Bacterial Expression and Characterization of Chicken Apolipoprotein A-I

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Apolipoprotein (apo) A-I is a 28-kDa exchangeable apolipoprotein that plays a key role in lipoprotein metabolism. It is widely distributed among animal species and is rich in \( \alpha \)-helical secondary structure. Unlike human apoA-I, which aggregates in the absence of lipid, chicken apoA-I is monomeric in the lipid-free state. To take advantage of this physical characteristic, a bacterial expression system for production of recombinant chicken apoA-I has been developed. The cDNA-encoding chicken apoA-I was cloned into the pET expression vector under the regulation of the lac operon and transformed into Escherichia coli. Recombinant apoA-I protein recovered from the soluble fraction of the bacterial cell pellet was purified to greater than 95% homogeneity by reversed-phase high-performance liquid chromatography. Although immunoblot analysis confirmed the identity of the overexpressed protein, its migration on denaturing polyacrylamide gel electrophoresis was slower than its natural counterpart. To determine if the vector-encoded 18 residue pelB N-terminal leader sequence was not cleaved by the bacterial leader peptidase, isolated recombinant chicken apoA-I was incubated with exogenous leader peptidase. This treatment resulted in an increased electrophoretic mobility, with migration to a position corresponding to plasma-derived chicken apoA-I. Electrospray mass spectrometry indicated a mass of 27,961 ± 4 Da, in agreement with that predicted for natural chicken apoA-I. Far-UV circular dichroism spectroscopy indicated an \( \alpha \)-helical content similar to apoA-I isolated from chicken plasma, suggesting that the protein is folded in solution. Fluorescence studies showed that the wavelength of maximum fluorescence emission of the two tryptophan residues in the protein was 331 nm, with no shift occurring following complexation with lipid. Recombinant apoA-I was shown to be functional in lipoprotein binding as well as to possess an ability to transform bilayer vesicles of dimyristoylphosphatidylcholine into discoidal complexes. This is the first report of bacterial expression of an avian apoA-I. Increased availability and the potential for site-directed mutagenesis of this protein will aid in further characterization of apoA-I and the mechanism whereby it functions in cholesterol transport. © 1998 Academic Press

Apolipoprotein A-I (apo-A-I)\(^{1}\) from human is a 243-amino-acid protein involved in cholesterol transport and homeostasis (1). ApoA-I is responsible for the activation of the plasma enzyme lecithin:cholesterol acyl transferase (LCAT; 2–4) and is the main protein component of high density lipoprotein (HDL). In plasma, apoA-I is believed to be present in both lipid-poor and lipid-rich states (5). In the lipid-poor state, apoA-I is an acceptor of cellular cholesterol and, as such, can induce cholesterol efflux from cells (6,7). ApoA-I has been proposed to be involved in the return of cholesterol from peripheral tissues to the liver (the reverse cholesterol transport pathway) and this may account for its beneficial effects in preventing heart disease (5). Study of these processes is hindered because apoA-I from mammalian sources self associates in the absence of lipid (8) and this tendency to oligomerize is a major impediment to obtaining structural information on this protein.

Chicken apoA-I (240 amino acid residues) is a close relative of human apoA-I, sharing 48% sequence identity and 66% sequence similarity (as determined by SEQSEE sequence homology program; 9). Chicken apoA-I is the major apolipoprotein component of chicken HDL (10,11). Unlike human apoA-I, chicken apoA-I was found to be monomeric in the absence of lipid. Recombinant apoA-I was shown to be functional in lipoprotein binding as well as to possess an ability to transform bilayer vesicles of dimyristoylphosphatidylcholine into discoidal complexes. This is the first report of bacterial expression of an avian apoA-I.

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\(^{1}\) Abbreviations used: apo, apolipoprotein; IPTG, isopropyl-\( \beta \)-D-thiogalactopyranoside; PLC, phospholipase C; LDL, low-density lipoprotein; LPC, lysophosphatidylcholine; TFE, trifluoroethanol; DMPC, dimyristoylphosphatidylcholine; LCAT, lecithin:cholesterol acyl transferase; HPLC, high-performance liquid chromatography; CD, circular dichroism.
lipid (12). Physical characterization revealed that, as with the 22-kDa N-terminal domain of human apolipoprotein E (apoE; 13) and insect apolipoporphin III (14,15), chicken apoA-I may exist as an amphipathic α-helix bundle (12). Likewise, it has been shown that chicken apoA-I is functionally analogous to human apoA-I in terms of LCAT activation (16), lipoprotein binding (12), and the ability to induce cholesterol efflux (unpublished observations). Thus, chicken apoA-I is a good candidate for further structural and physical characterization.

