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Polypeptides from Serum Low-density Lipoproteins of Pigs (*Sus domesticus*)

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Abstract

Low-density lipoproteins floating between densities 1·006 and 1·063 g cm^{-3} were isolated by centrifugation of blood serum obtained from 24-h fasted pigs (*Sus domesticus*). This lipoprotein fraction contained two components with S_F 1·063 values of 3·4 and 2·3 at 20°C when examined by analytical ultracentrifugation. Delipidation of the lipoprotein yielded 15% recovery of soluble protein whereas succinylation of the lipoprotein prior to delipidation gave 95% recovery of soluble protein. Chromatography on Sephadex G100 in 8 M urea of these delipidation products yielded three fractions of different sizes which were present in both native and succinylated apoproteins. These fractions from the succinylated apolipoproteins were further characterized. A polypeptide fraction comprising 70% of the total protein had an apparent molecular weight of 34 000 and contained greater amounts of amino acids with hydrophobic side chains than did the second fraction of apparent molecular weight 22 000 which contained 15% of the protein. The third fraction of apparent molecular weight 12 500 contained 15% of the protein.

Introduction

The amino acid composition of the serum low-density lipoprotein (LDL) obtained from the domestic pig (*Sus domesticus*) has been reported by Fidge (1973). However, the apoproteins from human LDL have been shown to contain two different polypeptides (Kane *et al.* 1970). Pig LDL has also been shown by Janado and Martin (1968) to contain two fractions of different density, LDL_1 and LDL_2. These workers showed that there is heterogeneity in the lipid content of pig LDL. We report here the presence of more than one protein in the LDL of density 1·006–1·063 g cm^{-3} obtained from pig serum.

Materials and Methods

Chemicals used were analytical reagent grade. Urea was further purified by passage of a 10 M solution through separate columns of Amberlite IRA 400 (OH^- form) and IRA 120 (H^+ form). The conductivity of the resultant solution was less than 10 μS. Proteins for calibrating the Sephadex G100 column were obtained from Sigma, St Louis, U.S.A. Solvents were freshly distilled and diethyl ether was peroxide-free.

Blood was obtained at the local slaughter house from pigs of both sexes of the Large White breed aged 120–180 days and fasted for 24 h. Pooled samples were used for the preparation of the serum. Sodium ethyl mercurithiosalicylate (Thiomersal, B.D.H. Chemicals Ltd, Poole, England) was added to the serum as recommended by Hatch and Lees (1968) and the very low-density lipoprotiens removed by centrifugation at a density of 1·006 g cm^{-3} as described by Hatch and Lees (1968). The remaining solution was adjusted to density 1·063 g cm^{-3} using KBr (Radding and Steinberg 1960) and centrifuged for 20 h at 100 000 g_{av}. The fraction floating at this density was washed by a further centrifugation at a density of 1·063 g cm^{-3}. The purity of this LDL preparation was tested using the analytical ultracentrifuge by flotation in a solution of density 1·063 g cm^{-3} using Schlieren optics.

The LDL was succinylated, using a method based on that of Hass (1964), by addition of succinic anhydride in small increments with stirring to a final molar ratio of succinic anhydride to lysine of 100 : 1. The pH was maintained at 8·9 by addition of 0·1 M NaOH. The reaction was considered complete when the pH had stabilized. Succinic acid was removed by dialysis against water.

Delipidation of both native and succinylated LDL was carried out by the method of Shore and Shore (1969) using diethyl ether and diethyl ether–ethanol mixtures. After each diethyl ether–ethanol wash, residual ethanol was removed from the aqueous phase using a diethyl ether wash.

Gel filtration chromatography was carried out on Sephadex G100 (40–120 μm bead size) (Pharmacia, Uppsala, Sweden). The column (0·9 by 100 cm) was equilibrated with 0·2 M tris–HCl buffer, pH 8·4, in 8 M urea and maintained at 25°C using a water jacket. The soluble apoproteins were dialysed against the buffer, applied in 2 cm^3, eluted at a rate of 4 cm^3 h^{-1}, and 2-cm^3 fractions were collected.

Elution of the protein from the Sephadex column was followed by absorbance at 280 nm and protein determination was by the method of Lowry *et al.* (1951). Total lipid was determined gravimetrically using the extraction procedure of Folch *et al.* (1957). Phosphate was determined by the method of Bartlett (1959) and this was used to estimate phospolipids by assuming 4% phosphorus in phospholipids. Disc gel electrophoresis in 8 M urea was carried out as described by Davis (1964) using 7·5% polyacrylamide gels and a current of 3 mA per gel. Amino acid compositions of polypeptides were determined after hydrolysis of the apoproteins with 6 M HCl in evacuated sealed tubes at 100°C for 24 h. Ammonia was removed from the hydrolysate by adjusting to pH 13 with 1 M NaOH followed by evaporation to dryness. The residue was dissolved in water (1 cm^3) and adjusted to pH 3·0 with 1 M HCl. Analyses were carried out on a Beckman 120C amino acid analyser. Norleucine was used as an internal standard with acidic and neutral amino acids and α-amino-β-guanidino propionic acid with the basic amino acids.

