JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2004; **47**: 79–89.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.799

Research Article

Microwave accelerated ^{68}Ga-labelling of oligonucleotides

I. Velikyan[1,2], G. Lendvai[2], M. Välilä[3], A. Roivainen[3], U. Yngve[1,2], M. Bergström[2] and B. Långström[1,2,*]

[1] *Department of Organic Chemistry, Institute of Chemistry, Uppsala University, Box 531, Uppsala SE-751 21, Sweden*
[2] *Uppsala Imanet, P.O. Box 967, Uppsala SE-751 09, Sweden*
[3] *Turku PET Centre, P.O. Box 52, Turku FIN-20521, Finland*

Summary

Oligonucleotides are extensively used for characterization of gene expression *in vitro* and have now been studied as inhibitors of gene expression *in vivo* in various diseases. Labelled antisense oligonucleotides are therefore of potential interest for possible *in vivo* imaging of gene expression, considering the biology of tumors and applications in designing novel molecule-targeted therapies. In the present work a method of microwave accelerated ^{68}Ga-labelling of oligonucleotides and analysis of the resulting tracers are described.

Four modified and functionalized 17-mer oligonucleotides with a hexylamine group in the 3′- or 5′-position were studied. The oligonucleotides were conjugated to the bifunctional chelator, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), and then labelled with ^{68}Ga($T_{1/2} = 68$ min) using microwave activation. The isolated decay-corrected radiochemical yields ranged from 30 to 52%. Labelled products were stable in water and ethanol for more than 4 h. The impact of the labelling procedure on the oligonucleotide probes was investigated using hybridization to a complementary 17-mer sense oligonucleotide in solution. Chemical modification did not influence either the labelling or hybridization ability of the oligonucleotides. The radiolabelled oligonucleotides will be used for the further *in vitro* and *in vivo* biology studies. Copyright © 2003 John Wiley & Sons, Ltd.

Key Words: ^{68}Ga; radiolabelled antisense oligonucleotides; DOTA; microwave activation

*Correspondence to: B. Långström, Uppsala Imanet, P.O. Box 967, Uppsala SE-751 09, Sweden.
E-mail: bengt.langstrom@uppsala.imanet.se

Contract/grant sponsor: Swedish Research Council; contract/grant number: K 5107
Contract/grant sponsor: Swedish cancer Society

Received 19 May 2003
Revised 23 June 2003
Accepted 15 September 2003

Introduction

Antisense oligonucleotides can inhibit gene expression.[1] It makes them of considerable interest for biological studies and particularly molecule-targeted therapies of cancer. Labelled antisense oligonucleotides may be used for *in vivo* imaging of gene expression using appropriate radionuclide and oligomer base sequence.[2,3] Labelling studies have been conducted with gamma emitters, such as [^{90}Y], [^{99m}Tc], [^{111}In] and [^{125}I][4–8] as well as with positron-emitting radionuclides, such as [^{11}C], [^{18}F] and [^{76}Br].[9–13] The reported methods modify the oligonucleotide by addition of labelled or radiolabel chelating groups. Compared to radiohalogenetion, radiometal ion complexation reactions have simpler chemistry and allow tracer production kits. Labelling with positron-emitting radionuclides offers a distinct advantage over gamma-emitting radioisotopes since positron emission tomography (PET) has a greater spatial resolution and allows not only imaging of biological processes but also biodistribution quantification. Generator available radiometals are preferable to costly cyclotron produced ones. To improve the stability and maintenance of the hybridization properties of nucleic acids, a number of modified oligonucleotides like phosphorothioate,[14] 2′-*O*-methyl,[15–17] methylphosphonate, phosphoramidate, morpholino oligonucleotide, mixed-backbone oligonucleotide and peptide nucleic acid (PNA) has been studied.[3] End-modification increases the stability of the oligonucleotide to degradation by exonucleases[18] and allows introduction of an amine group for the further conjugation of the oligonucleotide to a chelator.[19] The resulted conjugate can then be labelled with a metal radionuclide.

