Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXIV, No. 11

SYNTHESIS OF ^{123}I- AND ^{131}I-LABELLED DERIVATIVES OF LOW-DENSITY LIPOPROTEIN FOR RADIOPHARMACEUTICAL USE

S.M. Moerlein, K.B. Dalal and Y. Yano

Donner Laboratory, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720

Key words: low-density lipoprotein (LDL), tyramine-cellobiose-LDL (TC-LDL), radioiodination, ^{123}I, ^{131}I.

SUMMARY

The syntheses of two analogues of low-density lipoprotein (LDL) labelled with ^{123}I or ^{131}I was optimized for radiopharmaceutical application. Rabbit LDL was employed due to its usefulness in pre-clinical experimentation, although the parameters which were optimized also pertain to human LDL. LDL was iodinated directly with exchange-labelled [^{123}I]ICl to produce injectable [^{123}I]LDL in 40% radiochemical yield within 30 min. A derivatized version of LDL, [^{123}I]TC-LDL, was produced in about 30% radiochemical yield within 75 min by radioiodination of tyramine-cellobiose (TC) via in situ oxidation of $^{123}I^-$ followed by cross-linking to LDL using cyanuric chloride. Quality control tests indicated that both radioiodinated LDL and TC-LDL were very similar in character to the native LDL molecule.

INTRODUCTION

Low-density-lipoprotein (LDL) has an important function in human physiology as the major transport protein for cholesterol. The metabolism and plasma levels of LDL are altered in such disease states as cirrhosis, hepatitis, and nephrosis (1), and hyperlipidemia has been implicated as one of the major predisposing factors in

0362-4803/87/111325–15$07.50

Received November 26, 1986
Revised March 13, 1987

atherosclerosis and coronary heart disease (1-3). As a result of the pivotal role LDL plays in health and disease, a major goal of biomedical scientists has been to develop a radiopharmaceutical analogue of LDL which allows the non-invasive study of the metabolic status of this plasma protein. LDL has been labelled using [^{125}I]ICl for imaging studies of atherosclerotic lesions in man (4), as well as no-carrier-added (nca) $^{123}I^-$ or $^{131}I^-$ oxidized in situ (5). The preparation of ^{99m}Tc-labelled analogues of LDL has also been described (5,6).

Recent biochemical findings suggest that a radioiodinated tyramine-cellobiose adduct of LDL (TC-LDL) may have advantages as a radiopharmaceutical marker of LDL metabolism because the radioiodine label is intracellularly trapped following degradation of the protein (7,8). In the course of experiments which evaluated the use of ^{123}I (159 keV γ, $t_{1/2}$ = 13.2 hr)-labelled LDL and TC-LDL as radiopharmaceuticals for single-photon emission computerized tomography (SPECT), it was necessary to optimize the radiosyntheses of these compounds. The in vivo characteristics of these agents will be described elsewhere in a separate report. We present here the details of a systematic study of variables which affect the preparation of [^{123}I]- or [^{131}I]LDL and [^{123}I]- or [^{131}I]TC-LDL suitable for radiopharmaceutical use.

RESULTS AND DISCUSSION

The Watanabe WHHL rabbit (9, 10) has found widespread use in biomedical research as an animal model of human familial hypercholesterolemia. WHHL rabbits can be used in this way for the pre-clinical screening of potential radiopharmaceuticals for human applications, while the radiolabelling of animal rather than human blood components lessens the exposure of the radiopharmaceutical scientist to such human serum-transmitted diseases as auto-immune deficiency syndrome (AIDS) or hepatitis. Although these experiments were performed using rabbit LDL, the labelling parameters which were optimized also pertain to the radioiodination of human LDL. A major difficulty in the radiolabelling of rabbit rather than human LDL is the relatively low concentration of this protein in rabbit plasma. The plasma concentration of LDL in the normal rabbit is only 1.8 mg/ml, in contrast to the 24 mg/ml generally seen in man. This low concentration of rabbit LDL presents difficulties in the isolation of sufficient quantities of the plasma protein for radioiodination.

