Reprinted from *J. Mol. Biol.* (1988) **202**, 179–181

Crystallization and Preliminary X-ray Diffraction Studies on the Amino-terminal (Receptor-binding) Domain of Human Apolipoprotein E3 from Serum Very Low Density Lipoproteins

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Crystallization and Preliminary X-ray Diffraction Studies on the Amino-terminal (Receptor-binding) Domain of Human Apolipoprotein E3 from Serum Very Low Density Lipoproteins

Human apolipoprotein E is a component of several classes of circulating plasma lipoproteins. In addition to binding lipids, this apolipoprotein, which is composed of two structural domains, mediates some lipoprotein–receptor interactions by binding to the low density lipoprotein receptor. The receptor-binding function, as well as some lipid-binding capability, is contained in the amino-terminal structural domain of apolipoprotein E. Thrombin-catalyzed hydrolysis of apolipoprotein E yields a fragment (residues 1 to 191) that has the same properties as, and seems to be a good model for, the amino-terminal domain. Crystals of this amino-terminal fragment suitable for high-resolution X-ray diffraction experiments have now been grown. The crystals belong to the orthorhombic space group $P2_12_12_1$ and have unit cell dimensions of $a = 86{\cdot}0$ Å, $b = 40{\cdot}9$ Å, and $c = 53{\cdot}3$ Å (1 Å = 0·1 nm). This is the first human serum apolipoprotein to be crystallized.

Serum lipoproteins are supramolecular complexes in which many different types of lipids are non-covalently combined with proteins designated as apolipoproteins (Nelson, 1972). The apolipoproteins are located predominantly at the surface of the lipoprotein along with a monolayer of phospholipid and unesterified cholesterol, while cholesteryl esters and triglycerides form a non-polar core (Scanu *et al.*, 1979; Atkinson & Small, 1986). One function of these particles, intravascular lipid transport, involves cellular uptake of certain lipoproteins *via* specific, apolipoprotein-mediated binding to a cell-surface receptor. We report here on the first crystallization of one of the serum lipoprotein apoproteins that mediate this binding. Apolipoprotein (apo-)† E, which is a prominent constituent of plasma chylomicrons, very low density lipoproteins, and some high density lipoproteins, is one of two proteins (the other is apo-B) that mediate lipoprotein binding to the low density lipoprotein (LDL, or apo-B,E(LDL)) receptor (Goldstein *et al.*, 1985); thus, apo-E has a major role in triglyceride and cholesterol metabolism (Mahley & Innerarity, 1983). Apolipoprotein E ($M_r = 34{,}200$) is composed of 299 amino acids, as determined from the sequence of the protein and the cDNA (Rall *et al.*, 1982; McLean *et al.*, 1984; Zannis *et al.*, 1984). Structural heterogeneity at the gene level and variable post-translational glycosylation result in protein polymorphism; the E3/3 phenotype is the most prevalent form (Mahley *et al.*, 1984; Zannis & Breslow, 1985). Several apo-E structural variants with single amino acid substitutions have defective apo-B,E(LDL) receptor binding when assayed *in vitro*. These variants are associated with type III hyperlipoproteinemia (Mahley *et al.*, 1984).

Based on the position of amino acid substitutions in receptor-binding-defective apo-E variants, the receptor-binding properties of various apo-E fragments (Innerarity *et al.*, 1983), and the capacity of various anti-apo-E monoclonal antibodies having "known" epitopes to inhibit receptor binding (Weisgraber *et al.*, 1983), a region of the protein in the vicinity of residues 140 to 160 has been postulated to be crucial for receptor binding of apo-E. However, there has been no careful evaluation of the effects of single amino acid substitutions upon apo-E secondary and tertiary structure or upon apo-E–lipid interactions, effects that could also modify receptor binding.