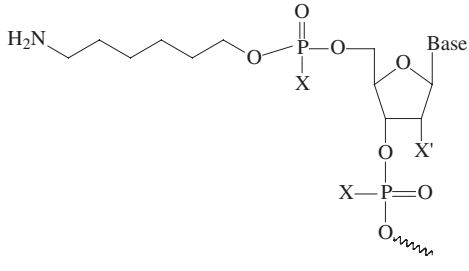
^{68}Ga meets the major requirements for a candidate in the labelling of oligonucleotides. It forms stable complexes with non-cyclic[20–22] and macrocyclic[23–25] bifunctional chelators containing nitrogen and oxygen donors. ^{68}Ga has favorable detection characteristics decaying 89% by positron emission. In addition, β^+_{max} energy of 1.9 MeV provides good resolution. ^{68}Ga is obtained from ^{68}Ge($T_{1/2}$= 270.8 days) in a generator-system with about 1.5 years life span. Its 68 min half-life is sufficient to follow certain biochemical processes.

Another factor that is crucial for radiochemistry and tracer production is the labelling synthesis time. Microwave activation providing the acceleration is an attractive tool. The technique has been used for labelling with [^{131}I], [^{11}C], [^{15}O], [^{18}F] and [^{13}N].[26] Microwave activation seems to be useful for microscale organic chemistry like radiolabelling where the size of the sample is comparable to the penetration depth of the microwave field.[26,27] The purpose of the present study was to develop a rapid oligonucleotide labelling method that would also give high radiochemical yields (RCY). Our study has shown that the microwave accelerated complexation method is applicable for the ^{68}Ga labelling of oligonucleotides and peptides. In this paper, we present ^{68}Ga labelling and product characterization of the following four modified antisense

oligonucleotides (**1** – **4**) specific for activated human K-*ras* oncogene: 17-mer phosphodiester oligonucleotide with hexylaminolinker at 5′ end (**1**); 17-mer phosphodiester oligonucleotide with hexylaminolinker at 3′ end (**2**); 17-mer phosphorothioate oligonucleotide with hexylaminolinker at 5′ (**3**); and 2′-*O*-methyl phosphodiester with hexylaminolinker at 5′ (**4**).

Results and discussion

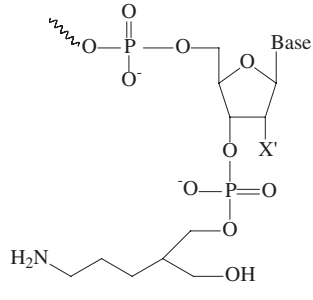
The structural formulas of the modifications introduced into the oligonucleotides are shown in Figure 1. The amine functionality introduced at the 3′- or 5′-end was used to covalently link the oligonucleotide to bifunctional complexing agents[28] which can form kinetically stable complexes with the radionuclides of interest. The chelator DOTA (**5**) has been shown to form stable complexes with Ga(III).[29] Conjugation protocols developed for antibody labelling[30] were shown to be applicable for DNA conjugation and radiolabelling.[31,32] The method originally developed for conjugation of monoclonal antibodies and proteins with *N*-hydroxy-sulfosuccinimidyl DOTA[33,34] was applied in this work for the oligonucleotide conjugation with (**6**) (Scheme 1, Table 1). The conjugate obtained was then used for the labelling with ^{68}Ga. As in the case of ^{76}Br labelling of oligonucleotides,[35] where the labelling procedure worked for oligonucleotides of different lengths (30, 20, 12 and 6-mer) equally well, the method used in the present work was shown to be applicable independent of the modification of the oligonucleotides. The oligonucleotides were reacted with the *N*-hydroxy-sulfosuccinimide ester of DOTA (**6**), generated *in situ* using water-soluble 1-ethyl-3(3-dimethylaminopropyl) carbodiimide (EDC) as



