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Preparation of no-carrier-added [^{124}I]A_{14}-iodoinsulin as a radiotracer for positron emission tomography

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Summary

A_{14}-Iodoinsulin is a close biochemical and pharmacological mimic of insulin. Human insulin was labelled in its A chain tyrosine-14 residue by direct iodination with the positron-emitter iodine-124 ($\beta^+ = 25.6\%$; $t_{1/2} = 4.15$ days) to provide a radiotracer for imaging with positron emission tomography (PET). Several reagents were compared for conversion of cyclotron-produced [^{124}I]iodide into a reactive species for the labelling reaction. Radiochemical yields from the use of Iodo-Gen®, Chloramine-T, *N*-bromosuccinimide or lactoperoxidase–hydrogen peroxide were similar [35% ($n = 1$), $33 \pm 9\%$ ($n = 10$), $32 \pm 11\%$ ($n = 25$) or 33% ($n = 2$), respectively]. [^{124}I]A_{14}-Iodoinsulin was separated from unreacted insulin and radioactive by-products by tandem reverse phase HPLC and rapidly formulated for intravenous injection by adsorption on a Sep-Pak ^{t}C18-Plus® cartridge, followed by elution with 10 mM hydrochloric acid–ethanol (1 : 1 v/v, 1 ml). This radiotracer can now be obtained in useful radioactivities at high effective specific radioactivity and is now being applied to PET studies of its biodistribution in living subjects. Copyright © 2001 John Wiley & Sons, Ltd.

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Introduction

Insulin is a well-known hormone that plays an important role in glucose and lipid metabolism. It is composed of 51 amino acid residues in two doubly inter-linked peptide chains (Figure 1). Absolute and relative lack of this hormone occurs in type I and II diabetes, respectively. Receptors for insulin are predominantly located in liver, muscle and fat and are also present in brain.[1] However, there is some controversy about whether insulin can cross the blood-brain barrier via transcytosis. Radiolabelled insulins may help to answer this question and may also allow conditions such as myocardial insulin resistance, cardiac hypertrophy, hypertension and type II diabetes to be investigated.

Hitherto, insulin has been labelled with iodine-131 ($t_{1/2} = 8$ days)[3,4] and iodine-125 ($t_{1/2} = 60$ days).[5–12] There have also been reports of insulin labelled with iodine 123 ($t_{1/2} = 13.2$ h),[13–15] but without explicit details on labelling methods. Insulin has also been labelled with the positron-emitter, fluorine-18 ($t_{1/2} = 109.7$ min), via a prosthetic group.[16] This procedure is however complex and low yielding.

In principle, generic labelling methods already established with iodine-123 and iodine-131 might be applied with the positron-emitter iodine-124 ($\beta^+ = 25.6\%$, $t_{1/2} = 4.15$ days) to provide radiotracers for clinical research with the powerful imaging technique of positron

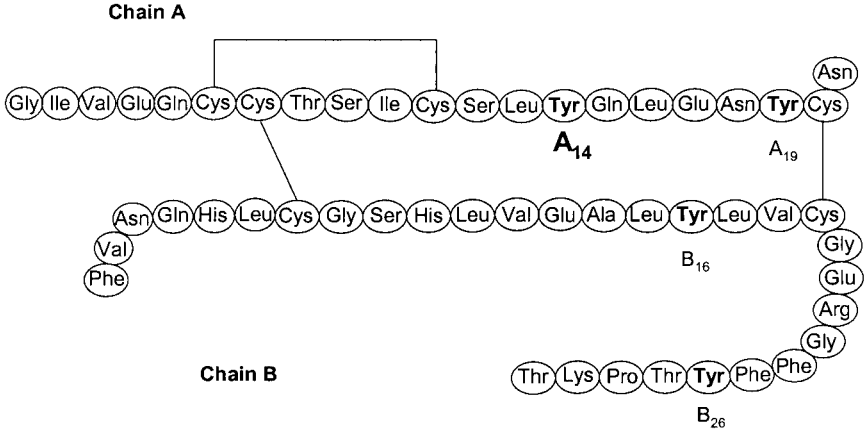

Figure 1. Primary structure of human insulin (from Reference 2)

emission tomography (PET). PET has the capability to deliver quantitative information on the biodistribution of a radiotracer in a living human subject with good spatial resolution ($\sim$4 mm full-width half-maximum) and high temporal resolution (a few seconds or minutes).[17] Iodine-124 is not an ideal radionuclide for imaging with PET. The proportion of decay of iodine-124 by positron emission is low. Also the emitted positrons have high average energy. Other modes of decay result in high-energy γ-rays. Despite these disadvantages, successful PET imaging with iodine-124 labelled tracers is possible.[18,19] Moreover, the long half-life of iodine-124 can be useful for assessing slow biochemical processes.

