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APOLIPOPROTEIN A-I ISOPROTEIN SYNTHESIS BY THE PERFUSED RAT LIVER

Giancarlo Ghiselli,[1] H. Bryan Brewer, Jr.,[1] and Herbert G. Windmueller,[2,3]

Molecular Diseases Branch, NHLBI,[1] and Laboratory of Cellular and Developmental Biology, NIADDK[2]

National Institutes of Health, Bethesda, MD 20205

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SUMMARY: The hepatic pattern of synthesis of the apolipoprotein A-I isoforms has been analyzed in the rat. After isolated livers were perfused with defibrinated rat blood and [^{3}H]leucine, the radioactivity associated with apolipoprotein A-I and other apolipoproteins was determined following two-dimensional gel electrophoresis of the perfusate d < 1.21 g/ml lipoprotein fraction. In rat serum, apolipoprotein A-I is a polymorphic system consisting of two major isoproteins and a series of minor species. Following liver perfusion, 72% of the radioactivity associated with apolipoprotein A-I isoproteins was recovered in the more acidic and quantitatively less abundant of the two major isoforms. Only 8% was associated with the major apolipoprotein A-I isoform, and similar or lower amounts were found in the other minor isoproteins. These results are consistent with the concept that, in the rat, the major apolipoprotein A-I isoforms differ in their pattern of biosynthesis and/or metabolism.

INTRODUCTION

The liver and intestine are the major sites of synthesis of plasma lipoproteins. The pattern of apolipoprotein synthesis by the liver is, however, different from that by intestine (1). Virtually all of the apoE[4] and nearly all of the apoC are produced by the liver, while both organs produce apoA-I, apoA-IV, and apoB (2). In these earlier studies, the newly secreted apolipoproteins, on the basis of their apparent molecular weight and their rate of migration in 8M urea polyacrylamide gel electrophoresis, were found to be similar or identical to those circulating in the plasma (1,2).

Recently, the polymorphism of apoA-I (3), apoA-IV (4), and apoE (5) has been investigated, and there are indications that the apolipoprotein isoforms

[3] To whom all correspondence should be addressed at Bldg. 10, Rm. 5N-102, NIH, Bethesda, MD 20205

[4] Abbreviations: Apo, apolipoprotein; HDL, high density lipoproteins; SDS, sodium dodecyl sulfate; IEF, isoelectric focusing.

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have functional importance in lipoprotein metabolism and are of clinical significance (6). Little information is, however, available regarding the mechanisms regulating their synthesis, and in particular, it has not been established to what extent the pattern of synthesis by liver and intestine resembles the pattern observed in the serum.

We have now perfused rat liver with [^{3}H]leucine and analyzed the distribution of radioactivity among the apoA-I isoforms in the perfusate. The data indicate that the perfusate contains a mixture of newly-synthesized apo A-I isoforms that is qualitatively similar to that observed in serum but quantitatively different in relative proportions.

MATERIALS AND METHODS

Liver perfusion: All rats were Osborne-Mendel males. Liver donors were fed a fat-free, sucrose-rich diet for 10-14 days (AIN 76 Purified Diet in which the 5% corn oil was replaced by an equivalent amount of additional sucrose) (7). Livers of 280 g animals were perfused *in situ* by a procedure modified slightly (8) from that of Mortimore (9). The perfusate consisted of 35 ml of defibrinated rat blood plus 35 ml of rat serum, both freshly prepared from 500-600 g rats fed NIH-07 open-formula stock diet, and was supplemented with 8300 units of penicillin G and 5 mg streptomycin sulfate. Perfusate at 37° C was continuously equilibrated with 95% O_2-5% CO_2 and was recirculated at 1.9 ml · min^{-1} · g liver^{-1}. At 5 min after the start of recycling, 1-2 mCi of L-[4,5-^{3}H]leucine (Amersham, Arlington Heights, IL, 60 Ci/mmol) in 1 ml of 0.9% NaCl was injected into the portal cannula and perfusion was continued for 3 h. The perfusate serum was then recovered and 3 mM EDTA plus 0.1% NaN_3 were added.

