Microcomplement fixation and particle size of chicken lipoproteins

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Abstract Conformational changes of the lipoproteins of chicken serum and egg yolk were studied with the use of microcomplement fixation. Egg yolk very low density lipoproteins and serum total low density lipoproteins were separated by 2% agarose gel chromatography into four and five fractions, respectively, with different average particle diameters. A correlation between percent complement fixed at equivalence and particle diameter was noted. Thus, smaller particles fixed less complement. The shortening of the radius of curvature could alter the steric fit of apolipoprotein with antibody and, as a consequence, reduce complement fixation. The patterns obtained after delipidation of very low density lipoproteins indicate a general distortion of apolipoprotein conformation supporting the view that lipids are essential for maintaining the conformation of these apolipoproteins. On the other hand, the general conformation of the high density apolipoproteins does not appear to depend on lipid content. It has been reported that lipids restrain the free movement of these apolipoproteins. After delipidation, the greater degree of freedom allowed the apolipoprotein may increase the strength of the antigen–antibody union and therefore an increase in complement fixation is observed. These studies demonstrate the applicability of quantitative microcomplement fixation to the eludication of lipoprotein structure and function.—**Hillyard, L. A., H. M. White, and S. Abraham.** Microcomplement fixation and particle size of chicken lipoproteins. *J. Lipid Res.* 1980. **21:** 913–920.

Supplementary key words complement fixation · agarose gel chromatography · very low density lipoproteins · low density lipoproteins · high density lipoproteins

There is a large body of information available on the composition, structure, immunology, and metabolism of serum lipoproteins (1–5). Although much has been learned about the interaction of apolipoproteins and lipids in these complex particles (5), it would be useful to have a procedure that could measure conformational changes for each specific apolipoprotein within intact lipoproteins, which result from either physical and chemical manipulations or from metabolic activity. We have examined the usefulness of quantitative microcomplement fixation for this purpose since it is a more discriminating method than is immunodiffusion, immunoelectrophoresis, or quantitative precipitation. Complement fixation is extremely sensitive to the three-dimensional configuration of an antigen (6, 7) and can distinguish between homologous proteins differing from each other by as little as one amino acid (8, 9). In addition, a single antigen-antibody system can be studied by this technique even in the presence of other such systems (6, 10).

The present report demonstrates the applicability of quantitative microcomplement fixation to the elucidation of some questions relating to lipoprotein structure and function. The chicken was chosen for study since fewer serum apolipoproteins have been found in this species than in mammals (11–14).

MATERIALS AND METHODS

Preparation of lipoproteins

Lipoproteins were isolated from the serum of white Leghorn chickens by ultracentrifugation at 17°C using a modification (11) of the procedure of Ewing, Freeman, and Lindgren (1). Egg yolk was diluted with three volumes of a sodium chloride solution (1.006 g/ml) and the yolk granules removed by centrifugation for 30 min at 15,000 *g* at 2–4°C. The protein concentration of the supernatant solution was adjusted to about 10 mg/ml and then the Ey-VLDL isolated by the same method used for serum VLDL (11).

Particle diameter estimation by agarose gel filtration

Gel filtration was carried out in a glass column (2.5 × 75 cm), with flow-adaptors fixed at each end, packed with 2% agarose gel (Bio-Gel, A-50m, 100–

Abbreviations: Ey-VLDL, egg yolk very low density lipoproteins; VLDL, serum very low density lipoproteins; LDL, serum low density lipoproteins; TLDL, serum total low density lipoproteins; CB, carbamylated; apo, lipid-free lipoprotein; EDTA, disodium ethylenediaminetetraacetate.

200 mesh) up to a volume of about 300 ml. The eluant was 0.2 M NaCl–0.001 M EDTA, pH 7.0 (15). The internal volume of the agarose gel column was calculated from the void volume, determined with lyophilized *E. coli*, and the total volume, determined with $NaNO_2$. The column was calibrated with human γ-globulin, equine ferritin, and bovine thyroglobulin. The Stokes diameters for the lipoprotein fractions were calculated using the formula and data published by Ackers (16).