1 X= O$^-$, X' = H

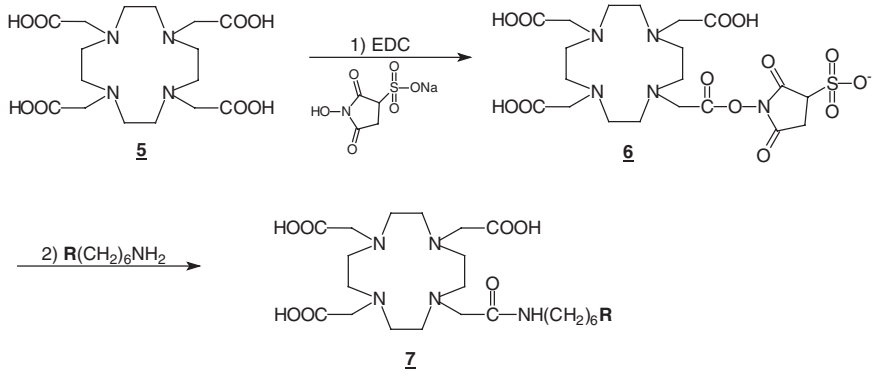
3 X= S$^-$, X' = H

4 X= O$^-$, X' = O - CH_3



2 X= O$^-$, X' = H

Figure 1. 1-phosphodiester oligonucleotide with 5′-hexylamine modification; 2-phosphodiester oligonucleotide with 3′-amino-C7 modification; 3-phosphorothioate oligonucleotide with 5′-hexylamine modification; 4–2′-*O*-methyl phosphodiester oligonucleotide with 5′-hexylamine modification



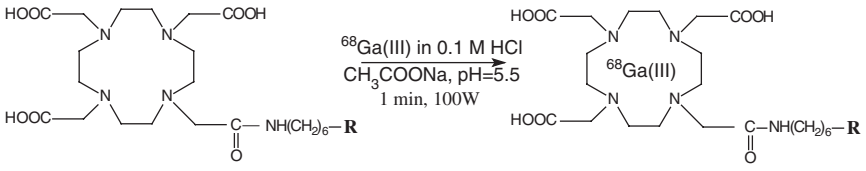
Scheme 1. Conjugation of DOTA (5) to the oligonucleotide bearing the hexylamine linker. R stands for four oligonucleotide counterparts (see Table 1 and Scheme 1)

Table 1. Isolated decay-corrected radiochemical yield (RCY) of the ^{68}Ga labelling of oligonucleotides (1–4) complementary to human K-ras oncogene

	R	RCY (%)
	Phosphodiester	
1	5′-CTA CGC CAC TAG CTC CA	50
2	CTA CGC CAC TAG CTC CA-3′	42
	Phosphorothioate	
3	5′-CTA CGC CAC TAG CTC CA	30
	2′-*O*–methyl phosphodiester	
4	5′-CTA CGC CAC TAG CTC CA	52

the coupling reagent, to give DOTA-oligonucleotide (7) (Scheme 1, Table 1). The advantage of adding *N*-hydroxy-sulfosuccinimide (Sulfo-NHS) to the EDC reaction is to increase the stability of the active intermediate, which ultimately reacts with the attacking amine.[19] The half-life of the intermediate activated carboxylate is crucial in our case where the target oligonucleotide molecule is at low concentration compared to water.[19] Purification of the conjugation product was performed taking particular care to ensure absence of free DOTA, since the latter would compete in the labelling with DOTA-oligonucleotide and decrease the radiochemical yield.

The maximum amount of ^{68}Ga available from the generator for the labelling reaction was in the pg range. The oligonucleotide amount was in the μg range. In order to provide the concentration necessary for the labelling reaction to take place, the volume of the ^{68}Ga generator eluate solution was minimized. The ^{68}Ga elution profile showed that over 50% of the maximum possible activity was obtained in the second fraction and the highest relative ^{68}Ge



7 **8**

Scheme 2. ^{68}Ga labelling of DOTA—oligonucleotides 7. For R see Table 1 and Scheme 1

breakthrough was in the first fraction of the eluate. Accordingly, the first part of the eluate was discarded and the next 1 ml of 0.1 M hydrochloric solution of ^{68}Ga(III) was collected and buffered with sodium acetate to a pH of 5.5 to avoid precipitation of gallium in the form of gallium trihydroxide.[36] After addition of the oligonucleotide to the latter mixture, the reaction was carried out in a microwave oven (1 min at 100 W, Scheme 2). Labelling of all four oligonucleotide counterparts gave isolated decay-corrected radiochemical yields in the range of 30–52% (Table 1). The specific activity of the antisense ^{68}Ga-DOTA-oligonucleotides (**8**) obtained ranged from 0.1 to 1.5 MBq/nmol.