We have recently shown that in aqueous solution apo-E3 contains two structural domains: an amino-terminal domain composed approximately of residues 20 to 165, and a carboxyl-terminal domain composed roughly of residues 225 to 299 (Aggerbeck *et al.*, 1987; Wetterau *et al.*, 1988). The two domains, which are capable of folding independently, are connected by a polypeptide segment that is highly susceptible to proteolysis and is presumed to be an exposed hinge region. The properties of the two domains are distinctly different when evaluated using an amino-terminal thrombolytic fragment (22,000 M_r, residues 1 to 191) and a carboxyl-terminal thrombolytic fragment (10,000 M_r, residues 216 to 299) of apo-E3

† Abbreviations used: apo-, apolipoprotein; LDL, low density lipoprotein.

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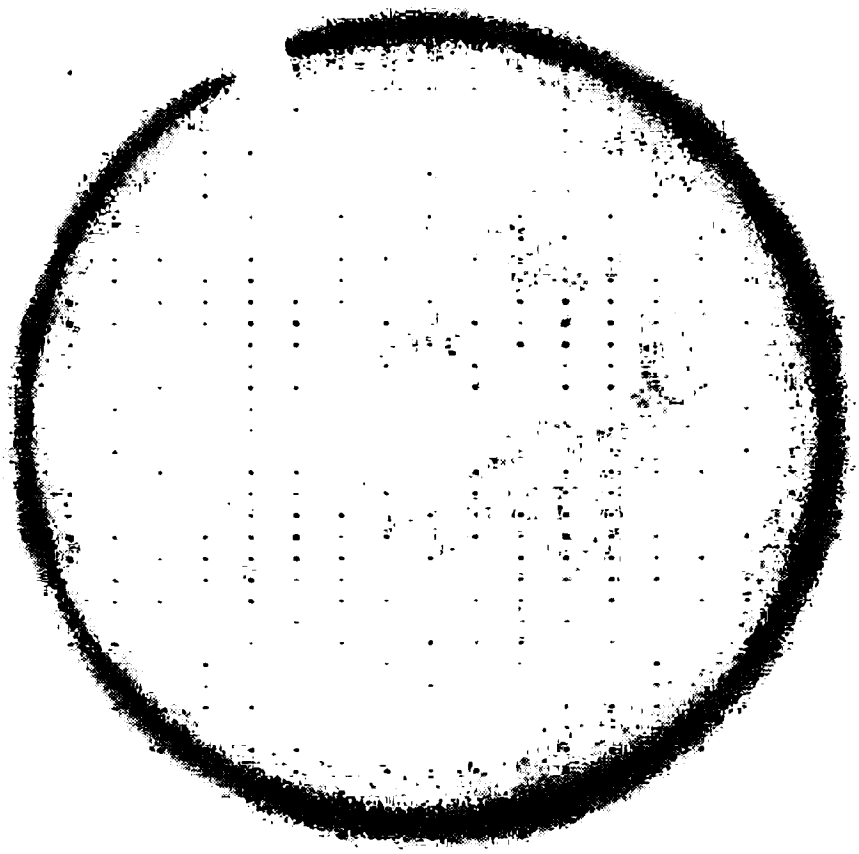

Figure 1. Precession photograph ($\mu = 10°$) of the *hk*0 zone of orthorhombic crystals of the 22,000 M_r model amino-terminal domain of apolipoprotein E3 grown from polyethylene glycol and *n*-octyl-β-D-glucopyranoside showing the systematic absences characteristic of the unit cell of the orthorhombic space group $P2_12_12_1$.