The lipoprotein solutions, before application to the agarose column, were dialyzed against 4 l of 0.2 M NaCl–0.001 M EDTA at 2–4°C for 24–48 hr. Each sample was introduced at the bottom of the column through a three-way valve and the eluant was pumped upwards at a flow rate of 17 ml/hr (approximately 3.5 ml/cm²/hr) at 20–22°C. The absorbance of the eluate was monitored at 280 nm and 5-ml fractions were collected automatically. Fractions to be rechromatographed were concentrated in Centriflo membrane cones (Amicon Corp.).

Total lipoprotein mass was determined by refractometry (17) with a Bausch and Lomb Precision Abbé refractometer maintained at 26.0 ± 0.02°C with a constant temperature circulating water bath. The contribution of salt to the total refraction was determined on an ultrafiltrate of each sample. The refractive increment of VLDL in 0.2 M NaCl was 0.000154 Δn/mg/ml and of HDL was 0.000172 Δn/mg/ml. These increments were calculated from the dry weight values for lipoprotein solutions of known refractive index. For this purpose, the lipoprotein solutions were washed free of salt in Centriflo membrane cones, centrifuged to dryness, and then taken to constant weight in vacuo at 40°C.

Protein was determined by the method of Lowry et al. (18) with bovine serum albumin as standard.

Calculation of particle weight and surface protein coverage

The minimum hydrated particle weight was calculated for each lipoprotein from the following formula: particle weight (daltons) = $0.3153\ d^3\delta$, where d is the diameter of the hydrated particle and δ, the density of the hydrated lipoprotein (19). The density of the hydrated lipoproteins, assuming a water content of 0.2 g/g lipoprotein, was determined from their protein and lipid contents and their respective densities. The partial specific volume for VLDL protein was calculated (20) as 0.7388 ml/g from its amino acid composition (11, 21) and yielded a density of 1.35 g/ml. The density for total lipid was assumed to be 0.95 g/ml.

The percent of the surface covered with protein was calculated as the ratio of experimentally determined percentage weight of protein to the theoretical percentage weight of protein for spherical particles, assuming the surface to be completely covered with a protein layer 20 Å thick (22). The value for complete protein coverage was calculated for each lipoprotein fraction from the density for protein (1.35 g/ml) and the volume of the outer 20 Å layer.

Immunological methods

The procedures used for the preparation of rabbit antisera, Ouchterlony double diffusion, and immunoelectrophoresis have been previously described (11). The microcomplement fixation experiments were performed according to the technique of Wasserman and Levine (23). Reaction volumes of 7 ml were used and all reagents were diluted in buffer consisting of 0.14 M NaCl, 0.01 M Tris, 0.5 mM $MgSO_4$, and 0.15 mM $CaCl_2$ at a final pH of 7.45. The reaction time, at 5°C, was 18 hr.

The sodium decylsulfate-solubilized apolipoproteins and carbamylated lipoproteins and apolipoproteins used in these experiments were prepared by methods reported previously (11).

Materials

Rabbit anti-phosvitin serum and lyophilized *E. coli* were purchased from Calbiochem, human γ-globulin from Pentex, bovine thyroglobulin from Sigma Chemical Co., equine ferritin from Mann Research Laboratories, standard solutions of crystalline bovine albumin from Armour Pharmaceutical Co., and Bio-Gel A-50m, 100–200 mesh, from Bio-Rad Laboratories. Hemolysin, guinea pig complement, and sheep red cells were obtained from either the Hyland Laboratories or the Sylvana Company.

RESULTS

Complement fixation with native lipoproteins

With the microcomplement procedure, we were effectively able to isolate a single antigen–antibody system for study for both VLDL and HDL by selecting the appropriate antigen concentrations. The antibodies developed with Ey-VLDL, VLDL, and LDL all produced single precipitin lines in immunoelectrophoresis and all three migrated more rapidly after reduction and carboxymethylation (11). The antiserum employed contained antibodies specific for the apolipoprotein consisting of two polypeptide chains linked together through a single disulfide bridge (11). The complete amino acid sequence for this specific VLDL apolipoprotein has been recently reported (24).

