1.P.11 Elucidating the role of the putative "apolipoprotein E-like" domain of chicken apolipoprotein A-I

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This study addresses the potential role of an "apolipoprotein E-like" domain in chicken apolipoprotein (apo) A-I. Cholesterol from chicken high density lipoprotein (HDL), but not human HDL, can be utilized by human fibroblasts with upregulated LDL receptors. This suggests a unique role for apo A-I in cholesterol delivery in chickens. Other circumstantial evidence (similar tissue distribution of apo E and chicken apo A-I; roles in nerve regeneration) indicate the possibility that, since chickens lack apo E, chicken apo A-I could be serving a dual function of both an apo E and an apo A-I. Apo A-I from chicken contains a 20 residue segment (residues 169–188; **EEARDRLRGHVEELRKNLAP**, one letter amino acid symbol) which bears considerable homology (70%, bold letters are homologous residues, compared to 30% for the remainder of the protein) with the receptor binding domain of human apo E (residues 131–150). The possibility that chicken apo A-I could bind to an apo E receptor via this "apo E-like" domain was investigated. We have cloned chicken apo A-I into the pET 22b expression vector and expressed the protein in *E. coli.* Recombinant protein was purified from the soluble fraction of the cell pellet by reversed phase high performance liquid chromatography. The recombinant protein was shown to be physically and functionally indistinguishable from native protein. Site-directed mutagenesis was performed on the chicken apo A-I construct in the "apo E-like" region and a double mutant (glutamates 180 and 181, underlined residues, substituted by arginine and lysine, respectively) was expressed. The double mutant was designed to confer enhanced low density lipoprotein (LDL) receptor binding characteristics to chicken apo A-I, by increasing the net positive charge. Both native recombinant and the double mutant will be assayed for LDL receptor binding in competition binding assays with ^{125}I LDL using cultured human and chicken fibroblasts. These studies will aid in elucidating the potential evolutionary role of chicken apo A-I as a link between apo E and apo A-I.

1.P.12 Hydrophobic residues between aminoacids 211–229 of apoA-I play an important role in binding to lipids and lipoproteins

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We have generated stable cell lines expressing mutant apoA-I forms and have developed strategies for large scale production and purification. Mutations were designed to affect specific residues or domains implicated in LCAT activation or lipid binding. HDL and DMPC binding assays have shown that replacement of specific carboxy terminal hydrophobic residues Leu^{222}, Phe^{225}, Phe^{229} with lysines as well as replacement of Leu^{211}, Leu^{214}, Leu^{218} and Leu^{219} with valines, diminished the ability of apoA-I to bind to HDL and to lyse DMPC liposomes. The findings indicate that Leu^{222}, Phe^{225}, Phe^{229} located in the putative random coil region, and Leu^{211}, Leu^{214}, Leu^{218}, Leu^{219} located in the putative helix 8, are important for lipid binding. In contrast, substitutions of alanines for specific charged residues in putative helices 7, 8 or 9 as well as various point mutations in other regions of apoA-I, did not affect the ability of the variant apoA-I forms to bind to HDL or to lyse DMPC liposomes. Cross-linking experiments confirmed that the carboxy terminal domain of apoA-I participates in the self association of the protein, since the mutants Δ185–243 and Δ209–243 were unable to form higher order aggregates in solution. LCAT analysis, using reconstituted HDL particles, has shown that mutants (Pro^{165}→Ala, Gln^{172}→Glu), (Leu^{211}→Val, Leu^{214}→Val, Leu^{218}→Val, Leu^{219}→Val), (Leu^{222}→Lys, Phe^{225}→Lys, Phe^{229}→Lys), and (Δ209–243), reduced LCAT activation (38–68%). Mutant (Glu^{191}→Ala, His^{193}→Ala, Lys^{195}→Ala), enhanced LCAT activation (131%), and mutant (Ala^{152}→Leu, Leu^{159}→Trp) exhibited normal LCAT activation as compared to the wild type apoA-I. The $Vmax_{app}/Km_{app}$ of the apoA-I mutants ranged from 18–107% of control due to variations in both the Km and/or the Vmax. These findings indicate that putative helices 6 and 7 and the carboxy terminal helices 8 and 9 contribute to the optimum activation of LCAT.

