In Vivo Hybridization of Technetium-99m-Labeled Peptide Nucleic Acid (PNA)

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Hybridization of a radiolabeled single-stranded DNA oligonucleotide with its single-stranded complement in vivo has not yet been convincingly demonstrated. A contributing factor may be unfavorable in vivo properties of the phosphodiester and phosphorothioate DNAs. Peptide nucleic acid (PNA) oligomers have been reported to possess in vivo properties more suitable for radiopharmaceutical applications. **Methods:** We have radiolabeled an amine-derivatized 15-base PNA oligomer with ^{99m}Tc through a modified MAG_3 chelator. **Results:** The ability of the PNA to hybridize in vitro with its complement appeared to be unimpaired after conjugation and radiolabeling. Size-exclusion, high-performance liquid chromatography (HPLC) analysis of 37°C serum after 24 hr of incubation showed the radiolabel to be present predominately as labeled PNA with indications of labeled serum proteins and a low molecular weight catabolite. Whole-body clearance in mice was rapid, with 50% of the label eliminated in about 2 hr. After 2.5 hr, the highest uptake (kidneys) was only 1.5% of the injected dose/g; less than 0.07%/g was present in all sampled tissues at 24 hr. To evaluate in vivo hybridization, beads were implanted subcutaneously in both thighs of normal mice. In the left thigh only, the beads were conjugated with complementary single-stranded PNA. At 23 hr following intraperitoneal administration of the labeled PNA, the left/right thigh radioactivity ratio was 6:1. Whole-body images at this time showed only bladder, kidneys and the left thigh. **Conclusion:** Unlike the radiolabeled DNAs investigated in this laboratory, ^{99m}Tc-PNA displays stability and pharmacokinetic properties suitable for eventual use as radiopharmaceuticals.

Key Words: technetium-99m-PNA; pharmacokinetics; hybridization

J Nucl Med 1997; 38:907–913

The property of oligomers (oligonucleotides) that best explains the interest these reagents now generate is an ability to hybridize specifically with their complementary sequence by the rules of Watson-Crick base pairing (*1*). This property of hybridization of single-stranded oligomers can be used to advantage in drug and radiopharmaceutical development. For example, oligonucleotide DNAs are currently under investigation for antisense applications (*2,3*). Accordingly, if radiolabeled, these oligonucleotides may usefully deliver radioactivity to targeted cells or tissues. Recently, the c-myc oncogene mRNA was targeted in mice with a radiolabeled antisense probe (*4*). Other possible applications include a novel method of radiolabeling large molecules by hybridization (*5*), pretargeting approaches based on oligonucleotides (*6*) and the amplification of radioactivity within a tumor or other lesion by sequential administration of complementary DNAs (*7*).

Although the native single-stranded phosphodiester DNA has been considered for these, and other, in vivo applications, these unmodified oligonucleotides are highly susceptible to degradation by nucleases (*8–10*) and, as such, may be an inappropriate carrier of radioactivity for in vivo imaging applications. Methods have been developed to chemically modify the phosphodiester DNA to improve its stability (*11*). Among the many possible modified DNAs, the most popular at present is the phosphorothioate, in which a nonbonding oxygen in the phosphate backbone is replaced with a sulfur (*12*). This laboratory has investigated a phosphorothioate DNA radiolabeled with ^{99m}Tc and reported a high affinity for serum and tissue proteins and a resulting unfavorable pharmacokinetics (*13*).

Peptide nucleic acid (PNA) is an oligomer in which the charged phosphate-ribose backbone has been eliminated and replaced with an uncharged polyamide backbone (*14*). These oligomers have been reported to resist nuclease and protease degradation (*15*). Furthermore, the binding affinities of PNA for its complementary single-stranded PNA has been shown to exceed that of comparable DNAs (*14*).

This laboratory reported earlier that amine-derivatized DNAs could be radiolabeled with ^{99m}Tc using a hydrazino nicotinamide (SHNH) chelator (*16*). Recently, we developed a synthesis of the N-hydroxysuccinimide derivative of mercaptoacetyltriglycine ($NHS\text{-}MAG_3$) chelator in which the thiol is protected by an acetyl group (*17*). We have established that the associated technetium-99m (^{99m}Tc) chelate is adequately stable for in vivo applications and that antibodies labeled in this manner do not bind nonspecifically to serum or tissue proteins at interfering levels (*18*). We report here on the use of this labeling technology to radiolabel with ^{99m}Tc an amine-derivatized 15-base single-stranded PNA. The pharmacokinetics of the labeled PNA were evaluated in normal mice. In particular, we demonstrate that the labeled PNA is capable of hybridizing to its complement in vivo in a mouse bead model.

MATERIALS AND METHODS

Two complementary 15-base single-stranded PNAs were synthesized for this investigation (PerSeptive Biosystems, Framingham, MA). One strand was derivatized with a primary amine on the amino terminus (i.e., 5′ equivalent) end through a 17-member ethylene-ether linkage. The complementary sequence was prepared with a biotin group on this end through the same linker. The base sequences were $NH_2\text{-}(CH_2)_2O(CH_2)_2OCH_2CONH(CH_2)_2O(CH_2)OCH_2CO$-TGT-ACG-TCA-CAA-CTA-$CONH_2$ and biotin-$(CH_2)_2O(CH_2)_2OCH_2CONH(CH_2)_2O(CH_2)OCH_2CO$-TAG-TTG-TGA-CGT-ACA-$CONH_2$. The melting temperature (i.e., the temperature at which half the base pairs have dissociated) in physiological saline of the duplex was calculated to be 72° C (*15*). The expected molecular masses were 4336 and 4634 Da, respectively, and were observed by mass spectrometry to be 4340 and 4635 Da. Purity of both chains was established by reverse-phase high-performance liquid chromatography (HPLC) (in both cases showing a single peak) and mass spectrometery (showing one predominant peak). The PNAs were lyophylized, stored dry and dissolved when needed in sterile water to a concentration of 4 mg/ml. After solubilization, aliquots of 20-1000 μg of PNA were added to plastic vials that were immediately frozen at −20° C for storage. Avidin (Sigma