This problem was circumvented by feeding the rabbits a diet containing 4% excess peanut oil and 1% cholesterol for a period of 8-20 weeks (11). The plasma concentration of rabbit LDL dramatically increased, to levels exceeding 17 mg/ml. The quality and viability of LDL isolated from rabbits which were fed this hypercholesterolemic diet was assessed using sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis, as shown in Table 1. The molecular diameter of diet rabbit LDL was equal to that of human LDL (265-266 Å), although its electrophoretic mobility differed slightly (R_f = 0.201 and 0.215, for the rabbit and man, respectively). Prolonged storage at 4°C for 7-7.5 months resulted in an increase in the electrophoretic mobility of rabbit LDL from R_f = 0.201 to 0.268, while its viability as indicated by molecular diameter was unaltered. Similar storage effects have been noted by others (13).

[^{123}I]LDL was prepared by direct radioiodination of rabbit LDL using

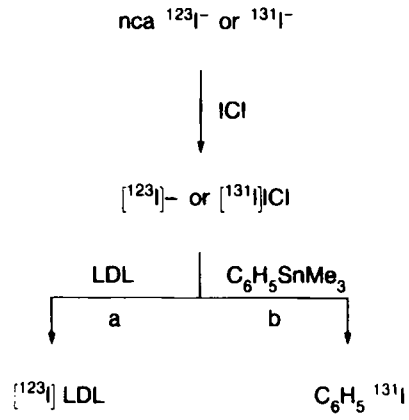
Table 1

Molecular Size and Electrophoretic Mobility of LDL Derivatives[a]

	Molecular Diameter (A)		R_f	
Plasma Protein	Fresh	Stored[b]	Fresh	Stored[b]
Human LDL	266	---	0.215	---
Rabbit LDL	265	265	0.201	0.268
[^{131}I]LDL	273	277	0.202	0.270
[^{131}I]TC-LDL	---	280	---	0.267

(a) 2-16% SDS-polyacrylamide resolving gel; running buffer 4.5 g Tris base, 21.6 g glycine, 1.5 g SDS in 1500 ml H_2O; constant current; 24 hr. Standards included in both apoferritin, thyroglobulin and carboxylated beads as previously described (12).
(b) 4°C under N_2 for 7-7.5 months.

exchange-labelled [^{123}I]ICl, as shown in Scheme 1, path a. The use of [^{123}I]ICl as the radioiodination reagent obviates the need for in situ oxidants which promote $^{123}I^-$ to electrophilic iodination species, which is beneficial because in the process of oxidizing radioiodide, damage to the sensitive protein may occur.



SCHEME 1

Using nca $^{131}I^-$ ($t_{1/2}$ = 8 d) for convenience, the exchange reaction $^{131}I^-$ + ICl → ^{131}ICl + I^- was optimized. Since the trimethyltin group of trimethylphenylstannane undergoes facile aromatic substitution by electrophilic iodine species in methanol to yield iodobenzene (14,15), this iododestannylation reaction was used to monitor the progress of the exchange process. As illustrated in Scheme 1, path b, any exchange-labelled iodine monochloride will react with trimethylphenylstannane to form $[^{131}I]$iodobenzene. Thus, determination of the radiochemical yield of $C_6H_5\ ^{131}I$ using radio-HPLC will give an indirect measurement of the degree to which $[^{131}I]ICl$ was formed from nca $^{131}I^-$.

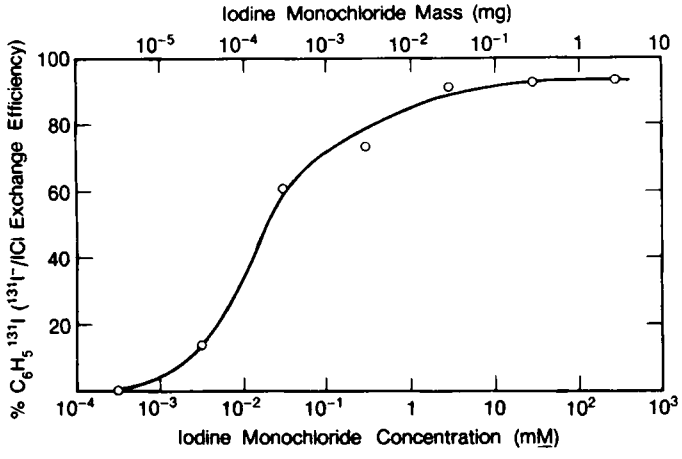


Figure 1. Radiochemical yield of $[^{131}I]$iodobenzene from the iododestannylation (30 min) of trimethylphenylstannane with exchange-labelled (5 min) $[^{131}I]ICl$. Due to the lability of the aromatic trimethyltin group to electrophilic substitution, the radiochemical yield of this reaction estimates the efficiency with which iodine monochloride is exchange-labelled by $^{131}I^-$. Data points represent the mean from 2-3 experiments.