1.P.13 Displacement of apo A-I by apo A-II results in a decrease of the LCAT activity and promotes the formation of pre-β HDL

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In the present study, we investigate the consequences of the displacement of apo A-I by apo A-II. We used reconstituted HDL (r-HDL) to study the influence of apo A-II on their structure and composition, and to investigate the influence of either apo A-II or its C-terminal helix on their LCAT activating properties. We show by gel filtration that, upon addition of apo A-II, the initial complex containing two moles apo A-I is remodeled into a mixed complex containing one mole apo A-I and two moles apo A-II. Moreover, the addition of more than two moles apo A-II per mole apo A-I, leads to the formation of an apo A-II rich complex of smaller size. The LCAT activation properties of r-HDL were further analyzed: the decrease of the LCAT activity was more important with r-HDL incubated with the apo A-II C-terminal helix than with the r-HDL incubated with entire apo A-II, as the Vmax of the reaction decreased from 28 to 16 and respectively 28 to 7 nmol cholesteryl ester/ml/hour, whereas the Km remained unchanged. The displacement of apo A-I from native HDL was further analyzed by two dimensional gel electrophoresis, using pyrene phospholipid labeled HDL. We demonstrate that the displacement of apo A-I generates pre-β migrating particles containing displaced apo A-I together with some phospholipids. We therefore propose that apo A-II has dual effects on the reverse cholesterol transport: shedding of apo A-I results in a negative control of the LCAT activity, but generates pre-β migrating particles, thus promoting the formation of potential acceptors of cell derived cholesterol.

1.P.14 Studies on cDNA sequences of tree shrew apolipoprotein AI and CI

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Tree shrew (TS) was known as the lowest primate and was discovered by us to be insusceptible to atherosclerosis for its high proportion of HDL (70–75%) in the total serum lipoproteins. In this paper, TS apo AI and apo CI cDNA sequences and their amino acid (AA) sequences deduced from the cDNAs were studied for the first time in order to obtain more important clues to elucidate TS's anti-atherogeniety as well as the role of apolipoprotein (apo) AI and CI played in HDL metabolism. TS apo AI, CI cDNA clones were identified in a TS hepatic cDNA library constructed in the phage λgt-11 with immunologic method by using rabbit anti-sera to TS apo AI, apo CI. The longest clone from each apo was sequenced. The complete cDNA sequences of TS apo AI and CI determined were 900 bp and 378 bp respectively. Apo AI cDNA contains 795 bp in the coding region predicting a 241 AA mature peptide with an 18 AA signal peptide and a 6 AA prosegment, two termination codons (TAA, TGA), 28 bp and 71 bp in 5′ and 3′ untranslated regions respectively. A polyadenylation signal is at 15 bases upstream of the polyA tail. The full length of TS apo CI cDNA includes 20 bp in the 5′-untranslated region, 264 bp in coding region, a termination codon TGA, then a 91 bp 3′-untranslated region. The coding region predicts a 88-AA protein (62-AA mature peptide whose length is the same as those of rat, mouse, and dog, but longer than those of human and baboon by 5 AA residues, and a 26-AA signal peptide). Comparisons of the AI and CI among different vertebrates indicated that TS apoAI has 69%, 67% and 71% homology with those of human, baboon and rabbit at AA level, but the identity in each apo AI prepropeptide is much higher (96%). The AA homology of apo CI with human, baboon, rat, dog are 66%, 67%, 65%, and 73% respectively. All Apo AIs of different species possess similar features, such as their N-terminals AA residues are Asp, have 11/22 AA tandem repeats of amphipathic α-helices separated by proline etc.. The results imply that all apo AIs have same functions, TS apo AI plays important roles in its lipid metabolism and is quite conservative during evolution.

1.P.15 Distinct apo B-100 conformation and surface charge are major determinants of the elevated atherogenicity of small-dense low density lipoprotein particles

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The plasma low density lipoprotein (LDL) profile in coronary artery disease patients and survivors of myocardial infarction is characterized by a