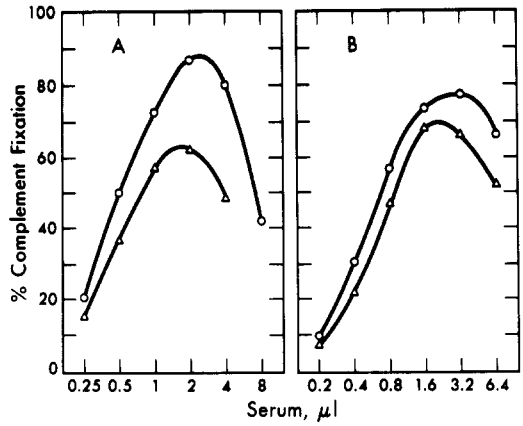

Fig. 1. Microcomplement fixation with (A) cockerel serum and (B) hen serum. The concentrations of antisera used were 1:400 for anti-Ey-VLDL serum (○) and 1:800 for anti-LDL serum (△).

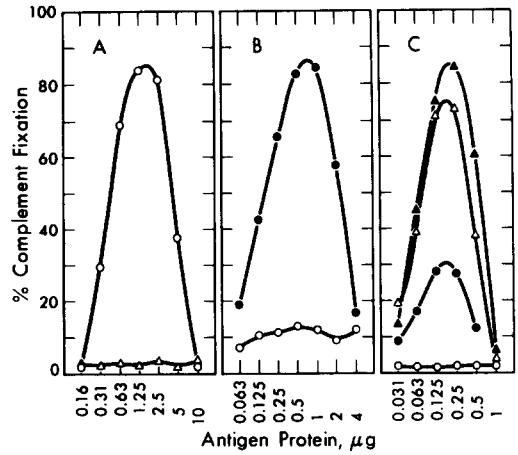

Fig. 2. Reactivity in the microcomplement fixation procedure of (A) anti-HDL serum, (B) anti-LDL serum, and (C) anti-VLDL serum. The concentrations of the antisera were 1:400 for anti-HDL serum, 1:1,000 for anti-LDL serum, and 1:10,000 for anti-VLDL serum. The lipoproteins reacted with antisera were HDL (○); LDL (●); VLDL (△) and Ey-VLDL (▲).

When anti-Ey-VLDL and anti-LDL sera were reacted with cockerel sera, the point of maximum fixation occurred at the same volume of cockerel serum (**Fig. 1A**). A similar relationship was found with hen serum (Fig. 1B). Since no shoulders or separate peaks were observed, a single antigen–antibody system must be involved which appears to be the same in cockeral and hen sera as well as in egg yolk. From these data, it is also apparent that both anti-Ey-VLDL and anti-LDL sera possess the same specificity.

When anti-HDL serum was reacted with HDL, a single complement fixation curve was obtained (**Fig. 2A**). No reaction occurred between anti-HDL serum and VLDL. Some samples of LDL did react with anti-HDL serum at very high antigen concentrations (25 μg/7 ml). Occasionally, we found that as much as 6% of the protein present in LDL was due to apoHDL. However, the presence of apoHDL did not interfere with the complement fixation analysis of LDL samples.

When anti-LDL serum was reacted with LDL, a single complement fixation curve was obtained (Fig. 2B). No complement was fixed when HDL was reacted with anti-LDL serum.

VLDL, LDL, and Ey-VLDL gave complement fixation curves with anti-VLDL serum which reached maximum values at about the same concentration of antigen protein (Fig. 2C). The maximum complement fixed was very similar for VLDL (78%) and Ey-VLDL (85%). No complement was fixed when HDL was reacted with anti-VLDL serum.

