Radioiodinated lipoproteins: absorption of ^{125}I radioactivity by high density solutions

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Summary Concentrations of potassium bromide commonly used for separation of lipoproteins were shown to cause absorption of ^{125}I and thus reduce the counting efficiency of the labeled lipoproteins. Chloroform was shown to cause a 50% reduction in counting efficiency of lipid from ^{125}I-labeled lipoprotein. No reduction of counting efficiency was observed in the presence of high density solutions when ^{131}I was used as label.

Supplementary key words ^{131}I · efficiency of radioactivity counting.

Labeling of proteins with radioactive iodine is an accepted procedure for the study of the metabolism of plasma proteins. This procedure is now often applied to follow the fate of plasma lipoproteins in humans (1–3), animals (4–6), and tissue culture systems (7–9). The isotope most commonly used in these studies is ^{125}I, which labels both lipoprotein protein and lipoprotein lipid. In most studies, labeled lipoproteins are separated according to density, and protein-bound radioactivity is calculated after lipid extraction and determination of radioactivity associated with the labeled lipids. Radioactivity is therefore measured in solutions of different salt concentration and composition and in various organic solvents. We report here our observations on the effects of various solutions on the counting efficiency of ^{125}I compared with that of ^{131}I.

Very low density lipoprotein (VLDL) and high density lipoprotein (HDL) of rat plasma were prepared by flotation in the Beckman L2-65B ultracentrifuge as described previously (4, 6). The lipoproteins were iodinated by a modification of McFarlane's iodine monochloride technique (10). Unbound iodine was removed by dialysis. Lipids were extracted in chloroform–methanol 2:1 (v/v) and washed as described by Folch, Lees, and Sloane Stanley (11). Radioactivity was determined in a Packard Auto-Gamma scintillation spectrometer, model 578, at an optimal setting for counting ^{125}I or ^{131}I in 0.9% sodium chloride solution. The value thus obtained (counts per minute, cpm) was taken as 100% counting efficiency. Care was taken to count all samples exactly in the same tubes or vials, using the same geometry in the counter.

TABLE 1. Effects of salts on efficiency of radioactive iodine counts

Salt	Density	^{125}I-labeled VLDL		^{131}I-labeled VLDL	
	g/ml	*cpm*	*%*	*cpm*	*%*
NaCl	1.006	14,040 ± 194	100.0	36,860 ± 235	100.0
NaCl	1.117	12,060 ± 187	85.9	35,450 ± 340	96.2
KBr	1.019	11,730 ± 206	83.5	36,880 ± 860	100.0
KBr	1.063	9,440 ± 217	67.2	34,160 ± 290	92.7
KBr	1.210	4,650 ± 250	33.1	36,200 ± 290	98.2

Values are means ± SE of six determinations. Radioactivity was determined in Packard counting tubes, at a volume of 2 ml.

Potassium bromide caused a marked decrease in the efficiency of counting ^{125}I-labeled VLDL (**Table 1**); at a salt concentration of density 1.21 g/ml, ^{125}I-labeled VLDL was counted at an efficiency of only 33.1%. In the presence of sodium chloride solution of density 1.117 g/ml, the efficiency of counting ^{125}I-labeled VLDL was also decreased, but to a lesser extent. With either salt, the efficiency of counting ^{131}I-labeled VLDL remained essentially unchanged. Similar results were obtained when iodinated HDL was used.

The attenuation of radioactivity emission by ions is a function of their mass absorption coefficient, which is dependent on the electron density of the ion and the energy of the radioactive source. The mass absorption coefficients for ^{125}I (energy of 27 keV) as calculated from standard tables (12) are 3.14 for chlorides and 23.5 for bromides; the corresponding figures for ^{131}I (energy of 364 keV) are 0.100 and 0.105. Therefore, with either cylindrical or globular sources, the absorption of ^{131}I irradiation is negligible even when using a high bromide concentration (13). The absorption of ^{125}I, however, is apparent with chlorides and is very pronounced with bromides. Moreover, the absorption of ^{125}I will vary considerably with the geometry and diameter of the radioactivity source. We have observed absorption of about 80% of ^{125}I-labeled VLDL radioactivity when using glass vials of 23 mm diameter and a KBr solution of density 1.21 g/ml.

The effects of organic solvents on the counting efficiency of dry samples of labeled VLDL and HDL lipids were de-

TABLE 2. Effects of organic solvents on efficiency of radioactive iodine counts

Solvent	^{125}I-labeled Lipid		^{131}I-labeled Lipid	
	cpm	*%*	*cpm*	*%*
Dry samples	30,250 ± 525	100.0	35,100 ± 311	100.0
Chloroform	15,320 ± 274	50.6	35,420 ± 242	100.9
Chloroform–methanol 2:1 (v/v)	18,730 ± 680	61.9	36,380 ± 172	103.6
Methanol	32,160 ± 1360	106.3	36,400 ± 217	103.7

Values are means ± SE of six determinations. Radioactivity was determined in Packard counting vials, at a volume of 5 ml.

Abbreviations: VLDL, very low density lipoprotein; HDL, high density lipoprotein.

termined. Chloroform caused a decrease of ^{125}I-labeled lipid counts to about 50% of that of the dry sample (**Table 2**), whereas methanol did not affect the efficiency of counting. Neither solvent changed markedly the counting efficiency of ^{131}I-labeled lipid.

These results emphasize the necessity of careful determination of counting conditions as a prerequisite in studies using ^{125}I for labeling of lipoproteins, especially if valid conclusions are to be drawn from double-label experiments.

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