A_{14}-Iodoinsulin has been characterized as an excellent biochemical and pharmacological mimic of insulin.[6,20] Here we compare methods for labelling insulin at the A chain tyrosine-14 residue with iodine-124 and describe an effective procedure for producing no-carrier-added [^{124}I]A_{14}-iodoinsulin as a radiotracer suitable for PET studies of insulin biodistribution in living subjects.

Results and discussion

Production of iodine-124

The yield of iodine-124 at the end of the proton irradiation of a [^{124}Te]tellurium(IV) oxide target was 12.5 MBq/μA h. This product (37–74 MBq) typically contained 0.5 and 0.07% of iodine-123 and iodine-125, respectively, at 2 days after irradiation. Iodine-124 was wholly in the form of iodide when trapped in sodium hydroxide solution and 80% in this form when trapped in sodium phosphate buffer.

Radiolabelling of insulin

Of the four tyrosine residues in human insulin (Figure 1), the A_{14} residue was the most reactive for direct radioiodination by the various tested methods; hence, the desired A_{14}-radioiodoinsulin was the main product in all reactions (Table 1). A_{19}-Radioiodoinsulin was always produced as a major by-product.

The development of a production method for [^{124}I]A_{14}-iodoinsulin was initially investigated using iodine-125 as a convenient surrogate for iodine-124.

Table 1. Recoveries of radioactivity from single pass HPLC of reaction mixtures, and corresponding isolated radiochemical yields (mean ± SD for $n > 2$) of [^{125}I]A_{14}- or [^{124}I]A_{14}-iodoinsulin, for different labelling methods

Radioisotope	Method	Recovery of radioactivity from HPLC column[a]	Isolated radiochemical yield[a]
		(%)	(%)
^{125}I	Iodo-Gen®	79 ($n = 1$)	36 ± 8 ($n = 5$)
	NBS	85 ($n = 1$)	39, 40 ($n = 2$)
	Lactoperoxidase–H_2O_2	84 ($n = 1$)	34 ($n = 1$)
^{124}I	Iodo-Gen®	89 ($n = 1$)	35 ($n = 1$)
	NBS	94 ± 4 ($n = 17$)	32 ± 11 ($n = 25$)
		92 ($n = 1$)[b]	19 ± 5 ($n = 8$)[c]
	CAT	92 ± 3 ($n = 6$)	33 ± 9 ($n = 10$)
	Lactoperoxidase–H_2O_2	84, 90 ($n = 2$)	12, 54 ($n = 2$)

[a] From first HPLC column (as shown in Figure 7), calculated from the radioactivity in the reaction mixture.
[b] Recovery of [^{124}I]A_{14}-iodoinsulin from tandem HPLC (as shown in Figure 7).
[c] After isolation following tandem HPLC (as shown in Figure 7).

[^{125}I]A_{14}-Iodoinsulin. Other investigators have used either Chloramine-T (CAT)[12,21] or lactoperoxidase-hydrogen peroxide,[8,12,22] to prepare [^{125}I]A_{14}-iodoinsulin. Some authors have reported the use of Iodo-Gen® but they did not undertake a separation of the A_{14}-iodo isomer.[23] We found that the Iodo-Gen® method[24] gave less unwanted [^{125}I]A_{19}-iodoinsulin than the lactoperoxidase method. For the Iodo-Gen® method, isolated radiochemical yields varied between 26 and 48%. Corresponding yields for the other methods were between these extremes (Table 1).