Other studies have shown a unique tissue distribution of chicken apoA-I mRNA compared to that of mammalian apoA-I. Chicken apoA-I mRNA is found in skin (17), muscle tissue (18), nerve tissue (19), kidney (20), and small intestine and liver (21), whereas human apo A-I is predominantly found in small intestine and liver (22). Interestingly, the tissue expression pattern of chicken apoA-I more closely resembles that of mammalian apoE than apoA-I (23). Indeed, nerve ablation studies indicate that chicken apo A-I resembles apoE in mammalian systems in terms of its expression at sites of nerve injury (24). Chicken apoA-I, like apoE, has been proposed to play a role in nerve regeneration. The fact that chickens do not possess apoE has led to the postulate that chicken apoA-I may fulfill functional roles in the chicken that are met by apoE in mammals (18,23). In order to address hypotheses related to the structural and functional properties of chicken apoA-I, we have established a bacterial expression system for production of full length recombinant apoA-I.

MATERIALS AND METHODS

Plasmid vector construction. A plasmid encoding the cDNA of chicken apoA-I (a kind gift of Dr. Alan Attie, University of Wisconsin) served as template for amplification of the coding sequence of chicken apoA-I. Oligonucleotides containing nonannealing MscI and HindIII restriction sites served as primers for DNA amplification. The amplification product was cloned into the pET-22b(+)-diluted plasmid (Novagen, Cambridge, MA) directly downstream of the plasmid-encoded pelB leader peptide (which functions to target the expressed protein to the periplasmic space of the cell). The final construct included a stop codon immediately following the nucleotides encoding the C-terminal amino acid of chicken apoA-I. Thus, no carboxyl tags or extensions were employed in the present strategy. The sequence of the chicken apoA-I/pET plasmid DNA insert was verified by the dideoxynucleotide chain termination method (24).

Bacterial expression of recombinant apoA-I. Escherichia coli BL-21 (de3) cells transformed with the apoA-I/pET plasmid were used to express recombinant protein. A single bacterial colony was used to seed a 3-mL culture of yeast tryptone medium with 200 µg/mL carbenicillin (Sigma, Oakville, ON) and grown at 37°C. After the culture reached an optical density of 0.6 units (measured at 600 nm) the cells were pelleted at 10,000g. The cells were resuspended in 20 mL medium containing 500 µg/mL carbenicillin and cultured at 37°C to an OD_600 = 0.6. Following this, the cells were pelleted at 10,000g, resuspended in 250 mL medium, induced with isopropyl-β-D-thiogalactopyranoside (IPTG; 1 mM final concentration) and cultured for 4 h. Cells were harvested by pelleting and resuspended in buffer (10 mM Tris–HCl, 1 mM glutathione, and 1 mM dithiothreitol). The suspension was sonicated with a tip sonicator until homogeneous. Cell debris was removed by centrifugation and the supernatant was retained.

To assess if the protein was targeted to the periplasmic space, the protocol of Tettamanti et al. was utilized (25). A 1.5-mL aliquot of induced culture was centrifuged at 13,000g for 1 min. The supernatant was decanted and 15 µL of chloroform was added. The cells were vortexed and, after 15 min at 22°C, 75 µL of 10 mM Tris–HCl, pH 8, was added and the sample was centrifuged at 13,000g for 15 min. The supernatant (soluble periplasmic space fraction) was then analyzed.

To cleave the pelB leader peptide from the recombinant protein, a soluble, truncated form of leader peptidase (a kind gift of Dr. Ross Dalbey, Ohio State University; 26,27) was incubated with the sonicated cell suspension (1:1000, 1:100, or 1:10, enzymesubstrate ratio) in the presence or absence of 0.1% Triton X-100 (BDH Chemicals, Toronto, ON) for 4 h at 37°C. Leader peptidase-treated protein was isolated by high-pressure liquid chromatography (HPLC) on a semipreparative, reversed-phase R XC-8 Zorbax 300SB column (9.4 mm × 25 cm) on a Beckman high-pressure liquid chromatography. Fractions were monitored at 210 nm and eluted with a linear gradient of 0.5% B/min, where solvent A was 0.05% trifluoroacetic acid in water and solvent B was 0.05% trifluoroacetic acid in acetonitrile. Fractions containing purified protein were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and analytical HPLC (R XC-8 Zorbax 300SB column, 2.1 mm × 15 cm) using a linear AB gradient of 2% B/min, pooled, lyophilized, and stored at −20°C until use.

