Measurement of apolipoprotein B radioactivity in whole blood plasma by precipitation with isopropanol

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Summary A method to measure apolipoprotein B radioactivity in whole blood plasma is described that is suitable for routine use in kinetic experiments in vivo. Radiolabeled apolipoprotein B is precipitated from plasma diluted 15- to 30-fold in the presence of carrier low density lipoproteins by 50% isopropanol. The amount of radioiodine in apoB is estimated from the difference between total radioiodine concentration in whole plasma and the fraction soluble in 50% isopropanol. Addition of up to 100 μl of plasma to radioiodinated lipoproteins did not alter the percent of radioiodine precipitated in 1500 μl of 50% isopropanol. The percent of radioiodine precipitated by isopropanol 3 min after intravenous injection of homologous radioiodinated very low density lipoproteins, intermediate density lipoproteins, and low density lipoproteins into rabbits was almost identical to that in the injected lipoproteins ($y = 1.009x \pm 0.462$; $r = 0.997$). — **Yamada, N., and R. J. Havel.** Measurement of apolipoprotein B radioactivity in whole blood plasma by precipitation with isopropanol. *J. Lipid Res.* 1986. **27**: 910–912.

Supplementary key words VLDL • IDL • LDL

Apolipoprotein (apo) B is a constant protein component of very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), and low density lipoproteins (LDL) in mammalian blood plasma. Each particle in these lipoprotein classes is thought to contain a single molecule (ca. 500,000 daltons) of apoB which, in contrast to other apolipoprotein components, does not move between the particles (1). Because of these properties, measurements of radioactivity in apoB are widely used in metabolic studies, in which apoB is usually separated from other apoproteins contained in isolated lipoproteins by selective precipitation in 50% tetramethylurea (2) or 50% isopropanol (3, 4). For measurement of radioactivity in apoB, use of isopropanol or other lipid solvents (5) has the advantage that labeled lipoprotein-lipids also remain in solution.

We show here that radioactivity in apoB can also be selectively precipitated from unfractionated blood plasma with isopropanol. This method permits a new approach to kinetic studies of interconversions and irreversible disposal of lipoproteins containing apoB.

MATERIALS AND METHODS

Lipoproteins

Blood obtained from male New Zealand white (NZW) or Watanabe heritable hyperlipidemic (WHHL) rabbits was mixed with disodium EDTA (1 mg/ml), sodium azide (0.2 mg/ml), and gentamycin (10 μg/ml). Lipoproteins were separated from fresh plasma by sequential ultracentrifugation in a 40.3 rotor of a Beckman ultracentrifuge at densities of 1.006, 1.019, and 1.063 g/ml to obtain VLDL, IDL, and LDL, respectively (6, 7), or by direct ultracentrifugation at a density of 1.063 g/ml. To obtain large VLDL, plasma was centrifuged in a Beckman SW 41 rotor at 39,000 rpm for 1 hr and the top 1 ml was obtained by tube slicing. Small VLDL were separated from the infranatant plasma in a 40.3 rotor at 38,000 rpm for 16 hr. Each lipoprotein fraction except for the combined lipoproteins of density < 1.063 g/ml was purified by recentrifugation at the upper density limit (large VLDL were recentrifuged twice), dialyzed against 0.15 M NaCl containing 0.01% disodium EDTA, 0.02% sodium azide, pH 7.4, and radioiodinated by a modification of the iodine monochloride method of McFarlane (8, 9). The labeled lipoproteins were dialyzed against 0.15 M NaCl.

Precipitation of apoB

ApoB contained in radioiodinated VLDL, IDL, or LDL was precipitated by addition of an equal volume of isopropanol (analytical grade, J. T. Baker Chemical Co., Phillipsburg, NJ). For measurement of apoB in lipoproteins or in whole plasma, a measured volume (up to 100 μl) was mixed with 50 μl of rabbit or human LDL (ca. 0.2 mg of protein), made to a volume of 750 μl with 0.15 M NaCl, and 750 μl of isopropanol was added. After 1 hr, the mixture was centrifuged at room temperature in a polypropylene centrifuge tube at 13,000 rpm for 10 min. Content of radioiodine was determined by gamma scintillation spectrometry in samples of the original lipoprotein or plasma and in a portion (usually 0.75 ml) of the supernatant fluid. Radioactivity in apoB was determined by difference.

In one experiment, the precipitated apolipoproteins of density < 1.063 g/ml were solubilized in 20% SDS and subjected to electrophoresis in 3–20% polyacrylamide gradient slab gels (10). The soluble proteins were also subjected to electrophoresis after removal of the isopropanol by evaporation.

