Molecular Properties of Radioiodinated Apolipoprotein A-I*

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Radioiodinated apolipoprotein A-I was separated into two pools by gel chromatography on Sephadex G-150 superfine resin or by high performance liquid chromatography using a TSK-2000 column. The first pool, pool 1, was indistinguishable from unlabeled apo-A-I as judged by sedimentation equilibrium, circular dichroic measurements, and radial immunodiffusion. This pool self-associated according to a monomer-dimer-tetramer-octamer scheme and underwent the expected conformation changes with dissociation as reported previously for unlabeled apo-A-I. The radioiodinated material in pool 2 had less secondary structure as compared to the unlabeled material and did not self-associate. Protein in this pool was immunologically less reactive than unlabeled apo-A-I. Kinetic studies, in vivo, demonstrated that these two pools of radioiodinated apo-A-I are also distinguished metabolically. Discussion centers around possible mechanisms of formation of the incompetent protein in pool 2 and factors that should be taken into consideration when using unfraccionated radioiodinated apo-A-I for experimental studies.

The in vivo metabolism of plasma lipoproteins has been investigated by several laboratories with the use of iodinelabeled plasma lipoproteins (1-8). In the majority of these studies, various density fractions of plasma have been labeled directly; since the majority, if not all, of the density fractions of plasma are heterogeneous with respect to apolipoprotein composition, several apolipoproteins are labeled simultaneously with this procedure. Because of the dynamics of lipoprotein metabolism the distribution of most apolipoproteins changes cyclically with time. Those labeled initially in one density fraction usually become unequally divided between several density classes, and the fate of a given apolipoprotein is quite difficult to follow quantitatively when the radiolabel is incorporated directly into the intact plasma lipoprotein. More recently, apolipoproteins have been labeled in their lipid-free form and then incorporated into plasma lipoproteins by in vivo incubations (9-13). With this latter procedure the kinetics of metabolism of individually labeled apolipoproteins is quantitated more easily, and models have been published describing the individual metabolism of apolipoproteins A-I and A-II (14, 15).

The assignment of physiological significance to any particular part of a model describing the kinetics of in vivo metabolism is dependent directly upon the relative validity of any assumptions postulated in the experimental design. A frequent assumption is the “tracer assumption” that the labeled material behaves physiologically like the unlabeled counterpart. Evaluation of the integrity of labeled apolipoproteins when plasma lipoproteins are labeled directly in the intact state is quite complex. The specific labeled species would need to be delipidated and purified to homogeneity prior to characterization. In addition, one must be concerned about selection of subsets of the labeled species, leading to erroneous conclusions about the integrity of the intact unfraccionated species. In the present report we compare the molecular properties of lipid-free iodinated apolipoprotein A-I with those of the corresponding unlabeled species. Information gained from these comparisons is used to evaluate the in vivo metabolic consequences of radioiodelabeling apolipoprotein A-I. Further, these studies are used to assess the degree of uncertainty in the tracer assumption.

MATERIALS AND METHODS

Apolipoprotein A-I was isolated from normal plasma by column chromatography as described previously (16). The purified apolipoprotein migrated as a single band upon gel electrophoresis in sodium dodecyl sulfate, formed a single immunoprecipitin line against antibodies to apo-A-I, and did not cross-react with antibodies to apo-A-II, apo-C-II, apo-C-III, apo-B, apo-E, apo-A-IV, apo-H, or albumin. Protein concentrations were determined by absorbance measurements on a Beckman Acta III spectrophotometer using a molar extinction coefficient of 32,350 at 280 nm. The buffer used for characterization of the physical properties of apo-A-I was 0.01 M Tris (Ultrapure, Schwarz/Mann), 0.001 M sodium azide (Fisher), and 0.1 M potassium chloride (J. T. Baker Chemical Co.), pH 7.4. All other chemicals were of reagent grade. A Radiometer model 38 pH meter was used for pH measurements, and glass-redistilled water was used in the preparation of all solutions.