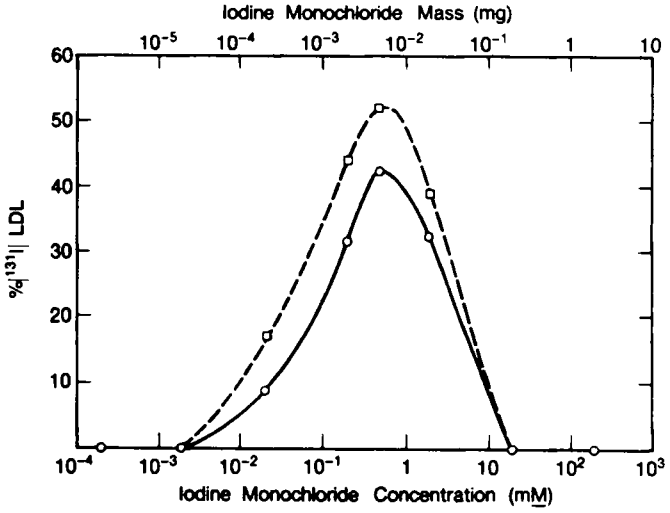
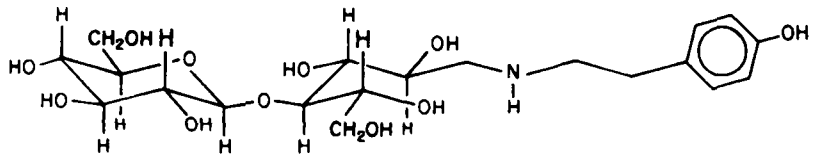


Figure 2. Radiochemical yield of [^{131}I]LDL produced by reaction (15 min) of exchange-labelled [^{131}I]ICl with 1 μM LDL. O , radiochemical yield as a percentage of starting $^{131}I^-$; □ , radiochemical yield as a percentage of available [^{131}I]ICl.

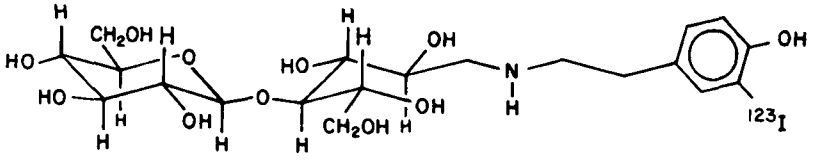
For an exchange interval of 5 min, the radiochemical yield of [^{131}I]ICl is dependent on the concentration of ICl (Figure 1). Despite the low carrier iodide concentration (ca. 50 nM resulting from 100 μCi $^{131}I^-$), exchange efficiencies exceeded 90% only with iodine monochloride concentrations of 10 mM or greater, and an exchange efficiency of 50% required approximately 0.1 mM ICl. These practical concentration limits for exchange place constraints on the radioiodination of LDL, since the mass of LDL which is required as a labelling substrate is dependent on the mass of ^{123}I– or ^{131}I-labelled iodine monochloride that is employed.

The relationship between the concentration of exchange-labelled ICl and of LDL is shown in Figure 2, where the radiochemical yield of [^{131}I]LDL from 1 μM LDL (2.3 mg/ml; MW = 2.4 - 3.9 X 10^6 (16)) is plotted as a function of the iodine monochloride concentration. The radiochemical yield of [^{131}I]LDL increased almost linearly to a maximum of 43% as the molar ratio of ICl to LDL (R) increased from 1 to approximately 10^3. This rise in yield may be attributed to an enhanced exchange efficiency as the iodine monochloride concentration increased from 10 μM to 1 mM, as well as to an increased fraction of non-specific oxidation of sulfhydryl groups by carrier (rather than radioactive) iodine monochloride. At higher specific activities of [^{131}I]ICl,