To isolate [^{125}I]A_{14}-iodoinsulin, we implemented an isocratic reverse phase HPLC method, based on that described previously.[7] This efficiently separates [^{125}I]A_{14}-iodoinsulin from insulin and major radioactive by-products. In order to inject this radiotracer safely into animals, it is necessary to remove the complex mobile phase, which contains organic solvents. Removal of the mobile phase by rotary evaporation adhered the radiolabelled insulin to the glass vessel surface in a form that could not be extracted into a physiologically acceptable media (e.g. 1% human serum albumin in phosphate-buffered saline). Pre-treatment of the flask with SigmaCote® allowed some resolubilisation, but only to provide [^{125}I]A_{14}-iodoinsulin in low isolated

radiochemical yield (10%). Therefore, we explored solid phase extraction for formulation.

It was found that [^{125}I]A_{14}-iodoinsulin that had been separated by HPLC could be quantitatively immobilized on a Sep-Pak C18-Plus® cartridge from which it could then be eluted in 80–90% efficiency with a small volume (1 mL) of 10 mM hydrochloric acid–ethanol (1 : 1 v/v). Other eluents were less effective. A later generation Sep-Pak ^{t}C18-Plus® cartridge proved to be superior in its performance and was therefore preferred (Figure 2). The formulated product was radiochemically stable for at least two days at room temperature.

[^{124}I]A_{14}-Iodoinsulin. The use of sodium phosphate buffer (25 mM, pH 7.6) rather than sodium hydroxide solution for trapping [^{124}I]iodide was explored and found effective. This simplified the control of reaction pH and reduced the number of required manipulations in the preparation of the [^{125}I]A_{14}-iodoinsulin. TLC analysis of the trapping phosphate buffer showed 80% of the radioactivity to be [^{124}I]iodide.

N-Bromosuccinimide (NBS) was found to be an effective oxidant (Table 1). Reaction parameters, such as amount of NBS, concentration of insulin, pH and reaction time, were investigated. The amount of NBS used was found to be critical. A low amount resulted in low overall radiolabelling of insulin (Figure 3). The use of 2 μg of NBS gave the

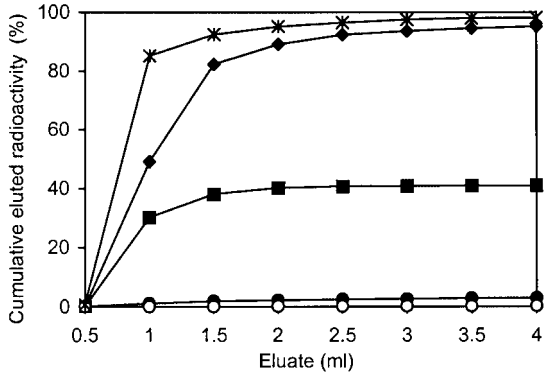

Figure 2. Cumulative percentage of [^{125}I]A_{14}-iodoinsulin (6 kBq) eluted from Sep-Pak C18-Plus® cartridges with increasing volumes of 5 mM hydrochloric acid in 25% aqueous ethanol (○), ethanol (●), acetic acid (■), and 5 mM hydrochloric acid in 50% aqueous ethanol (◆); elution of [^{125}I]A_{14}-iodoinsulin (3.9 MBq) from a Sep-Pak ^{t}C18-Plus® cartridge using 10 mM hydrochloric acid-ethanol (1 : 1 v/v) (*)

highest yield of radiolabelled insulins, with labelling predominantly at the A_{14}-position. Larger amounts of NBS produced radioactive products that were long retained on the HPLC column, or only elutable in mobile phase containing 32% acetonitrile (Figure 4). These products possibly arise from oxidative damage to the insulin.[25,26] Insulin at a concentration of 4.1 mg/ml gave optimal radiochemical yields at given molar ratios of NBS to insulin (Figure 5). The optimal pH for the NBS-promoted reaction in the range, 4.4–8.4, was 7.4 (data not shown). The labelling reaction was complete within 30 s.

The optimal reaction parameters (amount of insulin, reaction volume, reaction pH) for [^{124}I]A_{14}-iodoinsulin preparations with NBS were also used with other oxidants (Iodo-Gen®, CAT and lactoperoxidase–hydrogen peroxide) and gave similar radiochemical yields (Table 1). Radiochemical yields appeared independent of the initial amount of iodine-124 used in the reaction (up to the tested level of 33 MBq).