Received May 10, 1996; revision accepted Aug. 12, 1996.
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Chemical Co., St. Louis, MO) was used without further purification. The ^{99m}Tc-pertechnetate was obtained from a ^{99} Mo-^{99m}Tc radionuclide generator (Dupont, Billerica, MA). Streptavidin-conjugated magnetic polystyrene beads, 1 μm in size (BioMag, PerSeptive Biosystems, Framingham, MA), were stored wet at refrigerator temperatures as recommended by the manufacturer. The capacity of the beads for biotin was reported by the manufacturer to be 1.5 ng of biotin per milligram of beads. The NHS-MAG_3 used early in this investigation was synthesized according to published procedures and contained underivatized MAG_3 at a variable and unknown concentration (*17*). Subsequent studies used NHS-MAG_3 essentially free of contamination.

PNA Coupling and Labeling

The desired volume of the 4-mg/ml water solution of the amine-derivatized single-stranded PNA was made 0.36 *M* sodium bicarbonate, 1.4 *M* sodium chloride and 1.4 m*M* DTPA, pH 9.3. The NHS-MAG_3 was dissolved in dry DMF at a concentration of 20 mg/ml. A volume of the DMF solution representing a molar ratio of MAG_3 to PNA of approximately 20:1, was added to the PNA solution during vortexing. The solution (now containing no more than 10% DMF) was incubated at room temperature for 1 hr. The conjugated PNA was purifed over a 0.7 × 30 cm column of P4 (BioRad, Melville, NY) using 0.25 *M* ammonium acetate, 0.25 m*M* DTPA, pH 5.2 as eluant. The final PNA concentration was determined by UV absorption at 260 nm using an extinction coefficient determined in this laboratory of 33 μg/ml and was usually about 0.3 mg/ml.

The coupled PNA was usually stored frozen at −20° C for no more than 1 wk before use. Generally, 150 μg of the conjugated PNA was labeled on each occasion. To the PNA solution (about 300 μl) was added 2.35 mg of sodium tartrate (Sigma) from a fresh 50 mg/ml solution in 0.5 *M* ammonium bicarbonate, 0.25 *M* ammonium acetate, 0.18 *M* ammonium hydroxide, pH 9.4 buffer, followed by about 5 mCi of ^{99m}Tc-pertechnetate generator eluant (20 μl). Finally, 17 μg of tin(II) chloride (Sigma) from a fresh 1-mg/ml solution in 10 m*M* HCl was quickly added with agitation. The labeled PNA was purified on a 0.7 × 20-cm column of P4 using saline as eluant. The identical labeling procedure was performed on the native, uncoupled PNA as a control.

Each preparation of radiolabeled PNA was analyzed by size exclusion HPLC using a single 0.7 × 30 cm Superose 12 column (Pharmacia, Piscataway, NJ) with both in-line radioactivity and UV detection and 0.05 *M* phosphate, pH 7 eluant. Recovery of radioactivity was routinely determined. Confirmation of labeling was established by HPLC analysis before and after adding the sample to streptavidin-conjugated magnetic beads to which the biotinylated complementary PNA was bound (see below). Loss of radioactivity from solution was due to binding by hybridization of the labeled PNA to the beads.

Preparation of PNA-Bound Beads

Complementary PNA was bound to streptavidin on magnetic beads through its biotin moiety. The suspension of beads was rinsed three times with a washing buffer consisting of 20 m*M* tris, 2 *M* sodium chloride, 1 m*M* EDTA and 0.1% Tween 20, adjusted to pH 7.0 and six additional times with a 1:1 dilution of this buffer in water. The beads were manipulated for washing by using a magnetic separator (MPC, Dynal, A.S., Lake Success, NY). After the last wash, the beads were incubated for 30 min with biotinylated complementary PNA at 6 μg of PNA per milligram of beads (i.e., 100% of saturation) in the washing buffer. The beads were then washed five additional times with the diluted washing buffer.

Rate of Hybridization

The rate of hybridization of the labeled PNA to its complement under the conditions of this study was determined at room temperature by adding 1 μg of labeled PNA to 300 μl of complementary PNA attached to beads and suspended at a 1-mg/ml concentration in 10 m*M* tris, 1 *M* sodium chloride, 0.5 m*M* EDTA, 0.05% Tween 20, adjusted to pH 7.0 buffer. Samples were removed for analysis periodically over 24 hr. The beads in each sample were separated magnetically from the solution, washed five times in the washing buffer and counted in a NaI(Tl) well counter. As a control, the identical study was repeated with beads without the complementary PNA.

Serum and Whole-Blood Incubations

Labeled PNA was incubated at a concentration of about 5 μg/ml in fresh 37°C human serum from two healthy volunteers and in fresh mouse serum. Samples were periodically removed over 24 hr for HPLC analysis using a 0.1-*M* sodium phosphate, 0.15-*M* saline buffer, pH 7.0 eluant. The identity of labeled PNA peaks in the serum incubate was confirmed by HPLC analysis before and after the addition of 200 μg of complementary PNA beads to 100 μl of the serum.

To evaluate whether the labeled PNA accumulates in formed elements, the labeled PNA was also added to fresh human whole blood with EDTA anticoagulant. The whole blood was incubated at 37°C with gentle agitation every 15–20 min. Samples were removed at 1 and 24 hr and separated by centrifugation. The formed elements were washed three times with 0.1 *M* PBS, 0.15 *M* sodium chlorine, pH 7.4, and counted in a NaI(Tl) well counter.