TC

$^{123}I^-$ / Iodogen

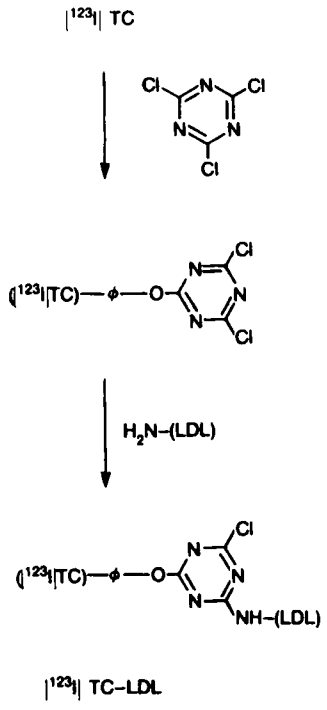


[^{123}I]TC

SCHEME 2

non-specific oxidation of exchange-labelled iodine monochloride can decrease incorporation of the radiolabel onto aromatic binding sites (17). Although higher radiochemical yields of [^{131}I]LDL may be afforded with greater concentrations of iodine monochloride in the exchange step (the 43% radiochemical yield of [^{131}I]LDL corresponds to 52% of the available [^{131}I]ICl), the use of more carrier ICl would require excessively large quantities of LDL substrate in the radioiodination step. As R increased above 10^3, the radiochemical yield of [^{131}I]LDL decreased, such that for R = 10^4 the yield was negligible. This effect may be due to saturation of aromatic binding sites (probably on tyrosyl residues (17)) for iodine on LDL. Figure 2 thus indicates that optimum radioiodination of LDL by [^{131}I]ICl occurs best when R is between 200 - 2000.

When these reaction conditions were applied to the preparative synthesis of [^{123}I]- or [^{131}I]LDL for in vivo application, isolated radiochemical yields of 38-40% were consistently obtained with an overall preparation time of 30 min (see Experimental). The radiolabelled LDL was purified by gel permeation chromatography to give the final [^{123}I]- or [^{131}I]LDL product in physiological saline. Quality control testing of the material indicated that the radioiodinated LDL was > 95% precipitated in 3% ethanolic



SCHEME 3

trichloroacetic acid, and the electrophoretic mobility and molecular size of both fresh and stored [^{131}I]LDL were very similar to that of native rabbit LDL (see Table 1). The distribution of radioactivity on the electrophoretic gel corresponded exactly with that of carrier I-LDL.

An alternative ^{123}I-labelled analogue of LDL was prepared by radioiodinating a tyrosine-cellobiose adduct (TC, Scheme 2) prior to conjugating this adduct to LDL to form radioiodinated TC-LDL, as shown in Scheme 3. There are several radiosynthetic advantages to this two-pot reaction procedure. An aromatic ring which is highly activated toward electrophilic substitution is employed, and the radioiodination step takes place in the absence of the sensitive LDL protein. Thus, in situ oxidation of nca $^{123}I^-$ or $^{131}I^-$ is possible without the risk of oxidative damage to LDL. Iodogen (1,3,4,6-tetrachloro-3α,6α-diphenylglycouril) is a more suitable in situ oxidizing agent in this case than other reagents which are available for electrophilic radioiodination (for a recent review, see reference 18) because its poor aqueous solubility allows for the reaction contents to be removed from the solid phase of Iodogen and placed into a

second vessel for conjugation to LDL (Scheme 3) without interfering reactions which otherwise result from the presence of the oxidant. This procedure was originally described by others for the preparation of [^{125}I]TC-LDL for biochemical applications (7), although a systematic examination of radiolabelling variables was lacking.

For convenience the parameters which influence the radioiodination of TC (Scheme 2) were examined using the relatively long-lived ^{131}I prior to the use of ^{123}I, and the radiochemical yield of [^{131}I]TC was determined using radio-HPLC. It was found that the mass of Iodogen which was employed for oxidation of nca ^{131}I$^-$ was not a critical parameter: radiochemical yields of 95.4 $\pm$ 2.9 % were achieved after 30 min when the Iodogen mass was varied from 1 μg to 10 mg. Subsequent experiments were performed using 500 μg (1.2 μmol) Iodogen.

The concentration of TC which was necessary for efficient incorporation of radioiodine was a second variable to evaluate, since the mass of TC present in solution with [^{123}I]– or [^{131}I]TC determines the amount of LDL that is required