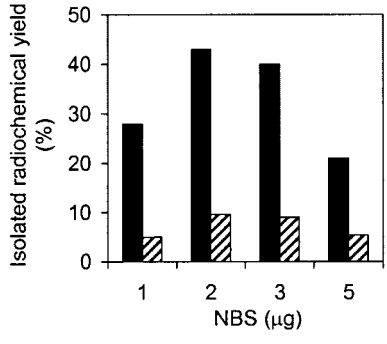

Figure 3. Effect of NBS amount on the isolated radiochemical yields of [^{124}I]A_{14}- and [^{124}I]A_{19}-iodoinsulin (solid and hatched columns, respectively)

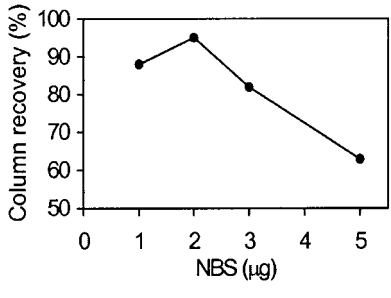

Figure 4. Effect of NBS amount on the recovery of radioactivity from the first HPLC column

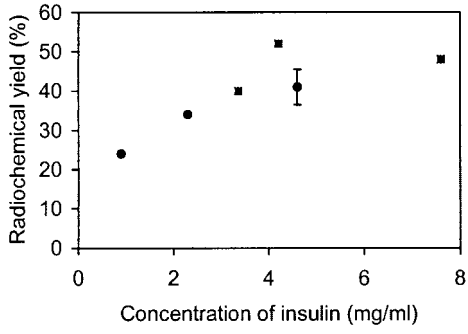

Figure 5. Dependence of the radiochemical yield of isolated [^{124}I]A_{14}-iodoinsulin on insulin concentration in NBS-promoted reactions. Product was separated by single column HPLC. Insulin was used in 30.7-fold (●) and 7.7-fold (■) molar excess over NBS. For an insulin concentration of 4.6 mg/ml the error bar is ± SD (n =4)

A minor radioactive by-product, with a slightly shorter retention time than [^{124}I]A_{14}-iodoinsulin, was observed in all single column HPLC separations (see, for example, Figure 6). This may be a B-chain labelled product.[11,22] This by-product was removed almost completely by peak-cutting and use of tandem HPLC.

The radiochemical purity of the product isolated by tandem HPLC (Figure 6) was >99% as established by ITLC and analytical HPLC. The formulation method developed for [^{125}I]A_{14}-iodoinsulin was similarly effective for [^{124}I]A_{14}-iodoinsulin (recovery efficiency, 73 ± 10%, n =20).

Unchanged insulin elutes ahead of A_{14}-iodoinsulin and therefore low amounts can tail into the labelled product. The amount of non-radioactive insulin in the preparations was reduced to a very low level by the use of tandem HPLC. Thus, analysis of formulated [^{124}I]A_{14}-iodoinsulin, prepared by the CAT method and collected after passing the first column, by HPLC revealed traces of A_{14}-iodoinsulin (1.78 μg/ml) and insulin (0.95 μg/ml) (detected by absorbance at 212 nm). The specific radioactivity, calculated on this basis, was 12.5 MBq/nmol. An antibody assay, that measures the amount of total biologically active insulin, showed a specific radioactivity of 13.1 MBq/nmol. An aliquot of [^{124}I]A_{14}-iodoinsulin obtained from the first column was then separately purified with the preparative column of the tandem system. After formulation, only A_{14}-iodoinsulin was detectable by analytical HPLC (0.34 μg/ml). The specific radioactivity was increased to 25.8 MBq/nmol. The antibody assay confirmed this observation showing a specific radioactivity of 21.5 MBq/nmol.