Our observation that LDL fixed less complement than VLDL at the same concentration of antiserum required further investigation (Fig. 2C). Since there was a marked difference in the complement fixed by VLDL and LDL at the same antibody concentration, the complement fixed at equivalence for various antibody and antigen combinations was determined for different dilutions of antibody. The height of the complement fixation peak was linearly related to the log of the antiserum concentration (**Fig. 3**). Since the slopes of the lines for the four low density lipoprotein systems were similar, we have inferred that a proportional relationship exists between antibody concentration and peak height. Therefore, the difference between the complement fixed by VLDL and LDL at the same antibody concentration has

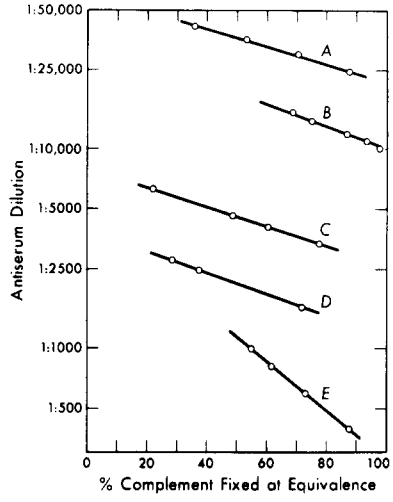

Fig. 3. Serial dilutions of egg yolk and serum lipoproteins were reacted with several concentrations of antiserum. The antigen–antibody systems were (A) Ey-VLDL reacted with anti-Ey-VLDL serum, (B) VLDL reacted with anti-VLDL serum, (C) Ey-VLDL reacted with anti-LDL serum, (D) LDL reacted with anti-LDL serum, and (E) HDL reacted with anti-HDL serum. Each point represents the peak height of a complement fixation curve for a particular antiserum concentration.

TABLE 1. Particle diameter, protein content and calculated surface protein coverage for chicken serum and egg yolk lipoproteins

Lipoproteins	Particle		Protein			Protein Coverage[b] (percent)
	Hydrated Diameter Å	Hydrated Weight (daltons × 10^{-6})	Weight (percent)	Weight (daltons × 10^{-6})	Relative Content[a]	
Ey-VLDL[c] (6)[d]	336 ± 5[e]	11.9 ± 0.5	11.5 ± 0.6	1.10 ± 0.05		29.0 ± 0.8
Fraction I (2)	416	22.6	10.9	1.97	100	32.6
Fraction II (2)	360	14.6	11.0	1.29	66	29.3
Fraction III (2)	328	11.1	12.1	1.08	55	29.9
Fraction IV (2)	298	8.4	13.1	0.89	45	30.6
TLDL[c] (4)	334 ± 3	11.7 ± 0.2	11.5 ± 0.5	1.08 ± 0.06		28.9 ± 1.4
Fraction I (4)	487 ± 12	36.0 ± 2.6	8.7 ± 0.4	2.50 ± 0.13	100	29.6 ± 0.4
Fraction II (4)	402 ± 12	20.3 ± 1.8	11.3 ± 0.4	1.83 ± 0.09	73	32.8 ± 1.1
Fraction III (4)	335 ± 13	11.8 ± 1.4	12.1 ± 0.8	1.15 ± 0.15	46	30.4 ± 1.4
Fraction IV (4)	267 ± 12	6.0 ± 0.8	14.2 ± 1.0	0.68 ± 0.09	27	30.1 ± 0.8
Fraction V (4)	220 ± 10	3.5 ± 0.3	17.0 ± 1.8	0.48 ± 0.05	19	31.5 ± 1.6
LDL[c] (4)	215 ± 10	3.2 ± 0.4	18.2 ± 1.8	0.47 ± 0.04		33.2 ± 1.0

[a] The protein content of Fraction I was assumed to be 100.
[b] Protein assumed to cover surface of lipoprotein in a layer 20 Å thick.
[c] Original lipoprotein fraction before separation on agarose gel columns.
[d] Number of experiments given in parentheses.
[e] Mean ± S.E.
The methods and assumptions used for calculation of these values are given in the text.

quantitative significance and can be used to compare particles of different sizes.

Ey-VLDL, isolated by ultracentrifugation, was separated into four fractions by agarose gel filtration. Fraction I contained the largest particles and fraction IV the smallest (**Table 1**). The individual fractions were concentrated and once again passed through the column to obtain accurate elution volumes for each fraction. Serum total low density lipoproteins were separated into five fractions using the same procedures. The particle diameter calculated for each fraction represent an average mean diameter (Table 1). When each Ey-VLDL fraction was reacted with anti-Ey-VLDL serum, we observed a direct relationship between the maximum complement fixed and the particle size (**Fig. 4A**). This relationship was also observed with the five serum fractions (Fig. 4B).