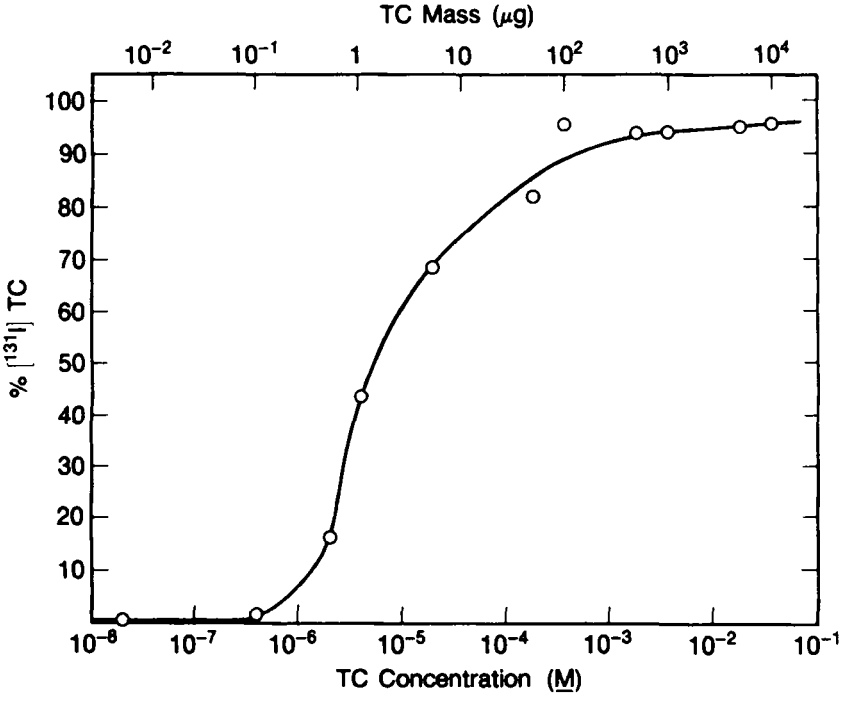


Figure 3. Radiochemical yield of [^{131}I]TC (after 30 min) as a function of TC concentration. Data points are the mean from 2-3 experiments.

for the coupling step that generates [^{123}I]- or [^{131}I]TC-LDL. Figure 3 illustrates that the mass of TC has a dramatic effect on the radiochemical yields of [^{131}I]TC which are achieved. At least 10 μM (5 μg/ml) TC are necessary for radiochemical yields to exceed 70%.

Nca radioiodination of the TC adduct occurred rapidly, as indicated by the data in Table 2. Due to the high susceptibility of the ortho position of the phenolic core of TC toward electrophilic substitution, nca radioiodination of TC took place within seconds, and the aromatic iodination reaction essentially reached completion by 5 min.

These optimized reaction conditions were applied to the preparative radiosynthesis of [^{123}I]- or [^{131}I]TC-LDL for in vivo applications. Nca $^{123}I^-$ or $^{131}I^-$ was incorporated into 100 nmol TC over a reaction period of 5 min (Scheme 2), and the vessel contents were subsequently transferred to a second vessel for coupling to LDL (Scheme 3). As outlined earlier (7), this coupling procedure involved reaction of [^{123}I]- or [^{131}I]TC with the cross-linking agent cyanuric chloride (2,4,6-trichloro-1,3,5-triazine) followed by conjugation of this radioiodinated TC-cyanuric chloride adduct to 10 mg LDL. Reaction between [^{123}I]- or [^{131}I]TC and cyanuric chloride occurred within 30 sec, and involved binding to TC either via an ether linkage of the phenolic oxygen, as suggested in Scheme 3, or to the secondary amine of TC (7).

Table 2

Effect of Reaction Time on the Nca Radioiodination of TC[a]

Reaction Time	% [^{131}I]TC [b]
10 sec	71.0 ± 4.4
30 sec	80.8 ± 4.7
1 min	88.1 ± 4.4
5 min	88.2 ± 0.4
15 min	89.9 ± 0.7
30 min	94.9 ± 1.5

(a) Reaction conditions: 50 μCi Na^{131}I, 500 μg TC, 500 μg Iodogen, 500μl 0.02 M $NaPO_4$ (pH 7.2), 25°C.
(b) Percentage of total radioactivity in solution; values represent the mean and range for 2-3 experiments.

Subsequent reaction with amino groups of LDL completed the cross-linking procedure within 30 min. Final purification of [^{123}I]– or [^{131}I]TC-LDL with gel permeation chromatography gave the radiolabelled LDL analogue in physiological saline with a radiochemical yield of 28-30% and an overall preparation time of 75 min. Quality control tests indicated that the radiolabelled TC derivative of LDL was > 95% precipitated by trichloracetic acid and was very similar in character to native and radioiodinated LDL, as shown in Table 1.