Analytical procedures. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was performed according to Laemmli (28) and gels were stained with Amido black 10B (Merck, Darmstadt, Germany). Immunoblot analysis was conducted by transferring SDS–PAGE gel contents to a polyvinylidene difluoride membrane (Millipore Corp.) and probing with a chicken apoA-I rabbit polyclonal antibody at a dilution of 1:5000. An enzyme-linked secondary antibody (horseradish peroxidase-linked goat anti-rabbit
IgG) and chemiluminescence reagents (Amersham) were used to detect antigen:antibody interactions. Electrospray ionization mass spectrometry was performed as previously described (29).

Spectroscopic studies. Circular dichroism (CD) spectroscopy was performed using a Jasco J-720 spectropolarimeter following protocols previously described (30). Fluorescence spectroscopy was conducted on a LS 50 luminescence spectrometer (Perkin Elmer, Beaconsfield, England) at 24°C. Tryptophan fluorescence emission spectra were recorded for plasma-derived and recombinant chicken apoA-I (10 μg/mL in 50 mM sodium phosphate buffer, pH 7.0) in the absence and presence of 0.4% lysophosphatidylcholine (LPC, Avanti Polar Lipids); emission spectra were obtained between 300 and 450 nm with excitation at 295 nm. Excitation and emission slitwidths were set at 4 nm.

Lipid binding assays. Three different binding assays were used to assess the ability of recombinant apoA-I to interact with lipids. In the first assay (31), human low-density lipoprotein (LDL, 150 μg protein) in 50 mM Tris·HCl, pH 7.5, 150 mM NaCl, 2 mM CaCl₂, was incubated with 80 million cells of Bacillus cereus phospholipase C (PLC; Sigma Chemical Co., St. Louis, MO), in the absence or presence of plasma-derived or recombinant chicken apoA-I (50 μg). Sample turbidity was measured at 340 nm on a Spectromax 250 microtiter plate reader (Molecular Devices, Sunnyvale, CA) at indicated time points. In a second lipoprotein binding assay, plasma-derived and recombinant chicken apoA-I were assayed for their ability to prevent aggregation of human LDL induced by facilitated lipid transfer, as described by Singh et al. (32). Incubations were carried out in a volume of 200 μL containing 250 μg Manduca sexta high-density lipophorin protein, 50 μg human LDL protein, 2 μg M. sexta lipid transfer particle, and 50 μg chicken apoA-I (plasma-derived or recombinant). Sample turbidity was monitored at regular time intervals at 340 nm. Finally, the ability of recombinant apoA-I to transform bilayer vesicles of dmyristoyl-phosphatidylcholine (DMPC) into disk complexes was studied as follows (33): DMPC (2.1 mg) was dissolved in chloroform:methanol (3:1, v/v) and dried to form a thin film in a test tube. Sodium cholate (2.7 mg) was added (cholate:phospholipid molar ratio 2:1), vortexed, and incubated at 37°C for 30 min until the solution was clear. Plasma-derived or recombinant chicken apoA-I (0.9 mg) in buffer A (10 mM Tris, pH 7.4, 140 mM NaCl, 0.15 mM sodium azide, and 0.25 mM sodium EDTA) was added and the solution incubated for 60 min at 37°C. The sample was then dialyzed exhaustively at 37°C against buffer A and analyzed by pore-limiting native gradient polyacrylamide gel electrophoresis (34).