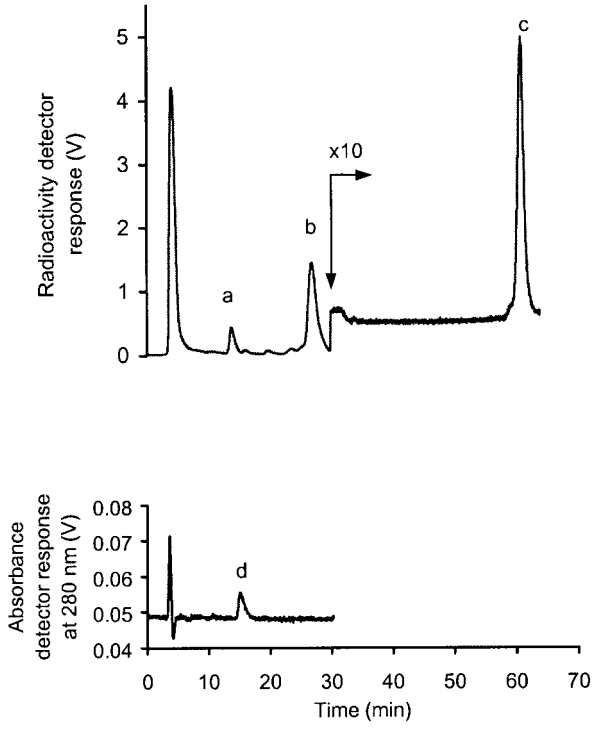

Figure 6. Chromatogram for the tandem column HPLC separation of [^{124}I]A_{14}-iodoinsulin prepared with NBS as oxidant. Key: a, [^{124}I]A_{19}-iodoinsulin (assigned according to Reference 7); b, [^{124}I]A_{14}-iodoinsulin (this peak was diverted to the second HPLC column); c, [^{124}I]A_{14}-iodoinsulin isolated from second column (the chromatogram between 30.5 and 65 min, was recorded with 10-fold increased gamma detector sensitivity); d, insulin. See Figure 7 for a scheme of the HPLC system and the text for elution conditions

Hence, the procedures developed with Iodo-Gen®, NBS, CAT or lactoperoxidase–hydrogen peroxide as oxidant, are similarly effective for producing [^{124}I]A_{14}-iodoinsulin for use in PET studies.

Experimental

Materials

Human insulin (recombinant, expressed in *E. coli*) and lactoperoxidase were obtained from Sigma. [^{125}I]Iodide (specific radioactivity: >0.6 TBq/mg iodide) was purchased from Amersham. A_{14}-Iodoinsulin was donated by Novo Nordisk A/S (Denmark). Iodo-Gen® was

purchased from Pierce. All other chemicals were supplied from Aldrich, Sigma or Merck and used as received. Labelling reactions were carried out in micro-reaction vessels (0.3 ml volume; Supelco) coated with SigmaCote. Autosampler vials (1 ml volume, Waters) were used for reactions using Iodo-Gen®. Indicator paper (pH range 1–14) was obtained from Whatman. C-18 Jupiter HPLC columns were purchased from Phenomenex.

Production and analysis of [^{124}I]iodide

[^{124}I]Iodide was produced according to the ^{124}Te(p, n)^{124}I reaction by irradiation of a [^{124}Te]tellurium(IV) oxide target (98% enrichment; diameter 10 mm; depth 0.5–1.0 mm) with a proton beam (9.8–12.3 MeV; 10 μA) from a Scanditronix MC40 Mk II cyclotron, usually for 2 h. Iodine-124 was recovered from the removed target by dry distillation in quartz apparatus.[27,28] For this purpose, a stream of oxygen was directed over the target at 760°C and passed into a trapping solution (300 μl) of sodium hydroxide solution (20 mM) or sodium phosphate buffer (25 mM; pH 7.6). When the [^{124}I]iodide was required in a smaller volume, this solution was evaporated under a stream of nitrogen (1 l/min).

The radionuclidic purity of iodine-124 was assessed using a co-axial GeLi detector and PC-based multi-channel analyser (GMX detector, ADC and Gamma-Vision software; EG & G). The system was calibrated for energy and efficiency using standardised sources of europium-132 and iodine-125 obtained from the National Physical Laboratory (Teddington, UK).

The radiochemical purity of [^{124}I]iodide was measured by TLC (methanol–water, 3 : 1 v/v) on cellulose layers (Polygram Cel 300 UV_{254}; Macherey-Nagel), with analysis by autoradiography (PhosphorImager 445 SI; Molecular Dynamics) R_f iodide, 0.8; R_f iodate, 0.3).

Radioactivity measurements

Iodine-124 was measured using an IG12 ionization detector (Centronics Ltd) coupled to a CP10 DC amplifier (Cooknell Electronics Ltd). The response of the system was measured using a source of iodine-124 that had been calibrated using the GMX detector.

The activity of iodine-125 was measured using a radioisotope calibrator (CRC®-10RB; Capintec) and a well-counter (1282 Compu-Gamma; Wallac).