Lipoprotein isolation and apolipoprotein analysis by two-dimensional gel electrophoresis: The perfusate d < 1.21 g/ml lipoprotein fraction was isolated by ultracentrifugation in a Beckman 65 Ti rotor (Beckman, Inc., Fullerton, CA) (10). The HDL, d = 1.063-1.21 g/ml, were similarly prepared except that a preliminary ultracentrifugation of the perfusate at d = 1.063 g/ml was performed (10). Sample densities were adjusted with solid KBr. Isolated lipoprotein fractions were desalted by passage through a PD 10 pre-packed column (Pharmacia, Uppsala, Sweden), equilibrated with 0.08% ammonium bicarbonate, and then lyophilized. Lipids were twice extracted with chloroform-methanol, 3:1 (v/v) and the protein pellet was dried under N_2. Apolipoproteins were dissolved in freshly-prepared 8.6 M urea and analyzed by two-dimensional gel electrophoresis (11). For the first dimension, 30 µg of protein was electrofocused on a 1.5 x 70 mm 7.5% acrylamide gel containing 2% (w/v) ampholines, pH 4-6 (Serva, Heidelberg, GFR). Gels were run at 4000 volts x h at 4° C, with 0.02 M NaOH and 0.01 M H_3PO_4 as the upper and lower tray buffers, respectively. For the second dimension, the focused gels were placed on the top edge of a 10 cm (long) by 14 cm (wide), 15% polyacrylamide slab gel containing 0.1% SDS. Electrophoresis was conducted at 30 mA per gel with cooling at 4° C. Slab gels were stained for 1 h with Coomassie Blue G-250 in methanol-acetic acid-H_2O, 50:5:45 (v/v/v) and destained in 7.5% acetic acid solution. To determine radioactivity, gel areas, usually < 0.5 cm^2, were excised and solubilized with 0.5 ml 30% H_2O_2 at 80° C for 4 h in a counting vial. After the addition of 2 ml of Ready-Solv HP (Beckman, Inc.) and shaking, 26,000 units of catalase (Sigma, St. Louis, MO) in 1 ml H_2O were added and, following an 18 h incubation, 15 ml Aquasolve (New England Nuclear, Boston, MA) was added. Samples were counted after the background decayed to 20 cpm.

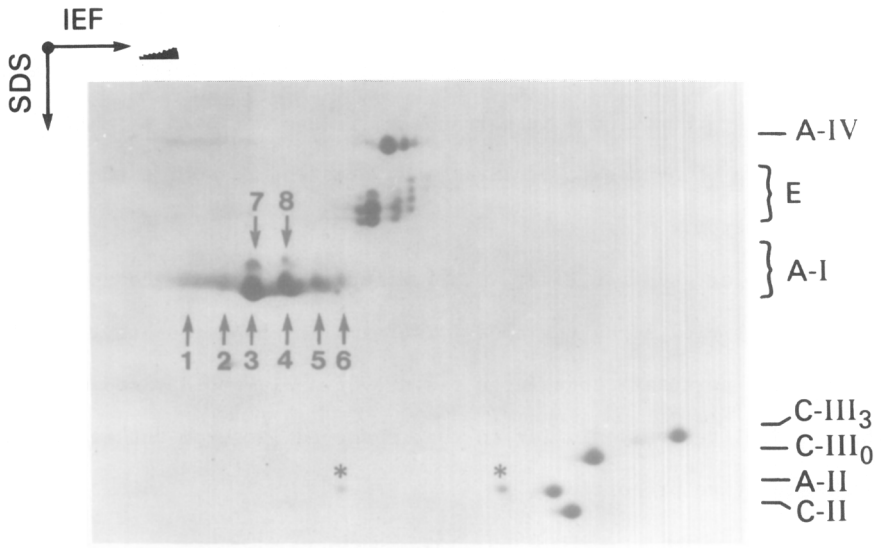

Fig. 1. Two-dimensional gel electrophoresis of rat serum apolipoproteins. The d < 1.21 g/ml lipoprotein fraction, isolated from the pooled serum of 27 rats used as blood donors for liver perfusion, was delipidated and analyzed. Apolipoproteins were identified according to Swaney and Gidez (12). The areas of the gel corresponding to the different apoproteins were cut out and digested for the determination of the associated radioactivity. For additional details, see "Materials and Methods." *, unidentified.

RESULTS

The apolipoprotein pattern of rat serum is shown in Fig. 1. Apolipoproteins were identified on the basis of apparent molecular weight and isoelectric point, as previously described (12). As in man, rat apolipoproteins include apoA-I, apoA-II, apoA-IV, apoC-II, apoC-III, and apoE (12, 13). ApoB and apoC-I, although identified in rat plasma, were not detected because of the high molecular weight of apoB and the isoelectric point of apoC-I, higher than the upper limit of the ampholine mixture used. As in man, apoA-I, apoE and apoA-IV circulate in the rat as polymorphic mixtures. Two major apoA-I isoforms were observed, the one with the more basic isoelectric point being the more abundant. At least six other minor apoA-I isoforms were also detected. Two of these corresponded to the two major isoforms in net charge, but their apparent molecular weights were slightly higher. For purposes of identification, these isoforms are numbered 1 through 8 (see Fig. 1).