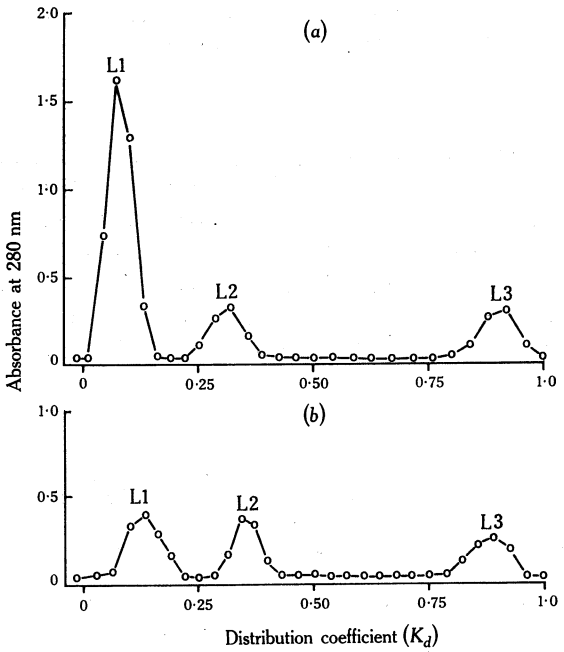

Fig. 1. Chromatography of (*a*) succinylated apoLDL and (*b*) native apoLDL on Sephadex G100 in the presence of urea. 30 mg of LDL protein was delipidated by solvent extraction and the soluble apoprotein in 2 ml of 0·2 M tris–HCl in 8 M urea, pH 8·4, was applied to a column (0·9 by 100 cm) of Sephadex G100 equilibrated with the same buffer.

Results and Discussion

The LDL from pig serum showed two components in the analytical ultracentrifuge, with S_F 1·063 values at 20°C of 3·4 and 2·3 respectively. These correspond to the LDL_1 and LDL_2 described by Janado and Martin (1968).

Following delipidation of succinylated LDL, 95% of the protein was soluble whereas using native LDL 15% of the protein was soluble in 0·2 M tris–HCl in 8M

urea at pH 8·4. Residual phospholipid in the low-density lipoproteins after lipid removal by solvent extraction (apoLDL) was less than 0·5% by weight. Native apoLDL and succinylated apoLDL showed three peaks on Sephadex G100 (Fig. 1).

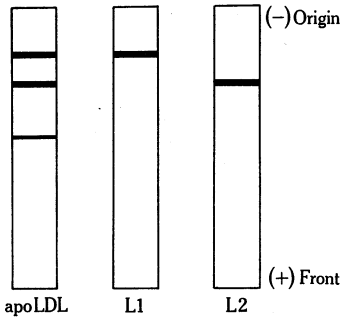

Fig. 2. Electrophoretic patterns of LDL apoprotein in the presence of urea. Samples of native LDL apoprotein were applied to 7·5% polyacrylamide gels containing 8 M urea at pH 8·9. Peak L3 did not fix in the gel. Details of electrophoresis and staining methods are given in the text.

Peak L1 was small in the run using native apoLDL showing a preferential loss of this component during delipidation. In the succinylated preparation of apoLDL peak L1 constituted 70% of the protein and peaks L2 and L3 15% each. The distribution coefficients (K_d) were calculated for ribonuclease (EC 3.1.4.22), myoglobin (horse heart), papain (EC 3.4.22.2), trypsin (EC 3.4.21.4), alcohol dehydrogenase (yeast) (EC 1.1.1.1) and for each of the apoproteins. L1 had a K_d corresponding to a molecular weight of 34000, L2 22000 and L3 12500. Electrophoresis in 7·5% polyacrylamide gel and 8 M urea gave three bands for native apoLDL. Two of these bands had relative mobilities that corresponded to those of L1 and L2 of the native apoLDL separated by Sephadex G100 gel filtration (Fig. 2). Peak L3 did not fix after separation from the other apoproteins, possibly due to the high-pH urea altering the protein.

Table 1. Amino acid compositions of LDL apoproteins

Values are expressed as moles of amino acid per 10^3 moles of total amino acid present in the sample and are the result of 24-h hydrolyses with 6 M HCl uncorrected for hydrolysis time. L1 is the mean of four determinations and L2 is the mean of two determinations

Amino acid	L1	L2	Amino acid	L1	L2
Lys	70·1	75·4	Ala	54·8	89·2
His	24·4	14·6	Cys	3·7	10·3
Arg	33·9	7·1	Val	61·5	49·9
Asp	107·8	104·9	Met	10·2	3·2
Thr	61·6	69·2	Ile	64·6	27·6
Ser	67·7	116·3	Leu	126·3	68·2
Glu	129·8	125·5	Tyr	37·4	30·9
Pro	42·7	49·1	Phe	48·0	41·0
Gly	55·6	117·5			

The amino acid composition of the polypeptides of peaks L1, L2 and L3 were determined using material prepared from succinylated LDL (Table 1). The amino acid compositions differed in that L1 contained more arginine, valine, methionine, isoleucine, leucine and less serine, glycine and alanine than did L2. Amino acid

analyses for peak L3 were not consistent between different preparations but the material in L3 had a different amino acid composition than either L1 or L2 with elevated levels of lysine and serine. A weighted average of the amino acid composition of peaks L1 and L2 gave an amino acid composition very similar to that reported by Fidge (1973) for the LDL of pig serum. Peak L1 contains many hydrophobic side chains and a polypeptide of similar composition has been reported by Kane *et al.* (1970) from human LDL. They also found a minor polypeptide with a greater number of hydrophilic residues than the major polypeptide. Human LDL shows only one peak on analytical ultracentrifugation and this peak must contain both polypeptides. LDL_1 and LDL_2 from pig serum have different S_F values and their apoprotein content could be investigated after separation by flotation.

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