as model domains (Aggerbeck *et al.*, 1987, 1988; Wetterau *et al.*, 1988). The carboxyl-terminal (10,000 M_r) domain has a low free energy of denaturation (4 kcal/mol; 1 cal = 4·184 J), self-associates as a tetramer, and exhibits a marked blue shift upon combination with detergent or lipid. Further, most of the 10,000 M_r domain is predicted to have an amphipathic α-helical structure. These properties suggest that the 10,000 M_r domain may be a major lipid-binding region in the protein. In contrast, the amino-terminal (22,000 M_r) domain has a high free energy of denaturation (8 to 12 kcal/mol), is monomeric even at very high concentrations (15 mg/ml), is compact and globular, and shows a small blue shift upon combination with detergent or lipid. The 22,000 M_r domain, but not the 10,000 M_r domain, has strong receptor-binding properties (Innerarity *et al.*, 1983). Finally, the 22,000 M_r domain is also capable of binding lipid, the effect of which may be to modulate the structure and receptor-binding properties of the protein (Innerarity *et al.*, 1979, 1983). The structure of apo-E is therefore of general interest as a model for understanding both protein–lipid and protein–protein (receptor) interactions, and it may contribute specifically to an understanding of the physiology and pathology of human lipid metabolism. Because of its remarkable characteristics compared with other apolipoproteins, and because it is capable of binding to the apo-B,E(LDL) receptor (Innerarity *et al.*, 1983), we attempted to crystallize the 22,000 M_r model domain.

Human apo-E3, from the very low density lipoproteins of a subject with the E3/3 phenotype, was isolated by preparative ultracentrifugation and liquid chromatography (Weisgraber *et al.*, 1981). The 22,000 M_r amino-terminal fragment (residues 1 to 191) was isolated by liquid chromatography after thrombin-catalyzed hydrolysis of apo-E (Innerarity *et al.*, 1983). For crystallization, solutions of the 22,000 M_r fragment were prepared at a concentration of 5 to 15 mg/ml in 0·1 M-NH_4HCO_3 (pH 7·8). The hanging drop vapor diffusion technique was used to screen precipitating agents and physicochemical conditions. Crystals were obtained from 10 to 20 μl droplets under the following initial conditions: 3 to 10 mg of

protein/ml, 33 mM-NH_4HCO_3 (pH 7·8), 0·042% (w/v) *n*-octyl-β-D-glucopyranoside, and 0·167% (w/v) polyethylene glycol 4000. The equilibrating reservoir consisted of 1 ml of 0·5 to 2·0% polyethylene glycol 4000. Crystals appeared within one to two weeks upon equilibration at 21 °C. These crystals were then used as seeds in similarly constituted larger drops (200 μl) contained in depression slides. Larger rod-shaped crystals grew very slowly (1 to 6 months), although there was considerable variation from batch to batch of protein. The largest crystals had dimensions of about 0·25 mm × 0·15 mm × 0·10 mm. The results obtained by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate and by immunoblotting with monoclonal antibodies were consistent with the crystals being composed of residues 1 to 191 of apo-E. The space group was determined to be $P2_12_12_1$ from inspection of the symmetry and systematic absences in screened precession films ($\mu = 10°$), and the unit cell constants of $a = 86·0$ Å, $b = 40·9$ Å, and $c = 53·3$ Å were also obtained from these films (1 Å = 0·1 nm). The diffraction limit was about 2·5 Å, as determined by unscreened precession films ($\mu = 0·5°$). Assuming average volume-to-mass ratios (V_m) of protein crystals (Matthews, 1968) there is one molecule per asymmetric unit and approximately 43% solvent content. This crystal form appears suitable for a detailed high-resolution crystallographic investigation.

We thank Lucia Kosik, Martha Kuehneman and Yvonne Newhouse for excellent technical assistance, Al Averbach and Sally Gullatt Seehafer for editorial assistance, James X. Warger for graphic arts, and Michele Prator for manuscript preparation. Monoclonal antibodies to apolipoprotein E were a gift from Drs R. Milne and Y. Marcel of the Clinical Research Institute of Montreal. This work was supported in part by research grant HL 18577-11 (L.P.A.) from the National Institutes of Health (U.S.A.). L.P.A. was a visiting scientist at the Gladstone Foundation Laboratories for Cardiovascular Disease.

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Received 23 December 1987

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