Abbreviations: VLDL, very low density lipoproteins; IDL, intermediate density lipoproteins; LDL, low density lipoproteins; NZW, New Zealand white; WHHL, Watanabe heritable hyperlipidemic.

RESULTS AND DISCUSSION

Radioiodinated lipoproteins were mixed with isopropanol in the presence of carrier LDL and varying amounts of rabbit blood plasma. As shown in **Table 1,** the percentage of radioiodine precipitated from VLDL, IDL, and LDL was unaffected by addition of up to 100 μl of plasma in a total volume of 1500 μl. Larger amounts slightly increased the amount precipitated.

To determine the extent to which labeled apolipoproteins other than apoB were coprecipitated by isopropanol, pellets were prepared from labeled total lipoproteins of density < 1.063 g/ml from blood plasma of an NZW rabbit, together with carrier LDL in the presence or absence of plasma. In each case, 78% of the total ^{125}I was precipitated by isopropanol. The pellets were vortexed thoroughly with 1 ml of 50% isopropanol, incubated overnight with continuous mixing, and then recentrifuged. Less than 1% of the ^{125}I in the pellets obtained in the presence or absence of plasma was found in the supernatant fluid. The distribution of ^{125}I in proteins that are soluble or insoluble in 50% isopropanol was assessed after separation in SDS polyacrylamide gradient gels (**Fig. 1**). Essentially all of the labeled apoB present in radioiodinated lipoproteins of density < 1.063 g/ml was found in the pellet, but only about 10% of the ^{125}I in apoE and 3–4% of that in the C apoproteins. Since the latter proteins comprised only about 5% and 10% of the total ^{125}I in the density < 1.063 g/ml lipoproteins, these data confirmed that 99% of the ^{125}I in pelleted apoproteins was in apoB. The presence of plasma during precipitation had no effect upon these results.

The labeled lipoproteins were injected into ear veins of rabbits and blood samples were obtained from the opposite ear vein. As shown in **Table 2,** the percentage of radioiodine precipitated in 50% isopropanol in samples

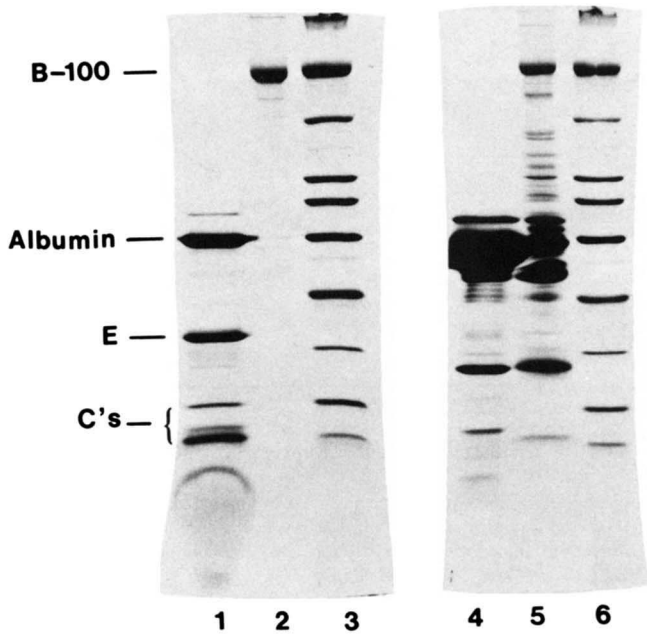

Fig. 1. SDS polyacrylamide gradient (3–20%) slab gel showing proteins (stained with Coomassie brilliant blue) from radioiodinated lipoproteins of density < 1.063 g/ml from blood plasma of NZW rabbits soluble (lanes 1 and 4) and insoluble (lanes 2 and 5) in 50% isopropanol in the presence of carrier density < 1.063 g/ml lipoproteins (lanes 1 and 2) or 3.75% plasma (lanes 4 and 5). The major soluble proteins found in the absence of plasma are albumin, apoE, and apoCs, whereas apoB is virtually the only visible protein component of the insoluble pellet. In the presence of plasma, nonlipoprotein proteins in addition to albumin are seen in both the soluble and insoluble fractions. Regions of the gels corresponding to apoB, apoE, and apoCs, respectively, were excised and counted. They contained the following percentages of ^{125}I in the total density < 1.063 g/ml lipoproteins: gel 1: 0.02%, 4.77%, 9.57%; gel 2: 76.7%, 0.45%, 0.35%; gel 4: 0.02%, 4.48%, 9.60%; gel 5: 76.6%, 0.57%, 0.39%. Gels 3 and 6 are marker proteins, from top: apoB-100, myosin, β-galactosidase, phosphorylase B, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor, lysozyme.