The labeled perfusate d < 1.21 g/ml lipoproteins contained a total of 2.2-3.0% of the administered [^{3}H]leucine dose. An average of 21.25% of the re-

TABLE I

RELATIVE DISTRIBUTION OF THE RADIOACTIVITY AMONG DIFFERENT RAT SERUM APOLIPOPROTEINS

	Apolipoprotein						
	A-I	A-II	A-IV	C-II	$C\text{-}III_3$	$C\text{-}III_0$	E
	(percent of total)						
Mean	21.25†	3.34	12.38	3.17	3.65	6.29	49.92
SD	2.32†	1.01	2.74	0.68	0.52	2.04	1.05

† Mean ± SD of data collected from four independent liver perfusion experiments.

covered radioactivity was in the apoA-I area of the gel, 49.92% was associated with apoE, 12.38% with apoA-IV, 3.34% with apoA-II, and 13.11% with the apoC (Table I). In the apoA-I region, 98.94% of the radioactivity was associated with the most heavily stained isoproteins (numbers 3 through 8). Most of this radioactivity, 72.86%, was found in the isoform designated number 4, the less abundant of the two major isoproteins. The remaining radioactivity was distributed among the other isoforms (Table II). Similar results were obtained whether perfusate HDL or d < 1.21 g/ml lipoproteins were analyzed. In preli-

TABLE II

RELATIVE DISTRIBUTION OF THE RADIOACTIVITY AMONG THE RAT apoA-I ISOPROTEINS

Perfusion Experiment	Apolipoprotein A-I isoprotein†							
	1	2	3	4	5	6	7	8
	(percent of total)							
1	1.36	0.18	11.30	73.06	7.09	1.31	2.16	3.99
2	0.01	0.38	7.17	70.55	8.71	7.45	2.39	3.34
3	0.40	0.72	7.84	74.43	9.03	2.80	1.35	3.43
4	0.64	0.87	6.95	73.43	8.99	3.41	1.79	3.92
Mean	0.60	0.54	8.30	72.86	8.45	3.74	1.92	3.67
SD	0.57	0.31	2.03	1.65	0.92	2.62	0.45	0.33

† See Fig. 1 to identify the gel region associated with each isoprotein.

minary experiments, similar results were also obtained when the liver donor rat was fed the stock diet (NIH-07) in lieu of the high-sucrose diet, and when a sucrose-fed rat was fasted for 24 h prior to perfusion. Also, a continuous infusion of porcine insulin (640 munits/h) and fructose (60 mg/h) into the perfusate in one experiment with a sucrose-fed liver donor (perfusion experiment 2, Table II) did not significantly alter the [^{3}H]leucine incorporation pattern.

DISCUSSION

ApoA-I is a major protein constituent of plasma HDL in man and in other animals (14). Plasma apoA-I polymorphism has been analyzed in man by isoelectric focusing, which separates two major isoproteins and a series of minor isoforms, all with similar apparent molecular weights (3). An analogous polymorphic series is observed in the rat, but in this animal species, at least two of the isoproteins have higher apparent molecular weights.

The structural and functional differences between the apoA-I isoproteins remain to be established. In man, it was reported that the two major apoA-I isoforms have indistinguishable amino acid composition and immunological properties (15, 16). Furthermore, cyanogen bromide fragments and their individual tryptic peptides were similar. No carbohydrate was detected on either isoprotein (15). The data presented in this report are consistent with the concept that, in the rat, the two major apoA-I isoforms differ at least in their metabolic behavior. As determined by rat liver perfusion, hepatic biosynthesis followed by exposure of the newly-synthesized apoA-I to serum cannot account for the isoform pattern observed in the circulation. The less abundant of the two major serum apoA-I isoforms accounts for more than 72% of liver apoA-I production while only 8% of the newly-synthesized apoA-I corresponded to the most abundant form in serum. These findings suggest that, in the rat, the two major serum isoforms may be interconverted or may differ in their site of synthesis or in their catabolic rate. It has been proposed that human liver and small intestine may not secrete the mature apoA-I proteins but rather a series of precursors that are rapidly transformed into the mature proteins in plasma or lymph (17, 18). Further studies

will be needed to evaluate these hypotheses and to clarify the functional significance of these findings and of apoA-I polymorphism.

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