EXPERIMENTAL

Chemicals. Unless otherwise indicated, all compounds and solvents were of analytical quality and were purchased from either Sigma Chemical Company (St. Louis) or Aldrich Chemical Company (Milwaukee). Trimethylphenylstannane was synthesized from chlorotrimethyltin and the Grignard reagent of bromobenzene and purified by fractionation (19). Tyramine-cellobiose (TC, Scheme 2) was synthesized by reductive amination of D(+)cellobiose(4-O-β-D-glucopyranosyl-D-glucose) by tyramine and purified chromatographically (7).

Preparation of LDL. New Zealand rabbits weighing 2-3 kg were fed a diet consisting of high fiber purina laboratory chow with 4% additional peanut oil and 1% cholesterol (11), whereas control animals were fed normal purina laboratory chow. The diets were followed for a period of 8-20 weeks.

Platelet-poor plasma (PPP) was prepared by drawing 10 ml of rabbit blood into 1.5% EDTA to give a final concentration ratio of 1:10. Pooled diet and control blood samples were centrifuged at 22°C for 30 min at 3000 rpm (Model J-6B, Beckman Instruments). The resultant PPP was carefully drawn into an acid-washed glass container and either used immediately or stored at 4°C (19).

LDL (d = 1.020-1.060) was prepared by differential ultracentrifugation of the diet rabbit PPP. Aliquots (6 ml) of the PPP were centrifuged at 40,000 rpm for 24 hrs at 15°C (Rotor Ti 50-3, Beckman Instruments). Very low density lipoprotein (VLDL) was removed from the top 2 ml aliquot of each tube and replaced with 2 ml NaBr/NaCl salt solution (124.755 g NaBr, 5.665 g NaCl, 500 ml deionized water, d = 1.1818). The contents of each tube were thoroughly mixed using acid-washed glass rods and centrifuged at 40,000 rpm at 15°C for 24 hrs. The particulate floatings in the diet sample were mixed into the supernate, and 1 ml of the top portion was subsequently

removed using a 0.4-0.6 mm capillary pipette. The LDL was then dialyzed exhaustively against a buffer (39 g NaCl + 0.39 mg EDTA / 3400 ml deionized water) at 4°C for 72 hr.

LDL was removed from the dialysis tubing and stored in glass vials under nitrogen at 4°C. Quantification of LDL was monitored using a precision refractometer (Model 33-45-01, Bausch and Lomb) (20). Total protein determinations were performed using the method of Lowry, et al. (21).

Native and labelled LDL preparations were subjected to SDS-polyacrylamide gel electrophoresis as described earlier (12). Sample volumes were adjusted to 25 µl per well prior to electrophoresis, and separation were performed at 20 mA constant current for 24 hr. Gels were then stained for 90 min with 0.1% Coomassie Brilliant blue R-250 in 40% methanol + 10% acetic acid for 0.5 hr and destained overnight with 10% methanol + 75% acetic acid. The migration distances were measured using a scanning densitometer (Model RFT, Transidyne General Corp., Ann Arbor), and the molecular diameter of each of the peaks was quantitated by comparison to standards of known diameters (12). The radioactivity distribution of [^{131}I]LDL and [^{131}I]TC-LDL was determined following electrophoresis by slicing the gel into 5mm sections, which were then counted in a NaI(Tl) well-type γ-scintillation counter.

Radiochemicals. Na^{131}I was obtained with a specific activity of 8.48 Ci/mg I (1077 Ci/mmol) in 0.1 N NaOH from New England Nuclear (Boston). This solution was diluted to 0.01 N NaOH with deionized distilled water prior to the initial optimization experiments to serve as a stock dilution. No-carrier-added Na^{123}I was purchased from the Crocker Nuclear Laboratory (University of California, Davis) in 0.1 N NaOH, and was used without further dilution.

Radiosynthesis of [^{123}I]- or [^{131}I]-LDL. In the optimization of conditions for the exchange-labelling of [^{131}I]ICl, 50-100 µCi (5 µl) of the Na^{131}I stock dilution was added to a tightly-sealed 2 ml glass reaction vessel which contained a magnetic stirring bar. Differing amounts of iodine monochloride (100 µl of 0.3 µM - 0.2 M ICl) were then added to the reaction vessel, and the ensuing exchange reaction was allowed to proceed at 25 C for 5 min. Following this interval, stoichiometric excess of trimethylphenylstannane was added to quench the exchange reaction. After an iododestannylation reaction period of 30 min, the radiochemical yield of

[^{131}I]iodobenzene was determined by direct injection of 10-20 µl of the reaction mixture into a Waters M-45 Solvent Delivery System with Model U6K Injector and Model 450 Variable Wavelength Detector. The radioactive reaction products were separated using a stationary phase consisting of a 3.9 mm X 30 cm µBondapak C_{18} column (Waters) and a mobile phase of methanol/water = 80/20 with a flow rate of 2 ml/min (k' = 12.1). The radiolabelled peaks were detected using a 2-inch well-type NaI(Tl) crystal and associated electronics connected to the HPLC effluent line. The peak areas were evaluated using a Hewlett-Packard 3390A Reporting Integrator.