The position of the ^{68}Ga-label was assessed by performing the labelling reaction with both conjugated and unconjugated oligonucleotides. No product was detected in the reaction with unconjugated oligonucleotide, indicating that the label was most likely attached to the chelator. The radiochemical yield was found to improve with increasing concentration of oligonucleotide. However, increasing the concentration of the oligonucleotide may render purification steps difficult and decrease the specific activity.

Compared to the synthesis procedure using conventional heating (radiochemical yield of 19%) the microwave application in the present work shortened the synthesis time considerably and improved the radiochemical yield. Owing to the shortened reaction time, the amount of radioactive material and the product specific activity was increased by 21%. Furthermore, microwave activation not only reduces the chemical reaction time, but also reduces side reactions, increases the yield, and improves reproducibility.[26] In the present case, the analytical radiochemical yield was increased two-fold compared to our previous results with conventional heating.

The stability of radiolabelled oligonucleotides (**8**) in water and 50% ethanol was monitored by radio HPLC with analysis of aliquots taken from the labelling reaction mixture every 15 min.^{68}Ga was used as the internal reference. The ratio of the areas of the free ^{68}Ga and labelled oligonucleotides radiosignals remained constant indicating that the loss of activity was only due to the decay of the radionuclide. Moreover, no additional radiosignals were detected in the stability study. The radiochemical purity of

^{68}Ga-DOTA-oligonucleotides (**8**) in water and 50% ethanol was >95% for more than 4 h. This time corresponds to 3–4 physical half-lives of ^{68}Ga and is the time required for biological experiments and diagnostical investigations.

The impact of the labelling procedure on the oligonucleotide probes was investigated using their specific hybridization to a complementary 17-mer phosphodiester sense oligonucleotide in solution. The results were analyzed using polyacrylamide gel electrophoresis in a cell-free system and the results were visualized by ethidium bromide staining and autoradiography. The results of the concentration-dependent hybridization of the phosphorothioate counterpart are shown in Figure 2. Nine samples with a constant concentration of the radiolabelled antisense oligonucleotide and a gradually increasing concentration of the sense oligonucleotide were analyzed (Figure 2, lanes 1–9). As a reference the sense oligonucleotide (lane 10), the antisense oligonucleotide (lane 11) and molecular weight marker (lane 12) samples were loaded onto the polyacrylamide gel. The gradual increase of the intensity of the hybrid bands, the absence of the free radioactive bands of antisense at the higher

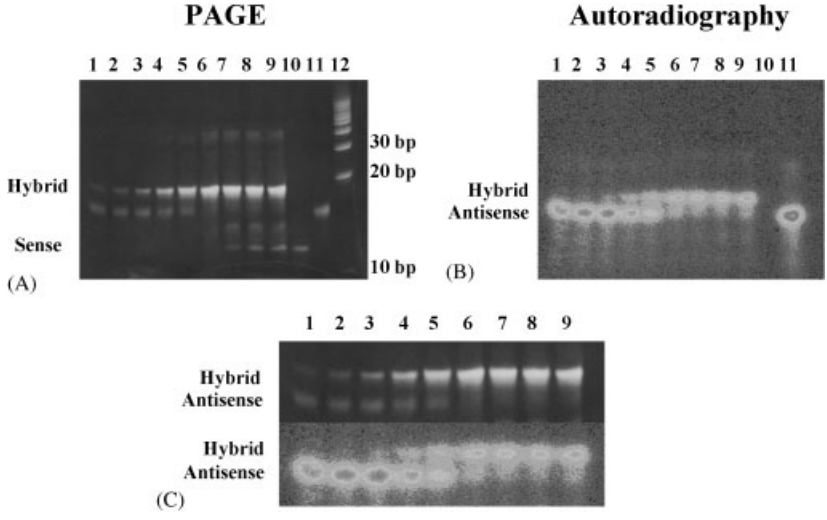


Figure 2. Concentration-dependent hybridization of 17-mer antisense phosphorothioate oligonucleotide (20 pmol in 1 µl) to the complementary 17-mer sense phosphodiester oligonucleotide in solution. The antisense:sense concentration ratios in the lanes are as follows: (1) 1:1/60, (2) 1:1/30, (3) 1:1/15, (4) 1:1/5, (5) 1:1/2, (6) 1:1, (7) 1:2, (8) 1:3, (9) 1:4 and the references are sense oligonucleotide (lane 10), antisense oligonucleotide (lane 11) and molecular weight marker (lane 12). (A) PAGE picture of the concentration-dependent hybridization study; (B) Autoradiography of the polyacrylamide gel (A); (C) The scaled up region of interest

concentrations of the sense oligonucleotide and the absence of free sense bands at the lower concentration of the sense oligonucleotide serve as an indication of the concentration-dependent hybridization. All four counterparts were able to hybridize to the complementary sense oligonucleotide.