Chromatography—Conventional gel permeation chromatography was performed using Sephadex G-150 superfine (Pharmacia Fine Chemicals) in the above buffer. The column (1.4 × 82 cm) was operated at room temperature (~24 °C) and at a pressure of 50 cm for all runs. The flow rate was 5.5 ml/h, and 0.5-ml fractions were collected. Initial sample volumes were 3.0 ml. Further details are given in the figure legends.

High performance liquid chromatography was performed with a Beckman 110A pump and a TSK 2000 SW column (30 × 0.75 cm, Toyo Soda, Tokyo, Japan). The column profiles were monitored at 280 nm using a Beckman 160 detector equipped with a flow through cell, and 20-ml fractions were collected using a LKB 2122 REDIRAC fraction collector. The buffer used was 0.01 M Tris/HC1, 0.10 M potassium chloride, 0.001 M sodium azide, pH 7.4, and the flow rate was 18 ml/h. Initial sample volumes were 250 μ1 and the columns were operated at room temperature (~24 °C). Additional details are given in the figure legends.

Sedimentation Equilibrium—Sedimentation equilibrium measurements were performed in a Beckman ultracentrifuge (model E) equipped with a photoelectric ultraviolet scanner and a temperature control system. Data were collected automatically via a computer-based data acquisition system. Double sector cells with quartz windows and charcoal-filled Epon centerpieces were centrifuged in an Ang-7Tr rotor. Samples were dialyzed exhaustively against buffer prior

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to centrifugation. Each cell contained 100 μl of sample and 110 μl of diffusate from the final dialysis. The rotor speed was 15,000 rpm, and the temperature was maintained at 21°C. Initial protein concentrations were measured by absorbance prior to each run. Initial scans at 280 nm and base-line determinations at 48,000 rpm after equilibrium had been established allowed extinction coefficients to be calculated for each run. Equilibrium was monitored by comparing scans 4-h intervals at 280 nm and base-line determinations at 48,000 rpm. The concentration profiles at equilibrium were fit to the following equation using a nonlinear least squares technique, MLAB, developed at the National Institutes of Health.

$$Y(x) = \frac{A + BX + CX^2}{1 + DX}$$  \hspace{1cm} (1)

where $Y(x)$ is the logarithm of protein concentration (mg/ml) and $x$ is the distance from the center of rotation in centimeters.

**Apparent weight average molecular weights** ($M_{\text{app}}$) were then obtained analytically using Equation 2.

$$M_{\text{app}} = \frac{2RT}{\omega(1 - \rho)} \left[ \frac{B + 2CX}{1 + DX} \right] \frac{(A + BX + CX^2)D}{(1 + DX)^2}$$  \hspace{1cm} (2)

where $R$ is the gas constant, $T$ is the absolute temperature, $\omega$ is the angular velocity, $\rho$ is the partial specific volume (0.737 ml/g), and $\rho$ is the solvent density.

**Circular Dichroic Measurements**—Circular dichroic spectra were obtained using a Cary model 61 spectropolarimeter equipped with a thermostated cell holder and standardized with d-10-camphorsulfonic acid. Temperature was maintained at 24°C, and sample spectra and appropriate blanks were obtained from 270 to 210 nm. Mean residue ellipticities were calculated according to the following equation.

$$[\theta] = \frac{\theta_{208} \times 115}{10 / I \times c}$$  \hspace{1cm} (3)

where $\theta_{208}$ is the observed ellipticity, 115 is the mean residue molecular weight, $f$ is the pathlength in centimeters, and $c$ is the protein concentration in grams/ml.

**In Vivo Studies**—The age, sex, height, weight, plasma lipid, and lipoprotein cholesterol concentrations for the subjects studied are given in Table I. Weights were determined daily during the course of each study on a metabolic scale, and plasma lipids were measured on blood samples obtained during the course of metabolic studies. Plasma lipid and lipoprotein cholesterol values were measured by established Lipid Reinetz (Clinics methodology utilizing the AutoAnalyzer II [17, 18]. Both subjects had normal hepatic, renal, and thyroid function, were not on medication known to affect plasma lipids or lipoproteins, and were studied while on an isocaloric, 16% protein, 42% fat (polyunsaturated fatsaturated fat ratio 0.1-0.3), 20% protein, 20% carbohydrate, 200 mg of cholesterol per 1000 calorie diet. This diet was begun 2 weeks prior to the start of a metabolic study utilizing the course of each study. Normal activity was permitted, and each subject was requested to adhere to a constant amount of physical activity each day. Subjects were given supersaturated potassium iodide (0.5-1.0 g/day) to decrease thyroid uptake of radioactivity and ferrous sulfate (900 mg/day) before and during each metabolic study. Informed consent was obtained from all the subjects.