The percent complement fixed at equivalence was plotted against the particle diameter for each lipoprotein fraction (**Fig. 5A**). For comparative purposes, the concentration of anti-Ey-VLDL serum and anti-LDL serum was adjusted so that the values for the peaks for Fraction I were almost the same for both

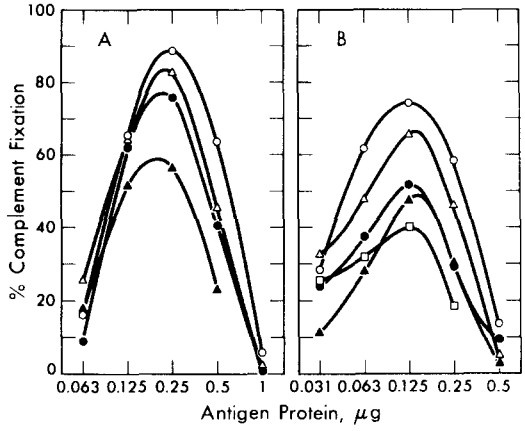

Fig. 4. Reactivity in the microcomplement fixation procedure of (A) four egg yolk VLDL fractions: I (○); II (△); III (●) and IV (▲) and (B) five serum TLDL fractions: I (○); II (△); III (●); IV (▲) and V (□). The fractions were prepared by agarose gel chromatography and the concentration of anti-Ey-VLDL serum (A) was 1:28,000 and of anti-VLDL serum (B) was 1:10,000.

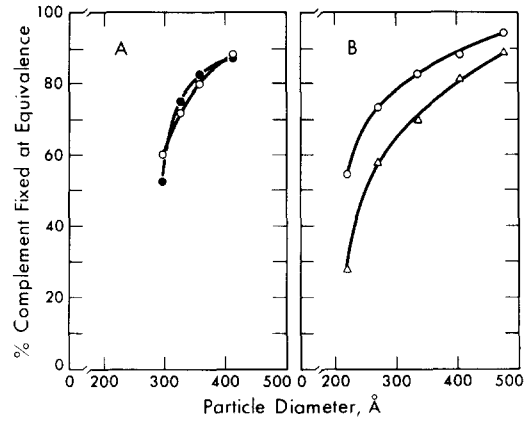

Fig. 5. Reactivity in the microcomplement fixation procedure for (A) four Ey-VLDL fractions and (B) five serum TLDL fractions of different particle diameter. The percent complement fixed at equivalence was determined for each fraction. The concentration of anti-Ey-VLDL serum (●) was 1:28,000; anti-LDL serum (○) 1:2,800; and anti-VLDL serum (△), 1:10,000.

antisera. We found a direct relation between the quantity of complement fixed and the particle size of the VLDL fractions. The percent complement fixed for each of the four Ey-VLDL fractions was similar when either anti-Ey-VLDL serum or anti-LDL serum was used. The complement fixation curve for serum TLDL is very similar although the peak height for anti-LDL serum was slightly higher than that for anti-Ey-VLDL serum (Fig. 5B). On the other hand, the percent complement fixed by the reaction of TLDL fraction V, equivalent in particle diameter to LDL, with anti-Ey-VLDL serum was about one-half that with anti-LDL serum (Fig. 5B).

Particle diameter and protein coverage

The calculated particle diameters for the various lipoprotein fractions are shown in Table 1. The particle weights calculated for the four Ey-VLDL fractions ranged from 22.6×10^6 to 8.4×10^6 daltons and the protein moieties ranged from 1.97×10^6 to 0.89×10^6 daltons. The particle weights for serum TLDL fractions ranged from 36.0×10^6 to 3.5×10^6 daltons and the protein moieties ranged from 2.5×10^6 to 0.48×10^6 daltons. Our calculations show that about 30% of the surface of each Ey-VLDL and TLDL fraction is covered with protein (Table 1). Lossow et al. (22) reported that three VLDL fractions isolated from human plasma had a protein coverage ranging from 20% to 29%. The chicken VLDL fractions studied here had particle sizes that were similar to those in humans which Lossow et al. (22) found to have 29% protein coverage.

