emitter ^{89}Zr bases on its complex formation with Df. Several versions of bifunctional desferrioxamine are used to covalently attach this ligand to e.g. monoclonal antibodies, followed by radiolabelling with ^{89}Zr [20,21]. Desferrioxamine thus is identified as chelator of choice to synthesize ^{90}Nb labeled biomolecules (antibody, antibody fragments or peptides) for *immuno*-PET. Bifunctionalization of Df as needed for covalently attaching Df to a targeting vector which should be even more stable than the ^{89}Zr-Df analogues does not decrease its potential for $Nb^{(V)}$ complex formation and stability. As proof-of-principle the Df-succinyl-(D)Phe^1-octreotide was successfully labeled with ^{95}Nb for 30 minutes at pH 5 at room temperature in more than 99% yield. The *in vitro* stability of this product was excellent over days in HSA at 37°C. The potential of bifunctional derivates of Df should retain also in the case of other bifunctional derivatives of Df such as *N*-succinyl-desferrioxamine (N-suc-Df) and p-isothiocyanato-benzyl-desferrioxamine (NCS-Bz-Df).

5. Conclusions

In summary, fast and almost quantitative complex formation of $Nb^{(V)}$ with desferrioxamine can be performed at a wide range of pH (4–7) and at room temperature, which opens a broad field for applications of Nb-Df for biomolecules. Bifunctionalisation does not affect the complex formation parameters of Df. Radiolabeled *Nb-Df-octreotide remains stable *in vitro*. With this Nb-specific bifunctional chelate in hands, the use of ^{90}Nb for *immuno*-PET may now be evaluated in adequate applications.

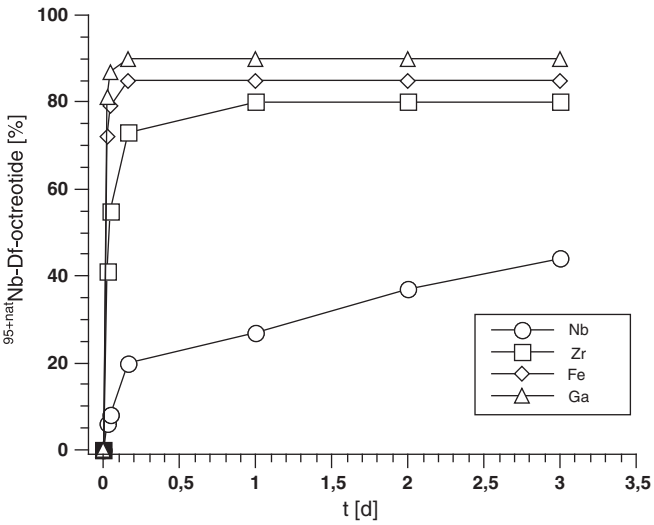

Fig. 6. Kinetics of the formation of $^{95+\ nat}$Nb-Df-octreotide in a system of M-Df-octreotide (M = Zr^{IV}, Fe^{III}, Nb^{V} and Ga^{III}) after addition of $^{95+\ nat}$Nb.

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