Experimental

Sodium acetate (99.995%, Aldrich) and doubly distilled HCl (Aldrich) were used in the labelling. 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (Sigma), *N*-Hydroxysulfosuccinimide (Sulfo-NHS) (Sigma) and 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) (Macrocyclics, USA) were used for the conjugation. The 17-mer phosphodiester (**1**) and phosphorothioate (**3**) oligonucleotides of antisense strand sequence with a hexylamine linker in the 5′-position, 17-mer phosphodiester oligonucleotide (**2**) of antisense strand sequence with a hexylamine linker in the 3′-position as well as 17-mer phosphodiester oligonucleotide of sense strand sequence were purchased from Scandinavian Gene Synthesis AB (Köping, Sweden). 2′-*O*-methyl phosphodiester oligonucleotide (**4**) was synthesized at Turku PET center, Turku, Finland and was used as received. The antisense and sense sequences used were, respectively, 5′-CTA CGC CAC TAG CTC CA and 5′-TGG AGC TAG TGG CGT AG. Deionized water (18.2 MΩ) was used in all reactions. The purchased chemicals were used without further purification.

Thin-layer chromatography (TLC) was performed on PEI-Cellulose F plates (Merck, Germany) with 0.4 M NaH_2PO_4, pH = 3.5 as the running buffer. Ultraviolet (UV) absorbance was visualized using short-and long-wave length ultraviolet light. Radioactivity on TLC-plates was measured by storage-phosphorous autoradiography using the Molecular Dynamics Phosphor Imager® (Sunnyvale, CA, USA).

Analytical liquid chromatography (LC) separations were performed using a Beckman (Fullerton, CA, USA) System (a 126 pump, a 166 UV detector and a radiodetector coupled in series). Data collection was performed using the Beckman System Gold Nouveau Chromatography Software Package. The columns used were; I: Vydac RP 300 Å high performance liquid chromatography (HPLC) column (Vydac, USA) 150 × 4.6 mm ID, 5 µm; II: Fast Desalting HR 10/10 fast protein liquid chromatography (FPLC) gel filtration column (Pharmacia Biotech, Uppsala, Sweden); System A (I): flow 1.5 ml/min, a = 20 mM triethylammonium acetate buffer (TEAA); b = 100% acetonitrile (MeCN), linear gradient 0–10% b 2–4 min, 10–30% b 4–9 min, 30–50% b 9–15 min; λ = 254 nm. System B (II): flow 1.5 ml/min, a= H_2O; b = phosphate buffered saline (PBS), 100% b 0–15 isocratic; λ = 280 nm.

Electrospray ionization mass spectroscopy (ESI-MS) was performed using a Fisons Platform (Micromass, Manchester, UK). Data were acquired in the negative ionization mode by scanning from m/z 500 to m/z 1100. Samples were

dissolved in 2.5 mM imidazole, 2.5 mM piperidine and aqueous 50% isopropyl alcohol.[37] For purification procedures C18 Supelclean LC-18 solid phase extraction (SPE) tubes 500 mg supplied by Supelco (Bellefonte, PA, USA) and NAP 5 columns (Amersham Pharmacia Biotech AB, Uppsala, Sweden) were used.

^{68}Ga generation and recovery: ^{68}Ga ($T_{1/2} = 68$ min, decay by β^+ 89% and by EC 11%) was available from ^{68}Ge ($T_{1/2} = 270.8$ days) in a generator-system (Cyclotron C., Obninsk, Russia) where ^{68}Ge is attached to an ion-exchange column and the ^{68}Ga is eluted in 0.1 M hydrochloric acid. ^{68}Ge activity is 1850 MBq (50 mCi). ^{68}Ga yield in 5 ml of 0.1 M HCl is 50%. Breakthrough of ^{68}Ge is < 0.01%. The shelf life of the generator is 2–3 years. Microwave activations were performed with a MicroWell 10 oven (Personal Chemistry AB, Uppsala, Sweden) with monomodal radiation.