Complement fixation with apolipoproteins

A comparison between the complement fixation curves for Ey-VLDL and its apolipoprotein (Ey-apoVLDL) is shown in **Fig. 6A.** We had to increase the concentration of anti-Ey-VLDL eight-fold (from 1:20,000 to 1:2,500) in order to enable Ey-apoVLDL to fix as much complement as the native Ey-VLDL. The antigen protein concentration at equivalence for Ey-apoVLDL was 1.5 μg/7 ml, whereas the concentration of Ey-VLDL protein was 0.25 μg/7 ml, a six-fold difference. Thus, a higher concentration of antigen protein is needed to fix the same quantity of complement required for the native lipoprotein. This lateral shift of the peak of equivalence indicates a general conformational change that results in a reduced affinity of antibody for a majority of determinants on the apolipoprotein (6).

The peak of equivalence for both native Ey-VLDL and CB-Ey-VLDL occurred at the same antigen protein concentration. However, a seven-fold increase

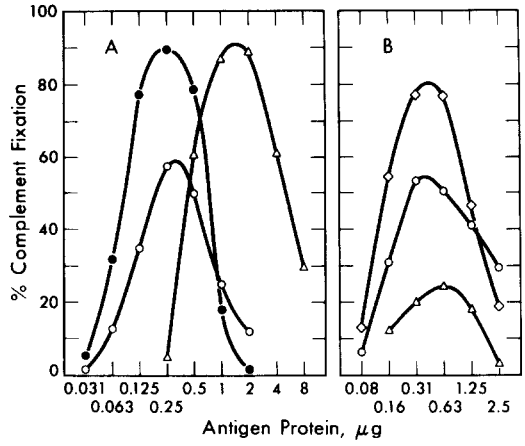

Fig. 6. Microcomplement fixation of (A) Ey-VLDL (●), CB-Ey-VLDL (○) and Ey-apoVLDL (△) with anti-Ey-VLDL serum at concentrations of 1:20,000, 1:6,000 and 1:2,500, respectively, and (B) HDL (○), apoHDL (◇) and CB-apoHDL (△) with anti-HDL serum at a concentration of 1:1,000.

in antibody concentration was required (from 1:20,000 to 1:3,000) in order to enable CB-Ey-VLDL to reach the same percent complement fixed as native Ey-VLDL (data not shown). The curve for the 1:6,000 antibody dilution is shown in Fig. 6A. Such a negative vertical shift suggests that carbamylation of Ey-VLDL weakens only a limited number of antigenic determinants.

When antibody was removed from the reaction mixture, CB-Ey-VLDL showed a peak of complement fixation at 16 μg of antigen protein (data not shown). This was the direct result of carbamylation since native Ey-VLDL did not fix complement at any concentration used. It appears that carbamylated Ey-VLDL particles formed aggregates, at antigen protein concentration of 16 μg/7 ml, which have the capacity to bind or inactivate complement.

An unexpected pattern emerged when complement fixation curves were plotted for sodium decylsulfate solubilized apoHDL. In this case, delipidation resulted in a marked increase in the amount of complement fixed (Fig. 6B), although the peak of equivalence for HDL and apoHDL occurred at the same antigen concentration. The unusual positive vertical shift could not be explained within the present theoretical framework. This result did not appear to be an artifact caused by the presence of sodium decylsulfate, since all preparations were dialyzed to remove the detergent and apoEy-VLDL did not show the same pattern.

The complement fixation curve for CB-apoHDL (Fig. 6B) showed a negative vertical shift indicating that a limited number of antigenic determinants had been effected by carbamylation.