The radiosynthesis of [^{131}I]LDL was optimized by placing 50-100 µCi (5 µl) of the Na^{131}I stock dilution into a sealed 2 ml glass reaction vessel which contained a magnetic stirring bar. Iodine monochloride was then added as 100 µl of 2 µM - 2 M ICl and allowed to exchange with the nca $^{131}I^-$ at 25°C for 5 min. While stirring the vessel contents, 800 µl of 0.2 M glycine (pH 8.6) was added, followed by 100 µl of LDL solution (23.32 mg/ml). Reaction between exchange-labelled [^{131}I]ICl and LDL was permitted for 15 min, after which [^{131}I]LDL was isolated using gel permeation chromatography (stationary phase: 1.5 X 28 cm Sephadex G-50; mobile phase: 0.2 M glycine pH 8.6; flow rate 1.5 ml/min). Effluent fractions were collected every 30 sec and counted in a NaI(Tl) well-type scintillation counter to assay the radiochemical yield of [^{131}I]LDL (elution time 1.5-2.0 min).

[^{123}I]LDL was synthesized at the preparative level for imaging studies by placing 10.4 mCi (70 µl) of Na^{123}I in 0.1 N NaOH into a 2 ml sealed glass reaction vessel which held a magnetic stirring bar. Iodine monochloride (100 µl of 10 mM ICl) was added. Following 5 min at 25°C, 800 µl of 0.2 M glycine (pH 8.6) was added, as well as 110 µl of LDL solution (23.32 mg/ml). The vessel contents were stirred at 25°C for 15 min, and [^{123}I]LDL was isolated using a mobile phase of 0.9% NaCl. The final product contained 4.2 mCi [^{123}I]LDL (40% radiochemical yield) and 1.6 mCi/mg, with an overall preparation time of 30 min. Identical reaction conditions and labelling results were obtained in the preparative synthesis of [^{131}I]LDL for in vivo studies.

Radiosynthesis of [^{123}I]- or [^{131}I]TC-LDL. In the examination of the parameters which affect the radioiodination of TC, 100 µl of 0.2 mM - 0.2 M Iodogen in methylene chloride was placed into an open 2 ml glass reaction vessel. After allowing the solution to dry completely by standing at ambient temperature for 30 min, the vessel

was sealed and 500 µl of 20 nM - 0.4 M TC in 0.02 M $NaPO_4$ (pH 7.2) was added. The nca radioiodination reaction was then initiated by the addition of 50-100 µCi (5 µl) of $Na^{131}I$ stock dilution. Following intervals of 5 sec - 30 min, the reaction was quenched by transferring the solution to a second sealed vessel which contained 500 µl of 1 M $NaHSO_3$. Care was taken during the transfer process to assure that the solid phase of Iodogen was not disturbed and remained in the intitial vessel. The radiochemical yield of [^{131}I]TC was determined by radio-HPLC as described above, using a 3.9 mm X 30 cm Carbohydrate Analysis column (Waters) as a stationary phase and a mobile phase of acetonitrile/water = 85/15, flow rate 2 ml/min (k' = 2.8).

For the preparative synthesis of [^{123}I]TC-LDL, 100 µl of 10 mM Iodogen in CH_2Cl_2 was dried in a glass reaction vessel. The vessel was then sealed, and 200 µl of 0.5 mM TC in 0.02 M phosphate buffer (pH 7.2) was added. The radioiodination reaction was started by the addition of 8.2 mCi (70 µl) of $Na^{123}I$ in 0.1 N NaOH, followed by 70 µl of 0.1 M HCl to neutralize the base in the radioiodide solution. A reaction period of 10 min was permitted before the resulting [^{123}I]TC solution was transferred to a second sealed 2 ml glass reaction vessel, which contained a magnetic stirring bar, for coupling to LDL (7). Cyanuric chloride (200 µl of a 0.5 mM solution in acetone) and 20 µl of 0.01 N NaOH were added to the vessel, and after 30 sec, 20 µl of 1.5 X 10^{-3} N acetic acid was added to quench the [^{123}I]TC – cyanuric chloride coupling reaction. The cross-linking reaction was completed by the addition of 600 µl of LDL solution (17.13 mg/ml) and 1 ml of 0.2 M glycine buffer (pH 9.2), and stirring the mixture for 30 min. The entire vessel contents were subjected to preparative gel permeation chromatography, as described above for [^{123}I]LDL. After an overall preparation time of 75 min, 2.3 mCi [^{123}I]TC-LDL (28% radiochemical yield) was isolated in physiological saline solution containing 0.2 mCi/mg. The preparative synthesis and radiochemical yield of [^{131}I]TC-LDL for in vivo use was identical to that described for [^{123}I]TC-LDL.