Conjugation of DOTA to oligonucleotides: DOTA (32 mg, 66 μmol) and Sulfo-NHS (14 mg, 65 μmol) in H_2O (250 μl) were added to EDC (13 mg, 68 μmol) in H_2O (250 μl) and stirred on ice for 30 min then warmed to room temperature to give DOTA-sulfo-NHS. A 100 fold excess of DOTA-NHS (**6**) solution was added dropwise to an oligonucleotide in 1 M carbonate buffer (pH 9) and then cooled on ice. The mixture was left at room temperature for 10 h. The reaction mixture was first purified by gel filtration on NAP 5 columns and 100 μl of 1 M TEAA (Triethylammonium acetate Buffer) was added to 1 ml of the product eluate (H_2O). The mixture was then applied to a C-18 SPE column (Supelco), the column was washed with 50 mM TEAA (5 ml), 50 mM TEAA containing 5% acetonitrile (3 ml) and the DOTA-oligonucleotide was eluted with water:acetonitrile 50:50 (1 ml). The water–acetonitrile fraction was dried using a vacuum centrifuge. The products were analyzed using electrospray ionization mass spectrometry. Analysis in negative mode after direct infusion resulted in the following data: 1. DOTA-phosphodiester: MS (ESI^-) m/z: 662.27 $[M\text{-}8H]^{8-}$; 756.36 $[M\text{-}7H]^{7-}$; 882.91 $[M\text{-}6H]^{6-}$. Reconstitution of the data gave $M = 5303.71$; 2. DOTA-phosphorothioate: MS (ESI^-) m/z: 656.58 $[M\text{-}8H]^{9-}$; 738.56 $[M\text{-}7H]^{8-}$. Reconstitution of the data gave $M = 5917.35$; 3. DOTA-2′-*O*-methyl phosphodiester: MS (ESI^-) m/z: 674.02 $[M\text{-}6H]^{9-}$; 770.19 $[M\text{-}8H]^{8-}$; 885.00 $[M\text{-}7H]^{7-}$. Reconstitution of the data gave $M = 6148.84$.

^{68}Ga–labelling of oligonucleotides: Sodium acetate was added to the eluate from the ^{68}Ge/^{68}Ga-generator (36 mg to 1 ml) to give a pH of approximately 5.5 and the mixture was vortexed well yielding the gallium acetate complex.[36] Then DOTA-oligonucleotide (**7**) (10–100 nmol) was added and the mixture was transferred into a Pyrex glass vial for microwave activation for 1 min at 100 W. The reaction mixture was cooled to room temperature then 1 ml of 150 mM TEAA in H_2O was added. The mixture was applied to a C-18 SPE-column (Supelco), which was then washed with 50 mM TEAA (1 ml), 50 mM

TEAA containing 5% acetonitrile (1 ml) and the product (**8**) was eluted with ethanol: water 50:50 (1 ml) or water:acetonitrile 50:50 (1 ml).

The reaction mixture was analyzed by HPLC using Vydac RP and Fast Desalting HR 10/10 FPLC gel filtration columns.

Synthesis of 2′-O-methyl phosphodiester oligonucleotide: 2′-*O*-methyl phosphodiester oligonucleotide was synthesized on an ABI synthesizer (392 DNA/RNA synthesizer; Applied Biosystems, CA) using 2′ methyl 5′ dimethoxytrityl cyanoethyl phosphoramidite RNA monomers (Glen Research, VI). After the synthesis the oligonucleotide was kept at 55°C in concentrated ammonia overnight to cleave the oligonucleotide from the solid support and release protecting groups from the bases. Subsequently the ammonia was evaporated and the residue was dissolved in water.

The oligonucleotide was isolated from the solid support by filtration using 0.2 μm size filter (Schleicher & Schuell, Germany) and purified from the protecting groups using HPLC with an anionic exchange column (SynChropak AX300, 250 × 4.6 mm; Eichrom Technologies, Inc, Il,). HPLC conditions: A/B gradient, where solution A was KH_2PO_4 (0.05 M, pH = 5.6) in formamide:water (50:50, v/v). Solution B was solution A + $(NH_4)_2SO_4$ (0.6 M). Gradient elution was conducted by increasing the concentration of B.