DISCUSSION

Quantitative microcomplement fixation has been applied successfully to the determination of the degree of dissimilarity among homologous proteins (6–10). Soluble, globular proteins were used in these comparative studies. Here, on the other hand, we have examined the more complex VLDL particle which has a triglyceride-rich lipid core covered with a monolayer of protein, phospholipid, and cholesterol. The VLDL particle possesses numerous apolipoprotein subunits in the surface layer and, hence, is antigenically multivalent. The usefulness of complement fixation data for the determination of conformational changes in the protein moieties of the VLDL particles must take into account the unique structure of these particles. Thus, changes in the following parameters may be responsible for the observed difference in complement fixed by VLDL particles with different diameters: a) concentration of apolipoproteins in the surface layer; b) ratio of various apolipoprotein species; c) degree of masking of antigenic determinants by the surface lipid; d) physical state of the surface lipid; and e) radius of curvature of apolipoproteins.

Our results show that about 30% of the surface of the VLDL particles is covered with protein, regardless of particle diameter. Since there were no changes in the apolipoprotein concentration in the surface layer, this factor could not account for the difference in complement fixation observed with particles of different diameters. No shift in the zone of equivalence was observed with native VLDL particles, suggesting that neither the ratio of variance apolipoproteins species nor the degree of masking of antigenic determinants by lipids was changed. Such shifts occur when the amount of a specific apolipoprotein relative to the total available protein changes.

In regard to the influence of the physical state of surface lipid, evidence is available from studies on the mechanism of complement-mediated attack on cells employing model membranes (25, 26). The use of model membranes allows the analysis of complement binding and activation under conditions in which the hapten characteristics and physical state of the membranes are controlled. Parce, Nicole, and McConnell (27) demonstrated that the binding of complement component Clq to antibodies on hapten-sensitized lipid vesicles showed little or no dependence on the dynamic state of the membrane lipids. Since the fluidity of the membrane influences complement-mediated lysis (25, 26), it was concluded that membrane fluidity must influence the activation, but not the binding, of complement (27). Since in our studies we measure the binding of complement, we may conclude that changes in fluidity of surface lipids cannot explain the differences in complement fixation observed.

On the basis of available physical and chemical evidence, Kamat et al. (28) proposed that egg yolk VLDL has a liquid, neutral lipid nucleus with loose packing of hydrocarbon chains at the interface. The apolipoproteins are arranged on the periphery of the lipid core and in a predominantly unordered and anti-parallel β-conformation with a low α-helix content. Such a structure would result in a high degree of conformational flexibility and hence the lipid core could play a significant role in maintaining apolipoprotein conformation. The interfacial tension between the aqueous medium and a VLDL particle tends to maintain a spherical shape. If the apolipoprotein in native VLDL conforms closely to the curvature of the lipid core, the steric alignment of some antigenic determinants on the apolipoprotein could be significantly different in VLDL with different diameters. Thus, the shortening of the radius of curvature of the apolipoprotein could result in the weakening of the antibody bond for a few antigenic determinants. Since complement fixation depends on the strength of the antigen-antibody union, a better steric fit may exist between a large VLDL particle and its antibody than between a small VLDL particle and its antibody. Brület and McConnell (29) have suggested that the major source of difference in antibody binding arises from differences in steric constraints that effect the accessibility of haptens or antigenic determinants to antibody binding sites. Such an hypothesis is consistent with our observations that a vertical shift occurs in the complement fixation curves in proceeding from large to small VLDL particles, and that anti-LDL serum fixed a greater percent complement when reacted with VLDL (94%) than with LDL (55%).

It is generally agreed that vertical shifts obtained in complement fixation studies with soluble proteins suggest that the antigen–antibody union has been weakened by an alteration of only a few antigenic determinants. However, the applicability of this interpretation to studies with lipoprotein particles has yet to be established. Therefore, in the case of lipoproteins, another possible explanation for a vertical shift merits consideration. Complement fixation requires two immunoglobulin G molecules bound to adjacent antigens in addition to a strong antigen–antibody union. Conceivably, the shortening of the radius of curvature of VLDL could turn the two bound and apposed immunoglobulin G molecules away from each other. Thus, the spatial relationship of these molecules bound to the surface of the smaller VLDL particles may be altered such that the accessibility of their binding sites to complement component Clq may be impaired. Such a change in the

geometry of the antibodies might occur whether or not changes in the conformation of the apolipoproteins have occurred.