Homogenate and Urine Analysis Studies

Normal CD-1 male mice (Charles River, Wilmington, MA) were injected through the tail vein with 0.1 ml of 0.15 *M* saline containing about 5–10 μg (about 100 μCi) of labeled PNA and, at 1 hr postadministration, samples of urine were obtained. The animals were killed by cervical dislocation at 2.5 hr and the kidneys removed. Homogenates were prepared in a 15-ml tissue grinder (Dounce, Wheaton, Millville, NJ) in ice-cold 0.2-*M* sodium acetate buffer, pH 5. After grinding, samples were sonicated for 0.5–1 min at 300 W on ice and then centrifuged at 3500 rpm at 4° C for 15 min. The pellet and supernatant were counted separately, and aliquots of the supernatant also were analyzed by HPLC.

Mouse urine collected 1 hr postadministration was analyzed by HPLC before and after adding the complementary PNA beads to establish the extent to which radioactivity in urine was ^{99m}Tc-PNA. Control studies consisted of the identical assay in which beads without PNA were added to the urine sample.

Animal Biodistribution and Imaging Studies

Biodistributions of ^{99m}Tc-labeled PNA were evaluated in normal CD-1 male mice. Each animal was administered by tail vein 0.1 ml of 0.15-*M* saline containing 5 μg (about 100 μCi) of ^{99m}Tc-labeled PNA. Whole-body activity was determined by repeatedly placing each animal momentarily in a dose calibrator. The anesthetized animals were killed by spinal dislocation either at 2.5 or 24 hr postadministration. Samples of organs were rinsed in cold saline and were weighed before being counted in a NaI(Tl) well counter along with a blood sample and an aliquot of the injectate. The biodistributions were reported as percent of the administered radioactivity per gram of tissue.

In a separate study, three male CD-1 mice were each injected intramuscularly in their left thighs with 150 μl of saline containing 1.0 mg of PNA-coupled beads. An exact equivalent of beads without PNA was injected into their contralateral thighs. Immediately thereafter, each animal received an intraperitoneal injection of 50–55 μg (about 1 mCi) of labeled complementary PNA. Animals

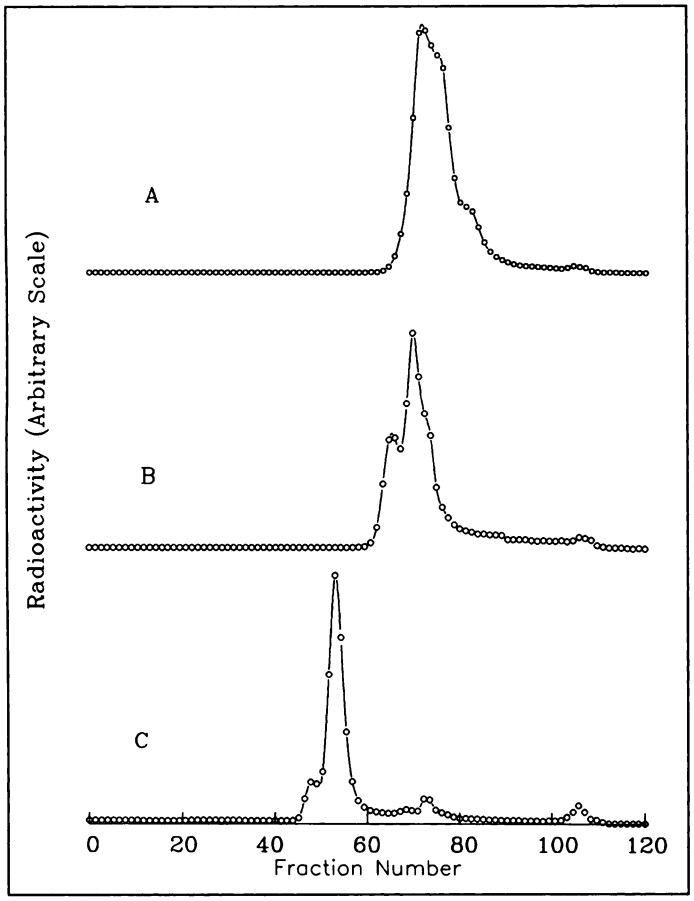

FIGURE 1. (A) Size-exclusion HPLC radiochromatograms of labeled PNA in buffer, (B) after the addition of biotinylated complementary PNA to form the biotin-PNA-PNA duplex and (C) after the addition of avidin to the biotin-PNA-PNA duplex.

were imaged simultaneously on an Elscint APEX 409-*M* portable gamma camera by resting the nembutal-anesthetized animals on the face of the upright collimator. Animals were imaged five times between 2 and 23 hr postadministration of the labeled PNA. At death, both whole thighs were excised for counting in a NaI(Tl) well counter. ROIs were drawn about the thighs and whole body in each image to obtain an estimate of the counts therein. Based on the counts in each image, the well counter counts of the thigh and the injected activity, the percent of the injected dosage in each thigh at each time point was estimated. The left thigh/whole-body radioactivity ratios were calculated without correction simply from the counts in each image.

RESULTS

PNA Labeling

Labeling efficiencies varied from 30%–70%, and specific activities as high as 100 μCi/μg of PNA were achieved. Control studies in which the unconjugated PNA was labeled showed less than 1% labeling under identical conditions. The HPLC radiochromatographic profile of the labeled and purified PNAs usually consisted of three distinct peaks, the relative intensities of which varied from preparation to preparation. Figure 1A shows a radiochromatographic profile of one such preparation. The UV profile (not shown) shows absorbency only with the earliest of these peaks. The recovery of radioactivity off the HPLC was occasionally as low as 25% and never exceeded 75%. The poor recovery was attributed to the lipophilicity of the uncharged PNA.