Desalting of the oligonucleotide was performed using HPLC with a reversed phase column (LiChroCART 250 × 10 mm, Hypersil ODS 5 μm; Merck KGaA, Darmstadt, Germany). HPLC conditions: A/B gradient, where solution A was water and B was water:acetonitrile (50:50, v/v). The collected separated fraction was evaporated and dissolved in water. The product was characterized with a mass spectrometer (PE Sciex API 365 Triple Quadropole LC/ESI-MS/MS; Perkin-Elmer).

Hybridization in solution: The ^{68}Ga-labelled antisense oligonucleotides (**8**) were obtained in 1 ml of water:acetonitrile (50:50) solvent. The concentration of the total oligonucleotide mass (labelled and unlabelled) was determined from standard calibration curves of the UV absorption at 254 nm. After evaporation of the solvent using a vacuum centrifuge (Labconco CentriVap, USA), the ^{68}Ga-antisense was dissolved in 1 × TES buffer (50 mM TRIS pH 8.0, 50 mM NaCl, 1 mM EDTA). The hybridization samples were prepared as follows: a gradually increased concentration of the sense phosphodiester oligonucleotide (from 0.33 to 80 pmol) was added to constant concentration of ^{68}Ga-antisense (20 pmol in 1 μl) and the total volume was adjusted to 10 μl with 1 × TES. As reference solutions 40 pmol of ^{68}Ga-antisense oligonucleotide and sense oligonucleotide were used. All hybridization mixtures were kept on ice and then at 40°C for 10 min for hybridization followed by gel electrophoresis.

Gel electrophoresis was performed using a 20% non-denaturing PAGE gel, run at 200 V for 1 h and 20 min. A 10 base pair (bp) DNA Step Ladder

(Promega, Madison, WI) was used as a molecular weight marker. After electrophoresis the gel was stained using ethidium bromide to visualize the DNA and photographed under UV-light. Subsequently, the gel was exposed to a phosphor imaging plate for 12 h, scanned using a Phosphorimager SI^{TM} device and analyzed using Image Quant 5.1 software (Molecular Dynamics Inc, Sunnyvale, CA, USA).

Conclusion

The 17-mer oligonucleotides were labelled with ^{68}Ga radionuclide using microwave activation to speed up the complexation of the metal with the DOTA bifunctional chelator. The modifications of the oligonucleotides like the introduction of hexylaminolinker either at the 3′- or 5′-end, substitution of non-bridging oxygens by sulfur or introduction of *O*-methyl group at sugar 2′ position did not influence the conjugation and radiolabelling result or the hybridization ability of the oligonucleotides. Labelled products were stable in water and ethanol for more than 4 h. All four oligonucleotide counterparts retained their ability to hybridize in solution. The ^{68}Ga-labelled oligonucleotides presented in this work will be used for the further *in vitro* and *in vivo* biological experiments.

Acknowledgements

This work was financially supported by Swedish Research Council, Grant K 5107 and the Swedish Cancer Society.

References

1. Agrawal S, Kandimalla ER. *Curr Cancer Drug Targets* 2001; **1**: 197–209.
2. Tavitian B. *Q J Nucl Med* 2000; **44**: 236–255.
3. Younes CK, Boisgard R, Tavitian B. *Current Pharmaceut Des* 2002; **8**: 1451–1466.
4. Liu C-b, Liu G-z, Liu N, Zhang Yu-m, He J, Rusckowski M, Hnatowich DJ. *Nucl Med Biol* 2003; **30**: 207–214.
5. Watanabe N, Sawai H, Endo K, Shinozuka K, Ozaki H, Tanada S, Murata H, Sasaki Y. *Nucl Med Biol* 1999; **26**: 239–243.
6. Dewanjee MK, Haider N, Narula J. *J Nucl Cardiol* 1999; **6**: 345–356.
7. Kobori N, Imahori Y, Mineura K, Ueda S, Fujii R. *Neuroreport* 1999; **10**: 1271–1274.
8. Stalteri MA, Mather SJ. *Nucl Med Commun* 2001; **22**: 1171–1179.
9. Kühnast B, Dollé F, Terrazino S, Rousseau B, Loc'h FV, Hinnen F, Doignon I, David C, Crouzel C, Tavitian B. *Bioconjugate Chem* 2000; **11**: 627–636.
10. Hedberg E, Långström B. *Acta Chem Scand* 1997; **51**: 1236–1240.
11. Hedberg E, Långström B. *Acta Chem Scand* 1998; **52**: 1034–1039.
12. Yngve U, Hedberg E, Lövqvist A, Tolmachev V, Långström B. *Acta Chem Scand* 1999; **53**: 508–512.