Some insights into the influence of VLDL particle size on complement fixation can be derived from data on the pattern of chicken serum lipoproteins (11, 30) and from studies on the action of lipoprotein lipase (31–33). LDL with particle diameters of 200–250 Å are the only low density lipoproteins present in significant amounts in the serum of cockerels and immature hens, and LDL particles with diameters less than 200 Å are not found in the circulation of these same animals (11, 30). The loss of activators for lipoprotein lipase from VLDL during lipolysis has been proposed to be responsible for the slow rate of removal of LDL from the circulation (31). In humans, a specific apolipoprotein which is a lipoprotein lipase activator is transferred to HDL during lipolysis (30). A similar lipoprotein lipase activator has been observed in chicken VLDL (32).

Another reason for the lack of LDL particles below 200 Å in the circulation was offered by Higgins and Fielding (33) on the basis of their experiments with rat lipoprotein lipase catalyzed hydrolysis of VLDL triglycerides. These workers observed that although the apparent Michaelis constant was about the same for various size VLDL remnant particles, the catalytic constant for the smaller sizes was reduced significantly. The lack of change in the Michaelis constant suggests that the affinity of lipoprotein lipase for VLDL is not effected by particle size. Higgins and Fielding (33) reported that a correlation exists between the changes in the catalytic constant and the triglyceride content. They suggested that this relationship may be another factor responsible for low lipoprotein lipase activity with LDL particles. We believe, on the basis of data presented here, that slight changes in apolipoprotein conformation on the particle surface, which are related to changes in triglyceride content, may provide a physical mechanism for the observed reduction in enzyme activity.

There are no changes in the zone of equivalence nor in the Michaelis constant for lipoprotein lipase for the various VLDL particle sizes. Therefore, the affinity of both antibody and lipoprotein lipase for VLDL is not different for various particle sizes even though the extent of complement fixation and lipolysis are affected. Accordingly, the steric fit of lipoprotein lipase with VLDL would be less precise with decreasing particle size with the result that the enzyme would be inactive with particles having diameters of less than approximately 200 Å.

We have calculated that 81% of the apolipoprotein content of VLDL would be removed from the surface during lipolysis if the largest serum TLDL particle (487 Å) was converted to the smallest (222 Å). In spite of this large loss, the apolipoprotein quantitated by complement fixation must be present in the same proportion in all VLDL fractions, since the peak of equivalence did not shift. Furthermore, if any lipoprotein lipase activator were present on the surface of any of these particles, it must either have been lost in direct proportion to the other apolipoproteins or have been present in such small quantities as to have no effect on the apolipoprotein ratio.

Our finding, that the delipidation of egg yolk VLDL results in a lateral shift in the complement fixation curve, and hence a severe distortion of all antigenic determinants on the apolipoprotein, is consistent with the structure proposed for low density lipoproteins (2, 5). The chicken HDL apolipoproteins have a high α-helix content which is only reduced by 20–30% upon delipidation (34). We have observed that delipidation of HDL does not result in a general conformational change which implies that lipids do not play an essential role in determining the conformation of the HDL apolipoproteins. Nevertheless, the loss of part of the α-helix content by delipidation suggests that lipids do exercise some degree of conformational restraint. The positive vertical shift we observed after delipidation of HDL cannot be explained by the unmasking of additional antigenic sites since this would result in the shift of the zone of equivalence to a lower antigen concentration, which did not occur. A possible mechanism for this unusual positive vertical shift may be that the antigen-antibody union becomes stronger after delipidation, due to a greater degree of freedom of the apolipoprotein in the absence of lipids.

The experiments in which Ey-VLDL and apoHDL were carbamylated show that it is possible to form derivatives which do not interfere with this very sensitive immunological procedure. Quantitation of conformational changes resulting from selective delipidations of lipoproteins, and other physical and chemical procedures, should also be feasible with this technique. This study thus demonstrates that quantitative microcomplement fixation can provide useful information regarding the interrelationships of apolipoproteins and lipids in intact lipoproteins.

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