That the label was on PNA was confirmed by adding to the labeled PNA solution an excess of complementary PNA. The

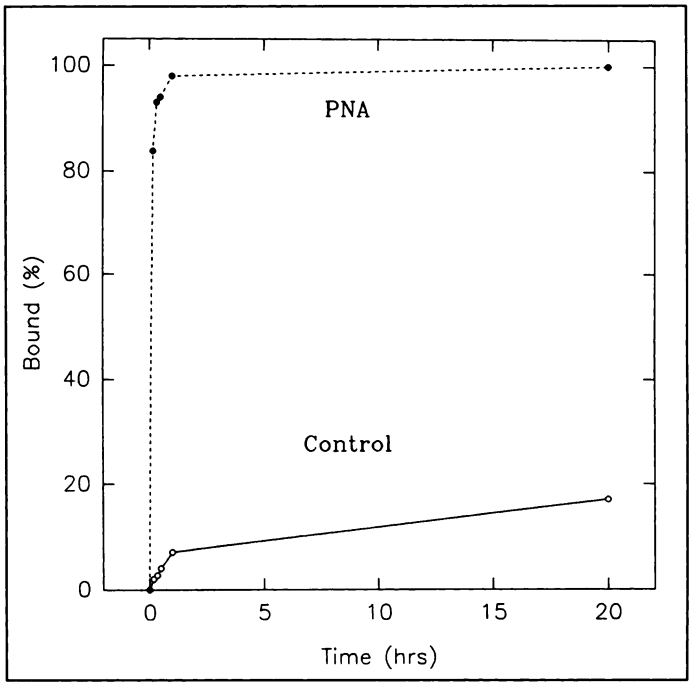

FIGURE 2. Rate of hybridization of labeled PNA in pH 7 buffer to complementary PNA immobilized on beads. Control refers to identical study using beads without PNA.

radiochromatogram resulting from this addition is shown in Figure 1B and may be compared to the radiochromatogram of the labeled PNA itself (Fig. 1A). A slight shift to higher molecular weight (i.e., earlier fractions) has occurred in the case of each peak in the triplet and is the result of PNA-PNA hybridization. Further evidence for labeled PNA is shown in Figure 1C. In this case, avidin was added to the PNA-PNA duplex to bind the complementary PNA through the biotin moiety. The shift in the radiochromatographic profile is now more pronounced, as expected, and is nearly quantitative. That this shift is not due to nonspecific binding of the label to avidin was established in a repeat study in which avidin was added to the labeled PNA without the prior addition of complementary PNA.

Rate of Hybridization

Figure 2 shows the percentage of labeled PNA bound to complementary PNA on beads versus time with early time points separated by 10 min. Under the conditions of this study, hybridization occurs rapidly and is completed within an hour. The extent of nonspecific binding of labeled PNA to the beads is minimal as shown by the control study in which identical beads, although without PNA, were used.

Serum and Whole-Blood Incubations

Figure 3 presents radiochromatographic profiles for labeled PNA after 1 and 24 hr of incubation in 37°C human serum and after 24 hr in saline. Multiple peaks are again apparent for the labeled PNA in Figure 3A. In serum, minimal binding of the label to serum proteins is apparent (Figs. 3B,C). The radioactivity ratios among the triplet PNA peaks have been consistently observed to change in serum in favor of the peak eluting in fraction 83. One peak, eluting in fraction 105 in the figure, is probably the result of catabolism. These general features were also observed during incubations in mouse serum and in the serum of another volunteer. A change in the radioactivity profile also occurs during incubation in room temperature saline (Fig. 3D).

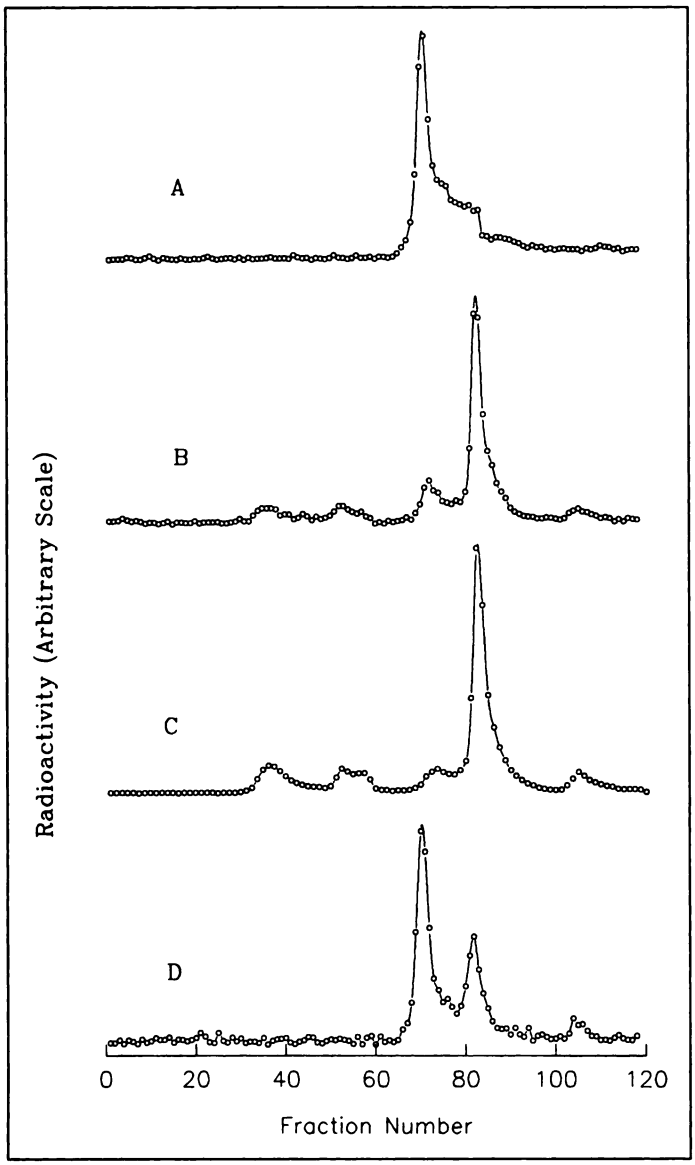

FIGURE 3. (A) Size-exclusion HPLC radiochromatograms of labeled PNA in buffer, (B) after 1 hr and (C) 24 hr of incubation in 37°C human serum and (D) after 24 hr in saline.