RESULTS AND DISCUSSION

Expression and purification of recombinant chicken apoA-I. The nucleotide sequence encoding chicken apoA-I (residues 1–240) was ligated into the pET-22b(+) expression vector and introduced into E. coli. Following induction and culturing, the cells were harvested and examined for recombinant apoA-I production. SDS–PAGE analysis of the soluble fraction of cell pellets from cultures harboring the parent pET vector or the apoA-I/pET vector is shown in Fig. 1. Comparison of the protein patterns revealed the presence of a prominent band at ~30,000 Da in induced cultures harboring the apoA-I/pET plasmid grown at three different temperatures. The slower migration of the enriched protein compared to plasma-derived chicken apoA-I standard (lane 5) indicated it has a higher apparent molecular weight. Given that the plasmid construct employed encodes an 18-amino-acid N-terminal leader sequence extension (the pelB sequence), we hypothesized that the apparent molecular weight difference was due to accumulation of an apoA-I-pelB fusion protein. This leader sequence is designed to target expressed proteins to the periplasmic space, where an endogenous leader peptidase should function to produce mature apoA-I. However, under the different culture temperature conditions examined, the molecular weight difference persisted, suggesting that either the recombinant protein was not a substrate for the leader peptidase or the protein was not targeted to the periplasmic space. Subsequently, the periplasmic space contents from an aliquot of cells expressing...
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of a protein product of approximately 28 kDa. Inclusion of Triton X-100 had no effect on the extent of cleavage at this ratio while increasing the enzyme concentration to 1:100 (enzyme: apoA-I) resulted in nearly complete cleavage. Importantly, at a 1:10 ratio, additional lower molecular weight cleavage products appeared, suggesting that nonspecific cleavage had occurred. On the basis of these data, subsequent cleavage experiments were performed at a ratio of 1:100 (peptidase: recombinant apoA-I) for 4 h at 37°C in buffer lacking detergent.

The ability of isolated leader peptidase to specifically cleave recombinant chicken apoA-I was incorporated into the purification scheme. Due to the relative hydrophobicity of recombinant apoA-I, reversed-phase HPLC was used to remove contaminating proteins present in the soluble fraction of the bacterial cell pellet, resulting in a >95% pure protein preparation. Electrospray mass spectrometry of the isolated recombinant protein revealed a molecular weight of 27,961 ± 4 Da, in agreement with that predicted from the amino acid sequence as described under Materials and Methods. Lane 1, plasma-derived chicken apoA-I; lane 2, soluble fraction from cells harboring pET vector; lane 3, soluble fraction from cells harboring chicken apoA-I/pET vector.

Chicken apoA-I were isolated. SDS-PAGE analysis of these fractions indicated no accumulation of protein (data not shown). It is likely, then, that the expressed apoA-I protein was not targeted to the periplasmic space and therefore could not be acted upon by the endogenous leader peptidase. It is probable that the nature of the expressed protein itself hinders its transport. It is known from previous displacement studies (unpublished observations) that chicken apoA-I has a higher lipid binding affinity than another protein produced with this expression system (34) and this characteristic may prevent apoA-I from efficient targeting and cleavage.

To confirm that the protein enriched in induced bacterial cultures harboring the apoA-I/pET vector was indeed chicken apoA-I, an immunoblot was performed. When the soluble fraction obtained from the bacterial cell pellet of induced cultures was probed with a polyclonal antibody directed against chicken apoA-I, the 30-kDa band was recognized along with a less intense reactive band, of mass corresponding to that of the chicken apoA-I standard (Fig. 2). Control immunoblots probing bacterial cell pellets harboring the parent pET vector with the same antibody failed to reveal cross reactive proteins. To evaluate if the slower mobility of the major reactive band was due to failure of the endogenous leader peptidase to efficiently cleave the N-terminal pelB sequence, an isolated, soluble, recombinant leader peptidase was employed in vitro cleavage experiments with recombinant apoA-I (Fig. 3). At an enzyme:substrate ratio of 1:1000, specific cleavage of the 30-kDa band occurred, resulting in appearance of a protein product of approximately 28 kDa. Inclusion of Triton X-100 had no effect on the extent of cleavage at this ratio while increasing the enzyme concentration to 1:100 (enzyme: apoA-I) resulted in nearly complete cleavage. Importantly, at a 1:10 ratio, additional lower molecular weight cleavage products appeared, suggesting that nonspecific cleavage had occurred. On the basis of these data, subsequent cleavage experiments were performed at a ratio of 1:100 (peptidase: recombinant apoA-I) for 4 h at 37°C in buffer lacking detergent.