**Radioiodinated Apo-A-I**—Apo-A-I was iodinated at a mean protein concentration of 3.6+1.1 mg/ml in sterile 1 M glycine, 0.1 M Tris (pH 8.5) buffer by the iodine monochloride method (19). Vortexing was not used in the labeling procedure. For all radioiodinated preparations the efficiency of iodination was assessed by precipitation of protein with 20% trichloroacetic acid following a 1:1 dilution with 5% bovine serum albumin solution. Unbound iodine was removed by extensive dialysis against sterile 0.85% NaCl, 0.01% EDTA, 0.1 M Tris (pH 7.4). The percentage of free iodine present in radioiodinated preparations following dialysis was assessed by precipitation of protein with 20% trichloroacetic acid following the addition of 5% bovine serum albumin and was also measured by descending strip paper radiochromatography (85% methanol on Whatman No. 1 filter papers).

**RESULTS**

**In Vivo Studies**—Apolipoprotein A-I was radiolabeled with iodine monochloride according to the procedure described by McFarlane (19). The efficiency of labeling was 61%, and the resulting specific activity was 5.27×10⁶ cpm/μg. Unbound radioactivity was removed by dialysis, and less than 1% of the final labeled material was soluble in trichloroacetic acid. The radiolabeled apolipoprotein was diluted with native apolipoprotein A-I, and the resulting mixture was analyzed by gel permeation chromatography through Sephadex G-150 superfiltering (5.1 cm) resin (Fig. 1, A and B). Apolipoprotein A-I self-associates according to a monomer-dimer-tetramer-octamer scheme under these conditions, and the elution profile depends critically upon the initial (loading) concentration of protein (20, 21). At high concentrations the major peak corresponds to octameric apo-A-I and with decreasing initial concentrations the elution profile shifts indicating the presence of slower migrating components, and at low initial concentrations of protein the elution profile corresponds primarily to monomeric apo-A-I. The elution profile of mixtures of native and radiolabeled apo-A-I (labeled/unlabeled, -6.5×10⁻⁶ to 2.2×10⁻⁹) were obtained at low (0.05 mg/ml, Fig. 1A) and high (1.7 mg/ml, Fig. 1B) initial concentrations of protein.

**TABLE I**

| Characteristic | Subjects |
|----------------|---------|
| Age (years)    | 22      | 21      |
| Height (cm)    | 175     | 169     |
| Weight (kg)    | 86      | 63      |
| Sex            | M       | M       |
| Plasma cholesterol (mg/dl) | 193 | 153 |
| Triglyceride (mg/dl)   | 29     | 69      |
| VLDL* (mg/dl) | 13      | 8       |
| LDL* (mg/dl)  | 100     | 77      |
| HDL (mg/dl)   | 80      | 68      |
| Plasma volume (ml) | 3797 | 2947 |

*Very low density lipoprotein.

† Low density lipoprotein.
The profiles as measured by radioactivity or protein both show the characteristic shift to the octameric species with increasing initial concentration of protein. It is emphasized that the concentration of radioiodinated apo-A-I is the same in A and B of Fig. 1; the increase in total initial protein concentration is due to the unlabeled species. Therefore, since the profile as obtained by radioactivity responds to changes in protein concentration (Fig. 1, A and B), the labeled material self-associates with native apo-A-I. The specific activity, i.e. counts/min/mg of protein, is not constant across the column profiles. This is especially evident for profiles obtained at high initial concentrations of protein, Fig. 1B, where the specific activity is much higher in the fractions corresponding to monomeric apo-A-I. Thus radiolaabeled apo-A-I does not behave the same as the unlabeled species upon column chromatography. Another sensitive criterion for evaluating the molecular properties of labeled apo-A-I is the effect of dilution on the overall folding of the polypeptide chain (22, 23). The mean residue ellipticity of unlabeled apo-A-I decreases with decreasing protein concentration as summarized in Fig. 2. Iodinated apo-A-I also undergoes a conformational change with dissociation, but the decrease in structure is more dramatic than that observed with unlabeled apo-A-I.