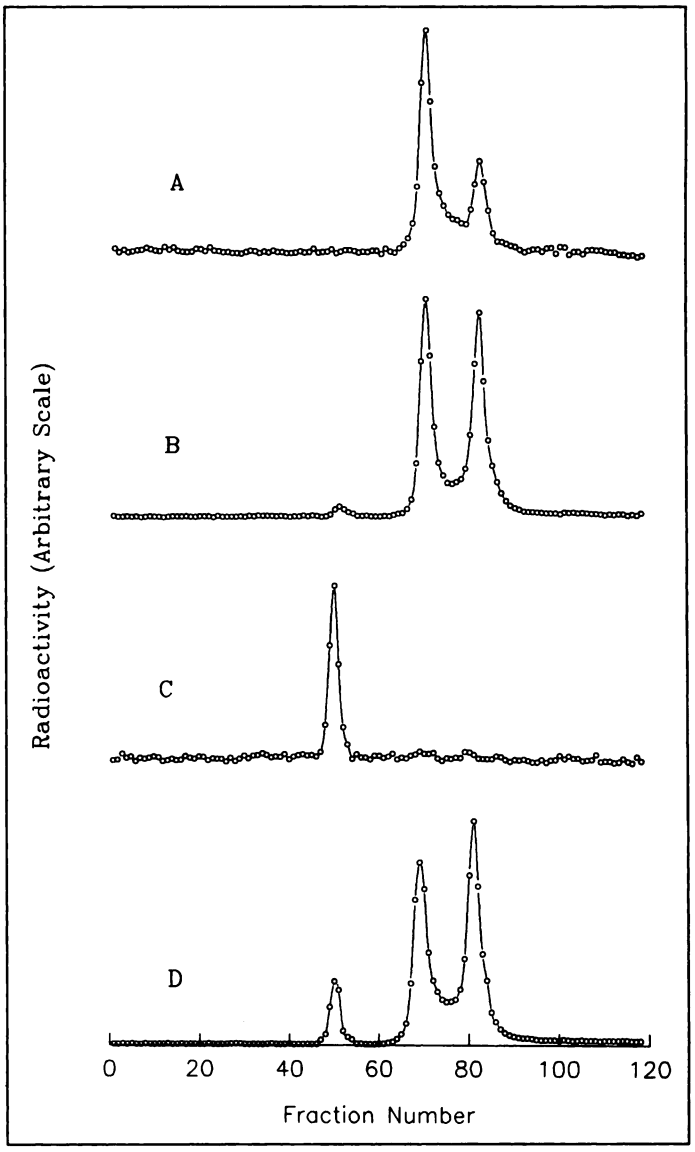

FIGURE 4. (A) Size-exclusion HPLC radiochromatograms of labeled PNA in buffer, (B) labeled PNA in 37°C human serum for 1.5 hr, (C) the 1.5 hr serum sample after removing labeled PNA by adding complementary PNA immobilized on beads and (D) the 1.5-hr serum sample after adding beads without complementary PNA as control.

To help identify radioactive peaks in serum due to labeled PNA, complementary PNA bound to streptavidin beads were added to a 1-hr serum sample and the sample reanalyzed after filtration to remove the beads. Figure 4 shows the radiochromatogram of the labeled PNA initially in saline (Fig. 4A) and after 1.5 hr in serum before (Fig. 4B) and after (Fig. 4C) extraction of labeled PNA. Only the serum-bound radioactivity remains after extraction. As a control against nonspecific binding, the identical serum sample was extracted under identical conditions except with beads without complementary PNA. As shown (Fig. 4D), the radiochromatogram is in this case unchanged.

Under the conditions of incubation of labeled PNA in whole blood described above, radioactivity bound to formed elements was 1.0% and 2.7% at 1 and 24 hr, respectively.

Homogenate and Urine Analysis Studies

Figure 5A presents HPLC radiochromatograms of labeled PNA administered to normal mice. The figure also shows the results of analyzing the serum (Fig. 5B) and urine samples (Fig. 5D) obtained at 2.5 hr postadministration. The serum sample shows only a single radiolabeled serum peak while the urine sample shows one of the labeled PNA peaks. Figure 5E is a repeat radiochromatogram (now presented on an expanded scale) obtained by analyzing the urine after the addition of complementary PNA beads. Virtually all radioactivity has been removed showing that the label in urine is present as labeled PNA. Figure 5C presents a radiochromatogram of the soluble fraction from the homogenate of a kidney obtained at this time showing labeled PNA and higher molecular weight, presumably labeled proteins. However, this analysis considers only about 20% of the radiolabel in the kidney since the remainder appeared in the insoluble pellet.

Animal Biodistribution and Imaging Studies

Figure 6 shows the whole-body radioactivity plotted separately for each of 10 mice receiving 5 μg each of labeled PNA. The half time of clearance is approximately 2 hr.