13. Pan D, Gamdhir S, Toyokuni T, Iyer M, Acharya N, Phelps ME, Barrio JR. *Bioorg Med Chem Lett* 1999; **8**: 1317–1320.
14. Ho PTC, Parkinson DR. *Semin Oncol* 1997; **24**: 187–202.
15. Zhang R, Lu Z, Zhao H, Zhang X, Diasio RB, Habus I, Jiang Z, Iyer RP, Yu D, Agrawal S. *Biochem Pharmacol* 1995; **50**: 545–556.
16. Larrouy B, Boiziau C, Sproat B, Toulme JJ. *Nucl Acids Res* 1995; **23**: 3434–3440.
17. Boiziau C, Larrouy B, Sproat BS, Toulme JJ. *Nucl Acids Res* 1995; **23**: 64–71.
18. Crooke ST. *Annu Rev Pharmacol Toxicol* 1992; **32**: 329–376.
19. Hermanson GT. *Bioconjugate Techn* 1996; **1**: 173–176.
20 Hnatowich DJ. *Int J Appl Radiat Isotopes* 1977; **28**: 169–181.
21. Otsuka FL, Welch MJ, Kibourne MR, Dence CS, Dilley WG, Jr SLW. *Nucl Med Biol* 1991; **18**: 813–816.
22. Smith-Jones PM, Stolz B, Burns C, Albert R, Reist HW, Fridrich R, Mäcke HM. *J Nucl Med* 1994; **35**: 317–325.
23. Broan CJ, Cox JPL, Craig AS, Kataky R, Parker D, Harrison A, Randall AM, Ferguson G. *J Chem Soc Perkin Trans* 2 1991; **1**: 87–99.
24. Prata MI, Santos AC, Geraldes CF, de Lima JJ. *J Inorg Biochem* 2000; **79**: 359–363.
25. Craig AS, Parker HA, Bailey NR. *J Chem Soc Chem Commun* 1989; 1793–1794.
26. Elander N, Jones JR, Lu S, Stone-Elander S. *Chem Soc Rev* 2000; **29**: 239–249.
27. Ohlsson T, Vengtsson NE, Risman PO. *J Microwave Power* 1974; **9**: 129.
28. Moi MK, Meares Cf, McCall JM, Cole WC, De Nardo SJ. *Anal Biochem* 1985; **148**: 249–253.
29. Heppeler ASF, Mäcke HR, Jermann E, Behe M, Powell P, Hennig M, *Chem Eur J* 1999; **5**: 1974–1981.
30. Hnatowich DJ, Layne WW, Childs RL. *Science* 1983; **220**: 613–615.
31. Chu BC, Orgel LE. *Proc Natl Acad Sci USA* 1985; **82**: 963–967.
32. Dewanjee MK. *Diag Oncol* 1993; **3**: 189–208.
33. Lewis MR, Raubitschek A., Shively JE. *Bioconjugate Chem* 1994; **5**: 565–576.
34. Lewis MR, Kao JY, Anderson A-LJ, Shively JE, Raubitschek A. *Bioconjug Chem ASAP published on Web 00/00/0000* 2001; page est: 4.9.
35. Wu F, Yngve U, Hedberg E, Honda M, Lu L, Eriksson B, Watanabe Y, Bergström M, Långström B. *Eur J Pharm Sci* 2000; **10**: 179–186.
36. Dymov AM, Savostin AP. *Analytical Chemistry of Gallium*. Ann Arbor Science Publishers: Ann Arbor, Moscow, 1968.
37. Greig M, Griffey RH. *Rapid Commun in Mass Spectrom* 1995; **9**: 97–102.