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FIG. 2. Immunoblot of recombinant chicken apoA-I. The immunoblot was probed with rabbit polyclonal anti-chicken apoA-I serum as described under Materials and Methods. Lane 1, plasma-derived chicken apoA-I; lane 2, soluble fraction from cells harboring pET vector; lane 3, soluble fraction from cells harboring chicken apoA-I/pET vector.

1 2 3

FIG. 3. Cleavage of the pelB leader peptide from recombinant chicken apoA-I by exogenous leader peptidase. The soluble fraction of the cell pellet was treated with leader peptidase for 4 h at 37°C. The extent of cleavage was evaluated by SDS-PAGE and immunoblotting. Lane 1, no exogenous leader peptidase; lane 2, enzyme:substrate ratio of 1:1000; lane 3, enzyme:substrate ratio of 1:1000 in the presence of 0.1% Triton X-100; lane 4, 1:100 enzyme:substrate ratio in the absence of Triton X-100; lane 5, ratio of 1:10 in the absence of Triton X-100.
RECOMBINANT CHICKEN APOLIPOPROTEIN A-I

Surface for apolipoprotein binding and provide a potential hydrophobic environment for the two tryptophan residues in the protein (residues 74 and 107; 35). Emission spectra (excitation 295 nm) showed no shift in tryptophan fluorescence emission maximum (331 nm) upon addition of LPC, indicating no change in the relative hydrophobicity of the environment of the two tryptophans. This trend is identical to the behavior of plasma-derived chicken apoA-I, which displayed a tryptophan fluorescence emission maximum of 330.5 nm (data not shown). Helical wheel modeling of chicken apoA-I indicates that the two tryptophans reside on the hydrophobic face of amphipathic α-helices. In the lipid-free state, it is conceivable that these tryptophans are sequestered from the aqueous environment by helix-helix contacts while, in the lipid-bound state, the tryptophans interact with the lipid surface. Another exchangeable apolipoprotein, L. migratoria apoLp-III, contains one tryptophan in the middle of an amphipathic helix and one tryptophan in an exposed loop region (14). Upon binding to lipid, a 15-nm blue shift in fluorescence emission maximum occurred, indicating a transition to a more hydrophobic environment (36). Although high-resolution structural data are not avail-

FIG. 4. Reversed-phase HPLC profile of recombinant chicken apoA-I. Soluble fractions from the sonicated cell pellet were chromatographed on a Zorbax C8 reversed-phase column as described.

Unlike our experience with expression of other exchangeable apolipoproteins using the pET expression vector (29,34), in the case of recombinant chicken apoA-I, the pelB leader peptide was not efficiently cleaved by the endogenous leader peptidase and the expressed protein did not specifically accumulate in the culture medium. These factors complicated the purification and limited the yield of recombinant protein. However, refinement of expression conditions have yielded expression levels of up to 50 mg apoA-I/L culture medium. Furthermore, addition of exogenous leader peptidase overcame the complication of the pelB leader peptide removal. As a result, adequate amounts of purified recombinant chicken apoA-I could be obtained using this expression system. The final yield was approximately 10 mg purified mature recombinant protein per liter of bacterial cell culture.

Characterization of recombinant chicken apoA-I. The structural properties of recombinant apoA-I were evaluated spectroscopically. Far-UV CD analysis of recombinant apoA-I revealed the characteristic high content of α-helical secondary structure (Fig. 5). Addition of 50% trifluoroethanol (TFE) resulted in a further induction of α-helix. The fact that this pattern was indistinguishable from that observed for plasma-derived chicken apoA-I (12) suggests that recombinant apoA-I adopts a folded conformation in solution similar to its natural counterpart.

Confirmation of proper folding of recombinant chicken apoA-I was obtained from tryptophan fluorescence emission spectra (Fig. 6) recorded in the absence and presence of 0.4% LPC. LPC micelles act as a lipid surface for apolipoprotein binding and provide a potential hydrophobic environment for the two tryptophan residues in the protein (residues 74 and 107; 35). Emission spectra (excitation 295 nm) showed no shift in tryptophan fluorescence emission maximum (331 nm) upon addition of LPC, indicating no change in the relative hydrophobicity of the environment of the two tryptophans. This trend is identical to the behavior of plasma-derived chicken apoA-I, which displayed a tryptophan fluorescence emission maximum of 330.5 nm (data not shown). Helical wheel modeling of chicken apoA-I indicates that the two tryptophans reside on the hydrophobic face of amphipathic α-helices. In the lipid-free state, it is conceivable that these tryptophans are sequestered from the aqueous environment by helix-helix contacts while, in the lipid-bound state, the tryptophans interact with the lipid surface. Another exchangeable apolipoprotein, L. migratoria apoLp-III, contains one tryptophan in the middle of an amphipathic helix and one tryptophan in an exposed loop region (14). Upon binding to lipid, a 15-nm blue shift in fluorescence emission maximum occurred, indicating a transition to a more hydrophobic environment (36). Although high-resolution structural data are not avail-