In order to quantitate the effects of iodination on apo-A-I, the labeled material was divided into two pools. The first pool corresponds to fractions 45-55 in Fig. 1B and represents the labeled material that elutes with octameric apo-A-I. The second pool corresponds to fractions 65–75 in Fig. 1B and represents those species of labeled apo-A-I that elute in the monomer position. The mean residue ellipticity of labeled apo-A-I taken from pool 1 is illustrated as a function of wavelength in Fig. 3A. The shape of the spectra and the decrease observed with dilution is the same as that reported previously for unlabeled apo-A-I (22, 23). In contrast, the mean residue ellipticity of material from pool 2 was quite different from that of unlabeled apo-A-I. The intensity at all wavelengths was somewhat lower at 0.28 mg/ml and at a concentration of 0.09 mg/ml, intensities were only 50% of that found for unlabeled apo-A-I (Fig. 3B).

The weight average molecular weight of each of the pools as obtained from sedimentation equilibrium measurements is given in Fig. 4. The increase in weight average molecular weight with increasing protein concentration found for pool 1 was the same as that reported previously for unlabeled apo-A-I (21). In contrast, the weight average molecular weight found for pool 2 increased only slightly with increasing concentration of protein and corresponded to that expected for monomeric apo-A-I.

In summary, we have fractionated iodinated apo-A-I into two pools, only one of which self-associates and resembles unlabeled apo-A-I in secondary structure. The material from the other pool does not self-associate and is less ordered in secondary structure than unlabeled apo-A-I. In addition, pool 2 is immunochemically different than unlabeled apo-A-I. The cross-reactivity of antibodies, prepared against unlabeled apo-A-I, toward pools 1 and 2 using radial immunodiffusion is not
Radioiodinated Apo-A-I

**Fig. 3.** A and B. mean residue ellipticity of iodinated apolipoprotein A-I (pool 1) as a function of wavelength at two different concentrations of protein. Pool 1 corresponds to fractions 45-55 in Fig. 1B. Pool 2 corresponds to fractions 65-75 in Fig. 1B. The buffer used was 0.01 M Tris/HCl, 0.1 M potassium chloride, 0.001 M sodium azide, pH 7.4, and the temperature was maintained at 24 °C. The concentrations of apo-A-I were 0.275 mg/ml (———) and 0.092 mg/ml (— — —). The same. Whereas the material in pool 1 behaved as native apo-A-I, quantitation of material in pool 2 using this technique resulted in a 75% underestimate of protein concentration (data not shown).

**In Vivo Studies**—The kinetics of metabolism in vivo of these two pools was measured simultaneously by using 125I- and 131I-labeled apo-A-I. Apolipoprotein was labeled with 125I or 131I as indicated under “Materials and Methods.” The labeled apolipoproteins were fractionated separately by high performance liquid chromatography using a TSK 2000 SW column. The peak corresponding to octameric apo-A-I was pooled from the 131I-labeled material (Fig. 5A), and the fractions corresponding to monomeric apo-A-I were pooled from the 125I-labeled material (Fig. 5B). The pools were tested for pyrogenicity and sterility, mixed, and injected intravenously (see under “Materials and Methods” for details). Samples of plasma were taken at 10 min, 6 h, 12 h, 24 h, and daily for a period of 7 days. Cumulative urine samples were taken at 6 and 24 h and daily for the duration of the study. The combined results for both subjects are plotted in Fig. 6, A and B, as well as the difference between data obtained using apo-A-I isolated from pool 1 and pool 2, respectively (Fig. 6C). The plasma volume was calculated by comparing the first sample point with the injected radioactivity and is given in Table 1. The plasma kinetics in both pools has a rapid decay and then a slowly decaying portion of the curve. This finding is complemented by the finding that 25% or more of the injected radioactivity appeared in the urine by the end of 24 h.