HPLC methods

The mobile phase component (mobile phase A) used in the reverse phase HPLC separation of A_{14}-iodoinsulin consisted of water (680 ml), sodium dihydrogen phosphate (537 mg; 4.48 mmol), ammonium acetate (11.5 g, 149 mmol), acetonitrile (220 ml) and *iso*-propanol (100 ml), adjusted to pH 3.0 with hydrochloric acid (12 M).

[^{125}I]A_{14}-Iodoinsulin was isolated by HPLC on a system comprising a pump (Model 110B; Beckman), injector (1 ml sample loop; Model 0594; Rheodyne), column (C18 Jupiter; 250 × 4.6 mm; 5 μm; 300 Å), detector for absorbance at 280 nm (Lambda-Max, Model 480; Waters), γ-counter (Flow-Count; Bioscan) and chart recorder (Model PM 8252A; Philips). The system was eluted with mobile phase A at 1 ml/min (retention times; A_{14}-iodoinsulin, 25.8 min; insulin, 14.5 min).

[^{124}I]A_{14}-Iodoinsulin was isolated by tandem HPLC (Figure 7). For the first separation, the system comprised a solvent delivery module (Model 127; System Gold®; Beckman), sample injector (1 ml sample loop; Model 7725; Rheodyne) and column (C18 Jupiter; 250 × 4.6 mm; 300 Å). For the second separation, the system comprised a pump (Model 110B; Beckman), injector (6 ml sample loop; Model 7125; Rheodyne) and column (C18 Jupiter; 250 × 10 mm; 5 μm; 300 Å). The product output from the first separation was switched into the loop injector for the second separation. The output of each column was monitored with a γ-counter (Flow-Count; Bioscan) and that of the first column also with an absorbance detector set at 280 nm (single path monitor UV-1; Pharmacia). Data were acquired by a home-made chart-recording PC program (LabView 5.0.1). The first column was eluted in single-column preparations at 1 ml/min with mobile phase A for 0–35 min and then with mobile phase A-acetonitrile (9 : 1 v/v) (36–65 min) (retention times: A_{14}-iodoinsulin 27.2 min; insulin, 13.9 min). The second HPLC column was eluted with mobile phase A at 4 ml/min (total retention times, A_{14}-iodoinsulin, 61.8 min; insulin, 40–45 min).

Analysis of [^{124}I]A_{14}-iodoinsulin

[^{124}I]A_{14}-Iodoinsulin was analyzed by HPLC on a Bondclone C18 column (250 × 3.9 mm;. Phenomenex) eluted at 1 ml/min with ammonium acetate (10 mM)-trifluoroacetic acid–acetonitrile (65 : 35 : 0.1 by vol), with eluate monitored for absorbance at 212 nm and radioactivity (Flow-Count γ-detector; Bioscan) (retention times; insulin, 4.2 min;

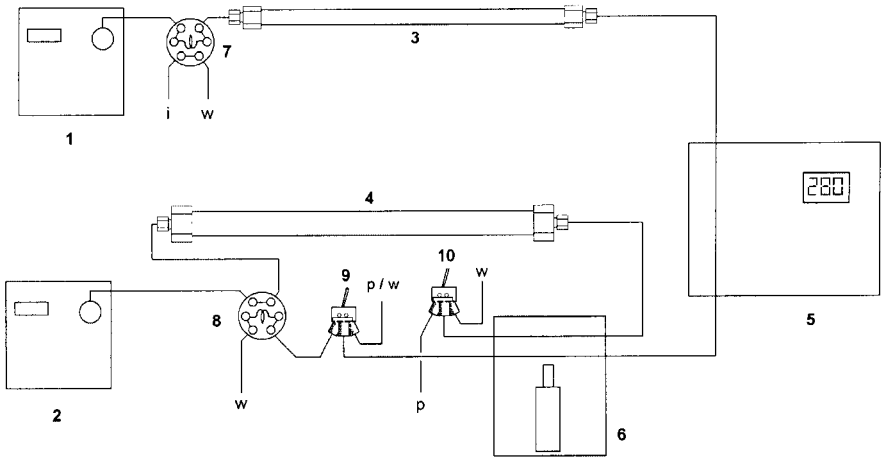

Figure 7. Line diagram showing the set-up for tandem HPLC separation of [^{124}I]A_{14}-iodoinsulin. Key: 1 and 2, solvent delivery modules; 3, 1st HPLC column; 4, 2nd HPLC column; 5, absorbance detector; 6, γ-detector; 7 and 8, injector valves; 9 and 10, peak-cutting valves; i, sample inlet; p, product collector; w, waste collector

A_{14}-iodoinsulin, 6.6 min). Radiochemical purity was also determined using ITLC plates (Gelman Sciences Inc., Ann Arbor) with methanol–water (4 : 1 v/v) as mobile phase.