TABLE 1. Effect of plasma on precipitation by isopropanol of radioiodine in labeled rabbit plasma lipoproteins

	% Radioiodine Precipitated		
Plasma Added	VLDL (n = 4)	IDL (n = 4)	LDL (n = 8)
μl			
0	45.1 ± 1.7	82.2 ± 0.14	84.7 ± 0.68
50	46.3 ± 0.28	82.0 ± 0.63	84.8 ± 0.75
100	46.7 ± 1.1	82.2 ± 0.15	85.1 ± 0.55
200	47.1 ± 0.84[a]	82.4 ± 0.21	85.3 ± 0.41[a]
500	48.6 ± 2.0[a]	83.4 ± 0.62[a]	85.5 ± 0.42[a]

Tracer amounts of radioiodinated lipoproteins were mixed with carrier LDL (0.2 mg of protein) and volume of plasma indicated in a total volume of 750 μl; 750 μl of isopropanol was added and the percentage of radioiodine precipitated was determined as described in the text. Values are mean and 1 SD.

[a] Significantly different from value obtained in the absence of plasma ($P < 0.05$).

TABLE 2. Percentage of radioiodine precipitated by isopropanol from labeled lipoproteins used for intravenous injection into recipient rabbits and in blood plasma obtained 3 min later

	% Radioiodine Precipitated	
Lipoprotein Fraction	Injected Lipoprotein	Plasma (3 min)
NZW LDL (5)	83.2 ± 5.6	82.6 ± 5.5
NZW IDL (7)	80.2 ± 3.3	82.3 ± 3.6
NZW VLDL (total) (6)	43.2 ± 5.8	42.9 ± 5.7
VLDL (large) (8)	23.3 ± 8.7	23.2 ± 9.0
VLDL (small) (7)	50.4 ± 8.6	51.8 ± 9.7
WHHL LDL (6)	78.8 ± 6.4	80.3 ± 6.5
WHHL IDL (7)	80.5 ± 4.9	81.6 ± 4.5
WHHL VLDL (3)	59.7 ± 12.5	59.8 ± 11.2

Lipoproteins from fed NZW rabbits or fed WHHL rabbits were injected into fed NZW and WHHL recipients, respectively, as described in the text. Values are mean and 1 SD. Numbers in parentheses indicate number of experiments.

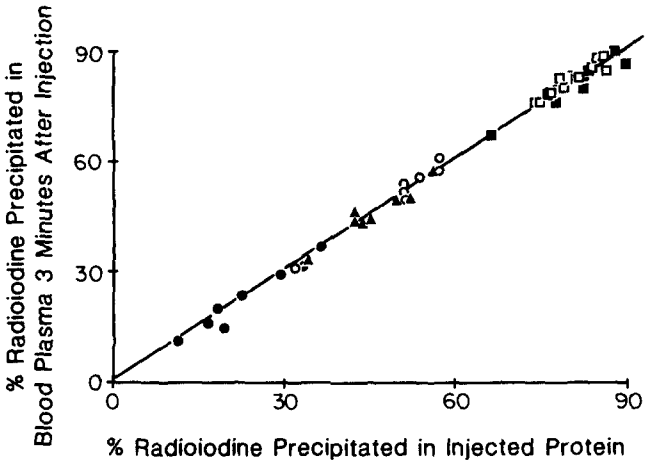

Fig. 2. Relationship between radioiodine precipitated by isopropanol in labeled lipoproteins and in blood plasma obtained 3 min after intravenous injection of these lipoproteins into recipient rabbits; (■), LDL; (□), IDL; (▲), total VLDL; (○), small VLDL; (●), large VLDL. The line represents the best fit by linear regression ($y = 1.009x \pm 0.462$; $r = 0.997$).

of whole blood plasma obtained 3 min after injection was almost the same as that in the injected lipoproteins. The correlation between the values obtained after 3 min and in the injected lipoproteins was highly significant (**Fig. 2**).

These results indicate that radioactivity in apoB can be measured simply and reliably in whole blood plasma during metabolic studies, so that lipoproteins need not be separated from plasma of recipients by ultracentrifugation or other procedures. We have used the method to measure radioiodine in plasma apoB after immunosorption of lipoproteins containing apoE (11), thereby obviating potential ultracentrifugal losses of apoE from these lipoproteins (12). The method does not permit measurement of the mass of apoB in whole plasma because small amounts of other plasma proteins are present in the isopropanol-insoluble pellet.

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