Apolipoprotein A-I is a major HDL2 protein and more than 95% of plasma apo-A-I can be isolated in the HDL density fraction (1.063 < ρ < 1.21 g/ml) of normal individuals. The

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The abbreviations used are: HDL, high density lipoprotein; PL, and PL2, plasma compartments; NP, and NP2, nonplasma compartments; I, iodine pool.
The protein A-I was radiolabeled with $^{125}$I and $^{131}$I as described under "Materials and Methods." The counts/min indicated in the figure correspond to the theoretical fit of the data, and it is clear that this model can account for the decay of both pools without topological changes. The major difference in the kinetic parameters describing the metabolism of these two pools of apo-A-I is the initial distribution of protein in the two plasma compartments and changes in the rate constants for removal of tracer from PLI. With pool 1, approximately 89% of the plasma protein is initially distributed in the slowly decaying compartment PLI, whereas less than 60% of the incompetent species is initially distributed in the compartment PLI of the model (Table II).

**DISCUSSION**

Apolipoprotein A-I, comprising approximately 65% of the total protein content of high density lipoproteins, has been investigated extensively over the past two decades (for recent reviews see Refs. 24–26). Studies have ranged from determination of primary structure, (amino acid sequences have been reported for the human and canine proteins (16, 27, 28)) to evaluation of the kinetics in vivo of metabolism (1–15). The human protein has a molecular weight of 28,000 and is composed of 243 amino acid residues. The sequence of this protein is unique in that the backbone can be modeled into multiple 22-residue segments that are amphipathic in character with nonpolar and charged residues on opposite sides of an $\alpha$ helix (29, 30). These amphipathic regions are believed to play a major role in the interaction between apo-A-I and lipid-water interfaces (31–34). In plasma, apo-A-I associates reversibly with several plasma lipoproteins; transfer and exchange of apo-A-I between plasma lipoproteins occurs presumably through lipid-free species and is governed by the laws of mass action (26, 35–37). In the lipid-free form apo-A-I is loosely folded and has a high degree of exposure of nonpolar groups to solvent. Minor changes in the polarity of the environment around apo-A-I result in major conformation rearrangements of the polypeptide backbone. Thus, the secondary structure of apo-A-I changes dramatically upon interaction with lipids, plasma lipoproteins, and other nonpolar interfaces and compounds including other apolipoproteins (23, 38, 39).

Our present concept of apo-A-I is that of a highly flexible pool. The plasma and urine data were fit simultaneously to the apo-A-I compartmental model published previously (14). In this model apo-A-I decays from plasma through two distinct pathways. The first has a residence time in plasma of 3.8 days and the second has a residence time of 6.2 days. Data for apo-A-I isolated from pool 1 and pool 2 were fit separately to this kinetic model, and the results are given in Fig. 6. The solid lines in Fig. 6 correspond to the theoretical fit of the data, and it is clear that this model can account for the decay of both pools without topological changes. The major difference in the kinetic parameters describing the metabolism of these two pools of apo-A-I is the initial distribution of protein in the two plasma compartments and changes in the rate constants for removal of tracer from PLI. With pool 1, approximately 89% of the plasma protein is initially distributed in the slowly decaying compartment PLI, whereas less than 60% of the incompetent species is initially distributed in the compartment PLI of the model (Table II).

**Fig. 5.** High performance liquid chromatography of radioiodinated apolipoprotein A-I. The column used was a TSK 2000 SW (30 x 0.75 cm) and the flow rate was 18 ml/h. The buffer used was 0.01 M Tris/HCl, 0.1 M potassium chloride, 0.001 M sodium azide, pH 7.4, and the columns were operated at room temperature. Apolipoprotein A-I was radiolabeled with $^{125}$I and $^{131}$I as described under "Materials and Methods." The counts/min indicated in the figure correspond to 5-$\mu$l fractions. A, the initial volume was 75 $\mu$l, and the initial concentration of $^{125}$I-labeled protein was 2 mg/ml. Fractions from 7.2 through 8.6 ml were combined (pool 1) for metabolic studies. B, the initial volume was 150 $\mu$l, and the initial concentration of $^{131}$I-labeled protein was 2 mg/ml. Fractions from 8.7 through 10.9 ml were combined (pool 2) for metabolic studies.