Table 1 presents the biodistribution results obtained at 2.5 and 24 hr postadministration of the radiolabeled PNA. The results reflect the rapid clearance of the label shown in Figure 6. The highest radioactive content is only 1.45% of the injected

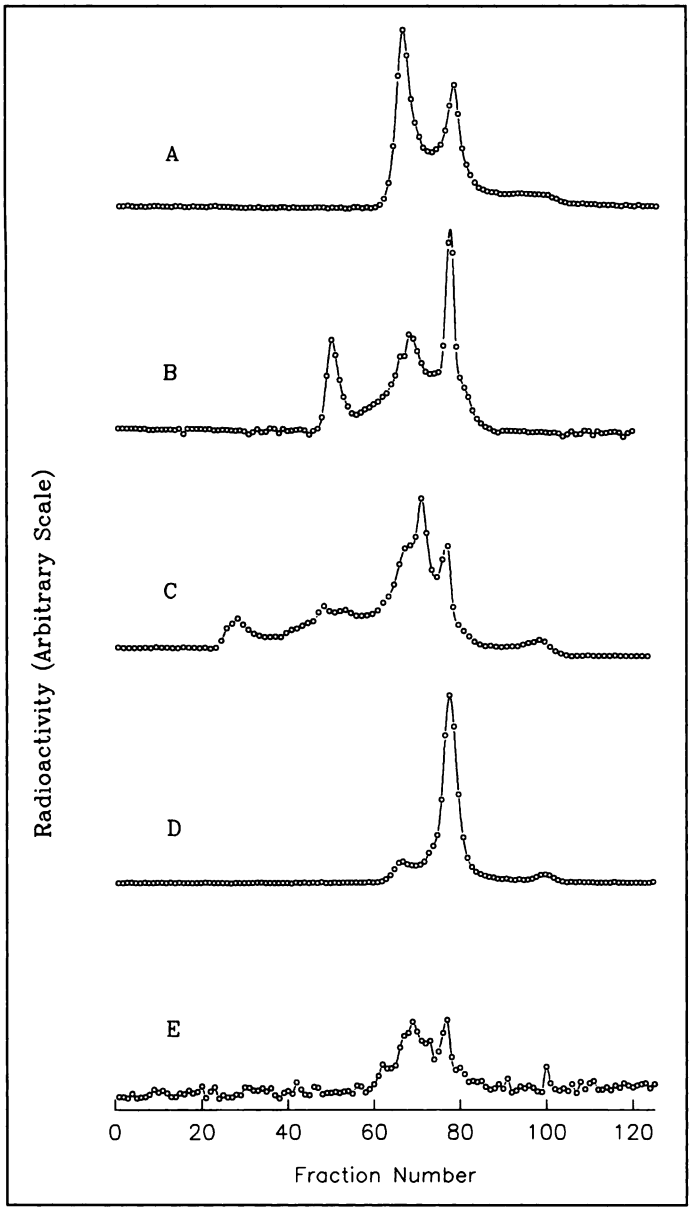

FIGURE 5. (A) Size exclusion HPLC radiochromatograms of labeled PNA in buffer and of several samples obtained at 2.5 hr postadministration of labeled PNA to a mouse that include (B) a serum sample, (C) the soluble fraction of a kidney homogenate, (D) the 2.5-hr urine sample and (E) the 2.5-hr urine sample after removing labeled PNA with complementary PNA immobilized on the bead.

dose per gram (ID/g) at 2.5 hr and 0.07% ID/g at 24 hr (in both cases in kidneys).

Three animals received identical administrations of beads subcutaneously in both thighs before the intraperitoneal administration of the labeled PNA. In all cases, only the beads in the left thigh contained the complementary PNA. Figure 7 presents the mean percentage of injected radioactivity in the left and right thighs along with the left/right thigh radioactivity ratios at each time point. The left thigh/whole-body ratio rose from 0.05 at 2 hr to 0.09 at 23 hr postinjection. As is evident from the figure, the left/right thigh radioactivity ratio rose from 2.7 to 5.8 during this period.

Figure 8 presents a composite whole-body anterior-posterior image of the three animals imaged simultaneously at 23 hr postintraperitoneal administration. Each image shows radioactivity essentially only in kidneys, bladder and the left thigh.

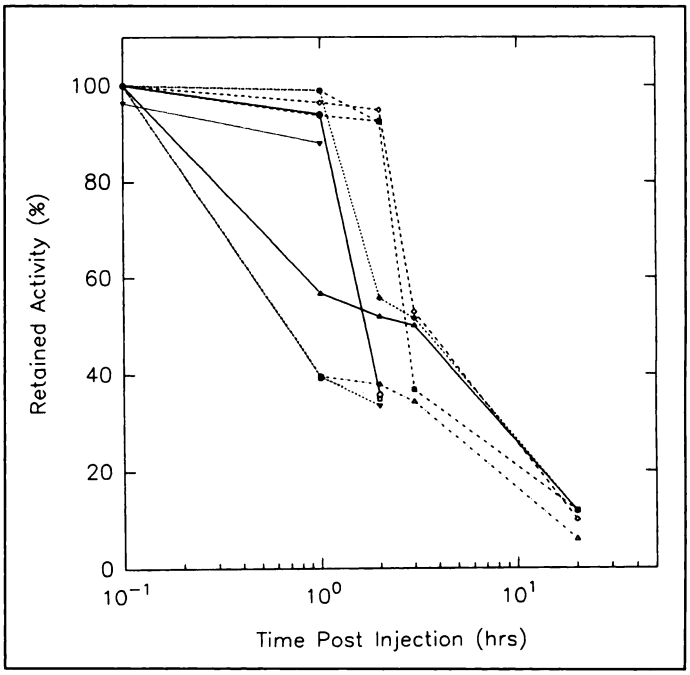

FIGURE 6. Whole-body radioactivity versus time plotted separately for mice receiving radiolabeled PNA.