Preparation of zinc-free insulin

Human Zn-insulin (9.6 mg, 1.647 mmol) was dissolved in hydrochloric acid (10 mM; 1 ml) and then EDTA solution (100 mM; 350 µl) was added. Cold sodium citrate buffer (200 mM, pH 5.6; 1 ml) was added to precipitate insulin, which was then centrifuged at 6000 g at 4°C for 10 min. The supernatant liquid was removed. The precipitate was washed with cold water (1 ml) and then dried under vacuum.

Preparation of [^{125}I]A_{14}-iodoinsulin using Iodo-Gen®

Hydrochloric acid (100 mM, 3 µl) was added to [^{125}I]iodide solution (10.5 MBq, 3 µl) followed by sodium phosphate buffer (250 mM, pH 7.4, 30 µl) plus zinc-free insulin solution (0.250 mg, 43.1 nmol) in sodium phosphate buffer (50 mM, pH 7.4; 10 µl). This solution was transferred to a conical auto-sampler vial (1 ml volume) that had been coated with Iodo-Gen® (10 µg). After 5 min, the reaction mixture was quenched

with a solution of *m*-tyrosine (2 μg, 11.0 nmol) in sodium phosphate buffer (250 mM, pH 7.4; 10 μl) diluted with mobile phase A (300 μl) and injected onto HPLC.

Preparation of [^{125}I]A_{14}-iodoinsulin using NBS

Hydrochloric acid (100 mM, 3 μl) and sodium phosphate buffer (250 mM, pH 7.4, 30 μl) were added to [^{125}I]sodium iodide solution (10.2 MBq, 3 μl). Zinc-free insulin solution (0.250 mg, 43.1 nmol) in sodium phosphate buffer (50 mM, pH 7.4, 10 μl) was then added to the stirred solution, followed by NBS (2 μg, 11.2 nmol) in sodium phosphate buffer (250 mM, pH 7.4, 20 μl). Stirring was continued for 5 min and the reaction terminated with *m*-tyrosine (as described above) and the mixture injected onto single-column HPLC.

Preparation of [^{125}I]A_{14}-iodoinsulin using lactoperoxidase–H_2O_2

Hydrochloric acid (100 mM, 3 μl) and sodium phosphate buffer (250 mM, pH 7.4, 30 μl) were added to [^{125}I]sodium iodide solution (9.6 MBq, 3 μl) followed by a suspension of zinc-free insulin (0.600 mg, 103 nmol) in citrate buffer (50 mM, pH 5.6, 72 μl). The solutions of lactoperoxidase (2.7 μg, 20.6 pmol, 20 μl) and hydrogen peroxide (0.56 mM, 10 μl) in sodium citrate buffer (200 mM, pH 5.6) were added with stirring. The reaction was terminated after 5 min *m*-tyrosine (as described above) and the mixing injected onto single-column HPLC.

Preparation of [^{124}I]A_{14}-iodoinsulin using Iodo-Gen®

Hydrochloric acid (100 mM, 12.9 μl) and then sodium phosphate buffer (250 mM, pH 7.4, 86.6 μl) were added to a stirred solution of [^{124}I]iodide (13 MBq) in trapping sodium hydroxide solution (273 mM, 5 μl). A sample ($\sim$0.4 MBq, 0.5 μl) was used to verify the pH as 7.4. Zinc-free insulin (0.5 mg, 86.1 nmol) in sodium phosphate buffer (50 mM, pH 7.4, 10 μl) was then added. This mixture was transferred into a vial plated with Iodo-Gen® (10 μg, 23.1 nmol) and left for 1 min. The reaction was then terminated with *m*-tyrosine (as described above) and the mixture injected onto single-column HPLC.