Radioactivity in total plasma decayed more rapidly than the radioactivity in HDL such that by 6 h approximately 70 and 55% of initial radioactivity remained in HDL and plasma, respectively. The radioactivity not associated with HDL decayed quite rapidly and was modeled as part of the iodine concentration of 13'I-labeled protein was 2 mg/ml. Fractions from 7.2 through 8.6 ml were combined (pool 1) for metabolic studies. The plasma and urine data were fit simultaneously to the apo-A-I compartmental model published previously (14). In this model apo-A-I decays from plasma through two distinct pathways. The first has a residence time in plasma of 3.8 days and the second has a residence time of 6.2 days. Data for apo-A-I isolated from pool 1 and pool 2 were fit separately to this kinetic model, and the results are given in Fig. 6. The solid lines in Fig. 6 correspond to the theoretical fit of the data, and it is clear that this model can account for the decay of both pools without topological changes. The major difference in the kinetic parameters describing the metabolism of these two pools of apo-A-I is the initial distribution of protein in the two plasma compartments and changes in the rate constants for removal of tracer from PLI. With pool 1, approximately 89% of the plasma protein is initially distributed in the slowly decaying compartment PLI, whereas less than 60% of the incompetent species is initially distributed in the compartment PLI of the model (Table II).

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protein which can rapidly refold to accommodate numerous different environments. This conformational adaptability is related presumably to the role(s) played by apo-A-I in lipoprotein metabolism. In view of these unique properties of apo-A-I, it is not surprising that covalent modification of amino acid residues can result in major changes in molecular properties. In the present report we have shown that the specific activity for slower migrating components.

For the first mechanism, the molecular properties of iodinated apo-A-I would be the same as the unlabeled species. We have separated iodinated apo-A-I into two pools by gel permeation chromatography. One of the pools (pool 1) elutes in the oligomeric position and is indistinguishable from native apo-A-I. This material self-associates according to a monomer-dimer-tetramer-octamer scheme with major conformational rearrangements upon oligomer formation, the monomer being less structured than the corresponding oligomer. The molecular weight and secondary structure of this pool as a function of protein concentration was the same as that found previously for native apo-A-I. In contrast, labeled material eluting in the protomer position did not resemble native apo-A-I in its solution properties. This material was incompetent in that it did not self-associate and was less highly structured than monomeric native apo-A-I. These combined data are most consistent with mechanism 3 given above.

The results of our kinetic studies in vivo demonstrate that pools enriched in the competent or incompetent forms of apo-A-I are also distinguished metabolically. The incompetent species is catabolized at a more rapid rate and appears more rapidly in the urine than the corresponding competent species. Residence times are frequently used to summarize the kinetics of a tracer substance or a tracee substance by a single number with the understanding that this single number may be a weighted average of several distinct residence times. Using a single value to describe the complex decay of radioactivity from plasma, pools 1 and 2 appear to be similar with residence times of 6.6 and 6.2 days, respectively. In the present analysis the initial conditions were divided into two plasma compartments, PL1, and PL2, and the iodide pool, compartment I in Fig. 7. The material in the iodide pool was not considered to be of physiologic significance, resulting in consideration of two physiologic components with their corresponding residence times. Compartment PL2 decays directly into the iodide pool (Fig. 7). The residence times for competent and incompetent species of labeled apo-A-I from this compartment were not statistically different from one another, and an average of 4.2 days was used in the data analysis. This residence time compares well with the value of 3.8 days found previously in the compartmental analysis of the kinetics of unfractionated apo-A-I (14). Initially 42% of the incompetent species of labeled apo-A-I, as compared to 11% of the competent species, is distributed into this compartment in plasma.

The remaining compartment in plasma, PL0, is connected to two non-plasma compartments, NP1, and NP2, and in this case comparison of residence times is quite complex. For instance, the residence time for material in compartment PL1 depends upon the relative masses of native apo-A-I in PL1, NP1, and the rate of irreversible loss to the iodide pool. The distribution of incompetent apo-A-I between PL1, NP1, and NP2 is not the same as the distribution of competent apo-A-I between these compartments (compare the rate constants given in Table II). According to the tracer assumption, discussed above, the distribution of label should correspond to the distribution of unlabeled counterpart. Competent and incompetent species of labeled apo-A-I are distinguished metabolically in that the distributions of their radioactivity in plasma do not correspond to the same pool(s) of unlabeled apo-A-I.