DISCUSSION

Of the potential imaging applications for radiolabeled oligonucleotides, perhaps the most obvious at present is antisense targeting. Antisense targeting in nuclear medicine may be defined as intracellular localization through hybridization of a radiolabeled oligonucleotide with a base sequence opposite (i.e., antisense) to a sequence (i.e., sense) on the messenger RNA coding for a protein relatively unique to the target (*19*). The obvious advantage of antisense strategies is the potential for extreme specificity as only those cells expressing a particular gene should, in principle, be targeted. Furthermore, with the explosion of information regarding the human genome in health and disease, the number of potential gene targets is, even now, quite large.

Several barriers to effective antisense targeting will need to be overcome (*20*). First, the oligonucleotide must survive in circulation long enough to accumulate in the target tissue. A second, and probably more intractable difficulty, is cell membrane transport. The antisense oligonucleotide must gain access to the cytoplasm and possibly even the nucleus. The results of tissue culture studies provide little cause for optimism by showing that generally only a few percent of DNA oligonucle-

TABLE 1
Biodistribution* Obtained in Normal Mice 2.5 hr and 24 hr Postintravenous Administration of Technetium-99m-Labeled PNA

Organ	2.5 hr	24 hr
Liver	0.19 (0.08)	0.010 (0.002)
Heart	0.05 (0.03)	0.001 (0.002)
Kidneys	1.45 (0.88)	0.065 (0.017)
Lung	0.11 (0.05)	0.002 (0.001)
Stomach	1.30 (0.82)	0.050 (0.027)
Spleen	0.05 (0.02)	0.000 (0.001)
Muscle	0.06 (0.04)	0.001 (0.002)
Intestine	0.18 (0.11)	0.007 (0.004)
Blood	0.17 (0.07)	0.000 (0.000)

*In percentage injected dose/mg of tissue.
Mean values (n = 5) with s.d. in parentheses.

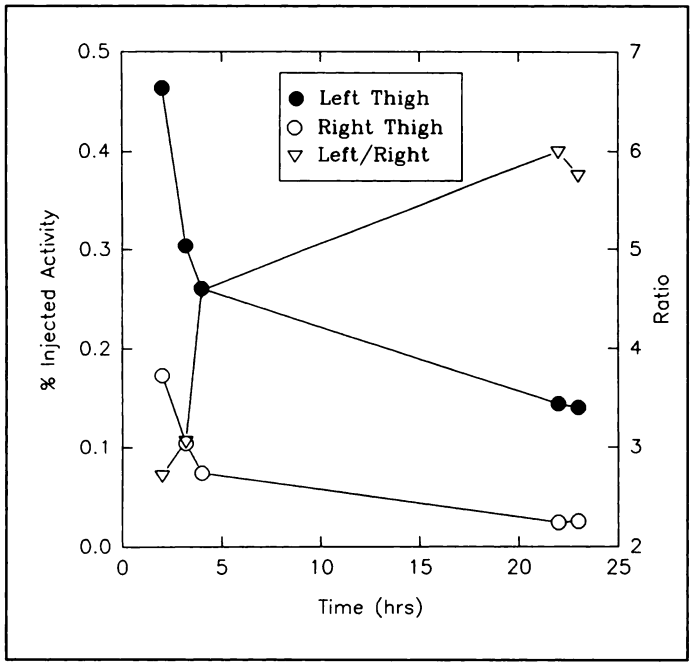

FIGURE 7. The percentage of injected radioactivity in the left thigh (closed circles) and right thigh (open circles) versus time postadministration of labeled PNA to mice implanted with complementary PNA-containing beads in the left thigh (left scale). Also presented is the left/right thigh radioactivity ratio versus time postinjection (right scale).

otides are incorporated in cells under the most favorable circumstances (*21*)

It is encouraging that some success has already been reported in the imaging of tumor by antisense. Recently Dewanjee et al. (*22*) have studied a ^{111}In-labeled 15-base phosphodiester and phosphorothioate single-stranded DNA oligonucleotides with an antisense sequence to that of c-myc, an oncogene thought to be expressed in a variety of malignant diseases. Under the conditions of the investigation, the uptake of label in cells in culture was higher for antisense (70%–80%) versus sense (3%–4%) DNA oligonucleotides and reached a plateau of uptake in only 45–60 min. The presence of mRNA-antisense DNA was confirmed by HPLC analysis of cytoplasmic extracts. Finally, whole-body images of tumor-bearing mice showed a slightly better tumor image with the phosphorothioate antisense versus the sense probe.

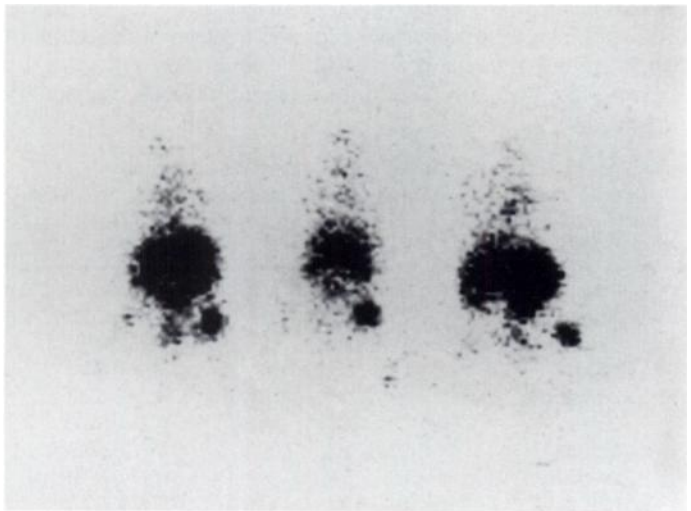

FIGURE 8. Whole-body anterior-posterior images of three mice obtained simultaneously 23 hr postintraperitoneal administration of labeled PNA. Underivitized beads implanted in the right thighs, beads with complementary PNA implanted in the left thighs (on the right in the image) of each animal.