ACKNOWLEDGEMENTS

The authors are indebted to Professor F. Lindgren and his associates for their assistance in the isolation and electrophoretic analysis of LDL samples. This work was supported in part by the Director, Office of Energy Research, Office of Basic Energy

Sciences, Biology and Medicine Division of the U.S. Department of Energy under Contract No. DE-AC03-76SF00098 and also by the National Insitutes of Health, National Heart, Lung, and Blood Institutes under Grant No. P01-HL25840.

REFERENCES

1. Brown M.S. and Goldstein J.L.- In: Harrison's Principles of Internal Medicine, 9th ed. Isselbacher K.J., Adams R.D., Braunwald E., Petersdorf R.G. and Wilson J.D. (Eds.). McGraw-Hill, New York, 1980, p. 507.
2. Miller N.E. and Lewis B. (Eds.)- Lipoproteins, Atherosclerosis and Coronary Heart Disease. Elsevier/North Holland Press, New York, 1981.
3. Reisel J.H.- Hypercholesterolemia and Clinical Atherosclerosis. Wyt Publishers, Rotterdam, 1968.
4. Lees R.S., Lees A.M. and Strauss H.W.- J. Nucl. Med. 24: 154 (1983).
5. Angelberger P., Hüttinger M. and Dudczak R.- J. Lab. Comp. Radiopharm. 23:1309 (1986)
6. Lees R.S., Garabedian H.D., Lees A.M., Schumacher D.J., Miller A., Isaacsohn J.L., Derksen A. and Strauss H.W.- J. Nucl. Med. 26: 1056 (1985).
7. Pittman R.C., Carew T.E., Glass C.K., Green S.R., Taylor C.A. and Attie A.D.- Biochem. J. 212: 791 (1983).
8. Carew T.E., Pittman R.C., Marchand E.R. and Steinberg D.- Arteriosclerosis 4:214 (1984).
9. Watanabe Y.- Atherosclerosis 36: 261 (1980).
10. Buja L.M., Kita T., Goldstein J.L., Watanabe Y., and Brown M.S.- Arteriosclerosis 3: 87 (1983).
11. Dalal K.B., Ebbe S., Mazoyer E., Carpenter D. and Yee T.- Blood (submitted).
12. Krauss R.M. and Burke D.J.- J. Lipid Res. 23: 97 (1982).
13. Larsen P.T., Blomhoff R. and Berg K.- In: Cardiovascular Disease '86. Molecular and Cellular Mechanism, Prevention, Treatment. Gallo N. (Ed.). Sixth International Washington Spring Symposium. Washington, D.C. May 20-23, 1986, p. 179.
14. Nasielski J., Buchman O., Grosjean M. and Hannecart E.- Bull. Soc. Chim. Belges 77: 15 (1968).

15. Moerlein S.M. and Coenen H.H.- J. Chem. Soc., Perkin Trans. 1, 1941 (1985).

16. Fisher W.R., Hammond M.G., Mengel M.C. and Warmke G.L. -Proc. Nat Acad. Sci USA 72: 2347 (1975)

17. McFarlane A.S.- Nature 182: 53 (1958).

18. Coenen H.H., Moerlein S.M. and Stöcklin G.- Radiochim. Acta 34: 47 (1983).

19. Moerlein S.M.- J. Organometal. Chem. 319: 29(1987).

20. Lindgren F.T.- In: Analysis of Lipids and Lipoproteins. Perkins E.G. (Ed.). Amer. Oil Chemists' Society, Urbana, 1975, p. 204.

21. Lowry O.H., Rosenbrough N.J., Farr A.L. and Randall R.J.- J. Biol. Chem. 193: 265 (1951).