FIG. 5. Far-UV circular dichroism spectra of recombinant chicken apoA-I. Spectra were obtained in the absence (--) or presence (---) of 50% TFE in 50 mM sodium phosphate, pH 7.
lipid transfer-induced LDL particle aggregation was prevented by inclusion of recombinant apoA-I through formation of a stable binding interaction with the modified lipoprotein (data not shown). Recombinant chicken apoA-I was functional and indistinguishable from plasma-derived chicken apoA-I in these lipid binding assays.

A hallmark property of exchangeable apolipoproteins is an ability to transform phospholipid bilayer vesicles into disk complexes. To examine the ability of recombinant chicken apoA-I to function in this manner, apoA-I was incubated with multilamellar bilayer vesicles of DMPC. Following incubation, the sample was analyzed by nondenaturing PAGE. The data revealed formation of a discrete population of particles in the range of 200 kDa, indicating disk particle formation.

In summary, the present results show that recombinant chicken apoA-I can be expressed in bacteria and

**FIG. 6.** Fluorescence emission spectra of recombinant chicken apoA-I. Recombinant chicken apoA-I in 50 mM sodium phosphate, pH 7, was excited at 295 nm and emission recorded between 310 and 450 nm in the absence (---) or presence (---) of 0.4% LPC. Slitwidths for excitation and emission were 7 nm.

able for apoA-I, the relative location of tryptophan residues in the protein and their fluorescence properties are supportive of the concept that chicken apoA-I exists as an α-helix bundle in the absence of lipid and, upon association with a lipid surface, substitutes helix-lipid interactions for helix-helix interactions which stabilize its lipid-free conformation.

Functional analysis of recombinant chicken apoA-I.

To examine apoA-I interaction with spherical lipoprotein particles a functional lipoprotein binding assay was employed. Human LDL aggregates following incubation with PLC due to hydrolysis of the phosphocholine head group from phosphatidylcholine molecules present in the surface monolayer of the particle, creating the membrane-destabilizing lipid, diacylglycerol. This results in LDL particle aggregation seen as an increase in sample turbidity which is conveniently monitored spectrophotometrically. When functional amphipathic exchangeable apolipoproteins are included in the incubation with LDL and PLC, however, particle aggregation is prevented by apolipoprotein binding with the lipolyzed lipoprotein surface (31). As shown in Fig. 7, recombinant apoA-I was effective in preventing aggregation of human LDL induced by treatment with PLC and this effect was comparable to plasma-derived chicken apoA-I.

A second lipoprotein binding assay was employed, in which the lipid content of human LDL was enriched by facilitated lipid transfer employing insect lipid transfer particle as the catalyst and insect high-density lipoprotein as lipid donor (32,37). As with PLC-treated LDL, lipid transfer-induced LDL particle aggregation was prevented by inclusion of recombinant apoA-I through formation of a stable binding interaction with the modified lipoprotein (data not shown). Recombinant chicken apoA-I was functional and indistinguishable from plasma-derived chicken apoA-I in these lipid binding assays.

**FIG. 7.** The effect of recombinant chicken apoA-I on the stability of phospholipase C-treated human LDL. Human LDL (150 μg protein) was incubated with 80 μL of phospholipase C for 2 h at 37°C in the absence or presence of plasma-derived or recombinant chicken apoA-I (50 μg). The absorbance at 340 nm was determined at the indicated time intervals. Open squares; LDL alone; closed squares, LDL plus PL-C; open circles, LDL, PL-C plus recombinant apoA-I; closed circles, LDL, PL-C plus plasma derived chicken apoA-I.
mechanistic details of its important biological roles. Studies are currently under way to employ site-directed mutagenesis to probe specific aspects of apoA-I function.

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