Most probably, PNA oligomers in the form used in this investigation will not be useful for antisense imaging studies. Although they appear to be stable to nuclease digestion, the results of this, and other (*23*) studies suggest that they are not transported across cell membranes to the degree required for antisense application. PNAs may be of more use for those imaging applications in which cell membrane transport is not required. For example, it is possible that pretargeting studies, in which PNA conjugated to antitumor antibodies at the tumor site are targeted with radiolabeled complementary PNA, may be improved over conventional pretargeting with (strept)avidin and biotin. For this, and other similar applications, the PNA will be required to hybridize in vivo with its complementary PNA rather than DNA or RNA.

Regardless of their use in radiopharmaceutical applications, oligomers must possess certain essential properties. Since diagnostic applications require only tracer quantities of drug, toxicity is unlikely to be an issue. Among other considerations, suitable stability of the oligomer in vivo is essential. In addition, the pharmacokinetic properties must be suitable for the intended application. For example, the oligomer should clear through the kidneys in a time consistent with the application to provide a favorable target/nontarget ratio. For use as radiopharmaceuticals, it must be possible to radiolabel with imagable radionuclides such as ^{99m}Tc such that the label is suitably stable in vivo. Finally, the labeled oligomer must be capable of hybridization in vivo with its complement in the target.

This laboratory has investigated the properties of both native single-stranded phosphodiester DNA and modified single-stranded phosphorothioate DNA after radiolabeling with ^{99m}Tc (*13*). Although the in vivo properties were influenced to some extent by the method of radiolabeling, the phosphodiester DNA was judged to be degraded by nucleases too rapidly for most applications. The phosphorothioate DNA, although stable towards nuclease digestion, showed a high affinity for serum and tissue proteins. As a consequence, background radioactivity in liver and other tissues were persistent at unacceptably high levels (*13*).

Peptide nucleic acids (PNA) are synthetic oligomers in which the sugar and phosphate backbone of oligonucleotides have been replaced with a polyamide linkage (*14*). Not only does this substitution provide an oligomer reported to be resistant to nuclease and protease attack, but the absence of charge improves the binding affinity of PNA-DNA heteroduplexes (*15*).

In this investigation, radiolabeling with ^{99m}Tc was achieved by means of an acetyl-protected MAG_3 chelator. This labeling strategy was developed to avoid nonspecific serum protein binding observed for DNA labeled using a hydrazino nicotinamide (SHNH) chelator (*16*). We have recently demonstrated very similar properties for ^{99m}Tc in vitro and in vivo in animals when labeled to two IgG antibodies by MAG_3 and SHNH chelators (*18*). Using MAG_3, respectable labeling efficiencies and specific activities were achieved for PNA. Furthermore, the stability of the label in 37°C serum was acceptable with minimal activity present on either higher or lower molecular weight species (Fig. 3).

Although the UV profile of PNA showed a single peak by HPLC analysis (data not presented), the radioactivity profiles always consisted of two to three distinct peaks with only the earliest eluting with UV absorbency. Furthermore, the HPLC radioactivity profile varied somewhat from preparation to preparation but showed a consistent and profound shift to the third (i.e., last) peak on incubation in saline and, especially, in

serum (Figs. 3, 4). That all three peaks were radiolabeled PNA was established by demonstrating that each shifted to higher molecular weight on addition of the complementary PNA (Fig. 1). The nature of these peaks was not established in this research.

PNA undergoes negligible transport across cell membranes (*23*). This property of ^{99m}Tc-labeled PNA was not seriously investigated in this research. Nevertheless, the negligible accumulation of radiolabel in formed elements after incubation of labeled PNA in whole blood supports this suggestion.

The properties of PNA labeled according to the methods used here may be suitable for imaging studies in vivo. Apart from the stability of the label discussed above, the pharmacokinetic properties appear to be favorable. Whole-body radioactivity in mice after intravenous administration showed a rapid decrease (Fig. 6). The biodistribution studies in normal mice (Table 1) also showed this rapid decrease. At 2.5 hr, the highest level of radioactivity was in kidneys at only 1.45 %ID/g. At 24 hr, radioactivity in several tissues was below detectability. These results are in sharp contrast to that observed in this laboratory for ^{99m}Tc-labeled phosphodiester and phosphorothioate DNAs of about the same chain length where tissue radioactivity levels (except for stomach) at 4 hr postadministration were about 2–10 times higher for the phosphodiester DNA and 10–400 times higher for the phosphorothioate DNA (*13*). The rapid clearance of the radiolabel in the PNA case would be problematic if hybridization were slow by comparison. However, as shown (Fig. 2), hybridization under one set of conditions is more than 50% complete in less than 10 min.

The ultimate test of in vivo suitability for oligomers may be in vivo hybridization. We have used a mouse model in which polystyrene beads of about 1 μm in size were implanted intramuscularly in both thighs of normal mice. The implanted beads were first bound with the complementary PNA in the left thigh. After IP administration of radiolabeled PNA, increased accumulation of label occurred in the left thigh due to hybridization, with the left/right radioactivity ratio increasing with time between 2 and 23 hr. Apart from radioactivity in the left thigh, the whole-body image (Fig. 8) shows radioactivity only in bladder and kidneys. Similar studies in this laboratory with ^{99m}Tc-labeled phosphodiester and phosphorothioate DNAs were unsuccessful (data not presented).

CONCLUSION

This investigation demonstrates that single-stranded PNA may be radiolabeled with ^{99m}Tc. More importantly, when administered to mice, radiolabeled PNA can be made to hybridize to its PNA complement in vivo and shows promise for radiopharmaceutical applications.

ACKNOWLEDGMENTS

The authors at the University of Massachusetts Medical Center are grateful to Dr. Cassandra Smith, Boston University, for originally suggesting PNA. This work was supported in part by U.S. Department of Energy Grant DE-FG02–93ER61656 and U.S. National Cancer Institute Grant CA59785.

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