Preparation of [^{124}I]A_{14}-iodoinsulin using NBS

Hydrochloric acid (100 mM, 9 µl) and sodium phosphate buffer (250 mM, pH 7.4, 30 µl) were added to a stirred solution of [^{124}I]iodide (27.3 MBq) in trapping sodium hydroxide solution (20 mM, 50 µl) in a coated vial (SigmaCote). A sample of this solution (0.6 MBq, 1 µl) was used to verify the pH as 7.4. Then zinc-free insulin (0.5 mg, 86.1 nmol) in sodium phosphate buffer (250 mM, pH 7.4, 20 µl), followed by NBS (2 µg, 11.2 nmol) in sodium phosphate buffer (250 mM, pH 7.4, 20 µl), was added to the stirred mixture. The solution was stirred for a further 5 min. The reaction was then terminated with *m*-tyrosine (as described above) and the mixture injected onto single-column HPLC.

In other preparations, the fraction containing the product peak (volume 4–5 ml) was diverted to the second column of the tandem HPLC system and the purified [^{124}I]A_{14}-iodoinsulin collected in 15–20 ml for formulation (see below).

In some experiments, the reaction time, pH, concentration of insulin or amount of NBS was varied.

Preparation of [^{124}I]A_{14}-iodoinsulin using CAT

Hydrochloric acid (100 mM, 9 µl) and sodium phosphate buffer (250 mM, pH 7.4, 60.6 µl) were added to a stirred solution of [^{124}I]iodide (12.5 MBq) in trapping sodium hydroxide solution (273 mM, 5 µl) contained in a coated vial (SigmaCote). A sample ($\sim$0.2 MBq, 0.5 µl) was used to verify the pH as 7.4. Zinc-free insulin (0.5 mg, 86.1 nmol) in sodium phosphate buffer (50 mM, pH 7.4, 10 µl) plus a solution of CAT (0.64 µg, 2.81 nmol) in sodium phosphate buffer (250 mM, pH 7.4, 20 µl) were added to the stirred mixture. The reaction mixture was stirred for a further minute. The reaction was then terminated with *m*-tyrosine (as described above) and the mixture applied to single-column HPLC.

Preparation of [^{124}I]A_{14}-iodoinsulin using lactoperoxidase–H_2O_2

A solution of [^{124}I]iodide (15.7 MBq) in trapping sodium hydroxide solution (273 mM, 5 µl) was mixed with hydrochloric acid (100 mM; 12.9 µl) in a coated vial (SigmaCote). A suspension of insulin (550 µg, 94.7 nmol) in sodium citrate buffer (50 mM, pH 5.6) was added. A sample (0.4 MBq, 0.5 µl) of this mixture was used to verify the pH as 5.6.

Then solutions of lactoperoxidase (2.7 μg, 20.6 pmol, 20 μl) and hydrogen peroxide (0.56 mM, 10 μl) in citrate buffer (200 mM, pH 5.6) were added with stirring. The reaction mixture was stirred for 5 min. Then the reaction was terminated with *m*-tyrosine (see above) and the mixture injected onto single-column HPLC.

Specific radioactivity of [^{124}I]A_{14}-iodoinsulin

The specific radioactivity of [^{124}I]A_{14}-iodoinsulin was estimated by antibody assay (human insulin-specific RIA kit, Linco Research Inc) and also by analytical HPLC with UV absorbance response calibrated for mass of A_{14}-iodoinsulin and insulin.

Formulation of [^{124}I]A_{14}-iodoinsulin

A Sep-Pak ^{t}C18-Plus® cartridge (Millipore) was conditioned with formulation solvent (ethanol–10 mM hydrochloric acid, 1 : 1 v/v, 20 ml) and then water (20 ml). [^{124}I]A_{14}-Iodoinsulin from the HPLC separation was diluted once with water and loaded onto the cartridge. The column was then washed with water (10 ml) and eluted with formulation solvent. The first 0.5 ml of eluate was discarded. Formulated [^{124}I]A_{14}-iodoinsulin was obtained in the next 1 ml of eluate.

Conclusion

No-carrier-added [^{124}I]A_{14}-iodoinsulin was prepared in moderate radiochemical yields from cyclotron-produced [^{124}I]iodide using various oxidants. Tandem HPLC followed by solid phase extraction provided [^{124}I]A_{14}-iodoinsulin in high radiochemical purity and specific radioactivity in a form suitable for intravenous injection. This radiotracer is now being applied in PET studies.

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