The kinetics of metabolism of apo-A-I in vivo has been investigated previously by labeling intact HDL or by labeling lipid-free apo-A-I with subsequent in vitro or in vivo recombination with plasma lipoproteins (1-15). There are two major problems in the evaluation of metabolic studies which em-

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**TABLE II**

| Parameter* | Pool of radiolabeled apo-A-I | Units |
|------------|-----------------------------|-------|
| IC (PL1)   | 68.5                        | %     |
| IC (PL2)   | 7.5                         | %     |
| IC (I)     | 34.0                        | %     |
| L (I, PL1)* | 0.24                       | 0.26 day⁻¹ |
| L (I, PL2)* | 0.14                       | 0.16 day⁻¹ |
| L (NP1, PL1)* | 0.34               | 0.54 day⁻¹ |
| L (PL1, NP2)* | 0.40               | 0.78 day⁻¹ |
| L (NP1, NP2)* | 0.07               | 0.07 day⁻¹ |
| L (NP2, PL2)* | 0.05               | 0.05 day⁻¹ |

*IC (x) is the percentage of total radioactivity placed initially in compartment x. Other parameters are defined under “Materials and Methods.”

*Values given for Pool 1 and Pool 2 are the average of values obtained for analysis of ¹³¹I and ¹²³I radioactivity, respectively. Control values were taken from Ref. 14.

* L (I, PL1) and L (PL1, NP1) were not statistically different for Pool 1 and Pool 2 and were set equal for the final model.

* Because of the duration of the studies (7 days) these values were fixed at the control values obtained in 14-day studies.

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**Fig. 7. Compartmental model for apo-A-I modified from Ref. 14.** PL1 and PL2 are plasma compartments; NP1 and NP2 are nonplasma compartments; and I is the iodine pool. Fractional rate constants associated with the arrows and initial conditions are given in Table II.
ployed labeling of intact plasma lipoproteins. First, with the exception of low density lipoprotein, which contains primarily apo-B, all plasma lipoprotein fractions are heterogeneous with respect to apolipoprotein composition, and decay of radioactivity from plasma corresponds to the kinetics of several different apolipoproteins, necessitating fractionation of plasma prior to analysis. Evaluation of the metabolism of a specific apolipoprotein requires daily fractionation of plasma, and kinetics is limited to short time periods due to decreasing specific activity over the course of the study. The second major problem in this type of study is the evaluation of the metabolic integrity of the labeled apolipoprotein. In order to derive meaningful conclusions about the behavior of the native apolipoprotein, the decay of the tracer must mimic that of the unlabeled endogenous species. In the present study, we have shown that labeling of apo-A-I leads to the formation of an incompeent species with physical and metabolic properties that differ from those of native apo-A-I. The incompetent form of labeled apo-A-I can be separated from competent labeled apo-A-I by gel permeation chromatography of the lipid-free labeled material at high initial concentrations of protein. We have shown previously that apo-A-II and apo-C-I can be labeled covalently with no detectable changes in molecular properties (40, 41). In each of these cases the labeled material was repurified prior to experimental characterization.

We do not feel that these combined results preclude the kinetic investigation of apo-A-I metabolism using intact radiolabeled plasma lipoproteins such as HDL. However, the effect of labeling on the properties of apo-A-I must be included in the data analysis. Previous models for the metabolism of apo-A-I have contained two kinetic pools for apo-A-I. While several explanations have been considered for the existence of several explanations have been considered for the existence of the incompetent form of apo-A-I, it is clear from these studies that the incompetent form of apo-A-I tends to increase the distribution of radioactivity into this kinetic component. Thus, at least a portion of the rapidly decaying pool of apo-A-I in plasma may be due to creation of an incompetent species with radioiodination. This should be taken into consideration, perhaps by emphasizing the terminal slope, when interpreting the complex decay of radiolabeled apo-A-I from plasma.

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