

RABBIT AND RAT C-REACTIVE PROTEINS BIND APOLIPOPROTEIN B-CONTAINING LIPOPROTEINS

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C-reactive protein (CRP)¹ is the classical acute phase plasma protein (1-3). Homologous proteins are present in the serum of all vertebrates studied (4, 5), and also in an invertebrate, *Limulus polyphemus*, the horseshoe crab (6, 7). CRP molecules from all these species share the property of calcium-dependent binding to phosphoryl choline and phosphoryl choline-containing substances (5, 8-10). However, the physiological functions and possible pathophysiological effects of CRP are not known.

We have recently reported that while human CRP in acute phase serum exists in a free state (11, 12), not significantly complexed with any potential ligands, CRP molecules that have been aggregated in vitro acquire the capacity to selectively bind to plasma low density (LDL) and very low density (VLDL) lipoproteins (12). Other workers have reported in contrast that rabbit CRP normally exists in acute phase serum as a complex with either LDL (13) or VLDL (14). In addition a so-called "novel phosphoryl choline-binding protein" that interacts selectively with rat VLDL has been described in rat serum (15). This protein is indistinguishable, immunochemically and in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (PAGE), from the protein we isolated and designated as rat CRP (16) and that has now been shown to have 79% strict residue for residue identity of amino acid sequence with human CRP at the amino terminus (17).

In view of the potential physiological importance of interactions between CRP and apolipoprotein B (apoB)-containing lipoproteins, we have undertaken in the

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¹ *Abbreviations used in this paper:* apoB, apolipoprotein B; BSA, bovine serum albumin; CNBr, cyanogen bromide; CRP, C-reactive protein; HRS, hypercholesterolemic rabbit serum; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; NRS, normal rabbit serum; PAGE, polyacrylamide gel electrophoresis; Tris-saline-Ca, 0.01 M Tris-buffered 0.14 M NaCl pH 8.0 containing 0.002 M CaCl₂; Tris-saline-EDTA, 0.01 M Tris-buffered 0.14 M NaCl pH 8.0 containing 0.01 M EDTA; VLDL, very low density lipoprotein.

rabbit and the rat the experiments that we have previously described in man. We report here that rabbit and rat CRP aggregated in vitro selectively bind LDL and VLDL from autologous serum and also from human serum. Furthermore we confirm the observation that rabbit CRP may exist in plasma and serum as a complex with apoB-containing lipoproteins. We extend this work in showing that the presence and quantity of such complexes depend on the plasma concentration of apoB-containing lipoproteins. These results demonstrate that the binding of apoB-containing lipoproteins by CRP is a stably conserved property and is likely to have important implications both for the function of CRP itself and for the metabolism and/or in vivo handling of lipoproteins.

Materials and Methods

Animals and Serum. Fresh rabbit serum was from fasting or nonfasting New Zealand White or Half Lop rabbits fed on a normal diet, a semi-synthetic diet to which 1% cholesterol had been added (18), or a standard rabbit diet with 1% cholesterol added (Special Diet Services Ltd., Witham, Essex, England). Acute phase responses were stimulated by subcutaneous injection of 5 ml of 10% vitamin free casein (ICN Pharmaceuticals, Cleveland, OH) in 0.05 M sodium hydrogen carbonate on two consecutive days before the rabbits were bled on the third day. Fresh rat serum was from Wistar rats, and fresh normal human serum was from nonfasting laboratory volunteers.

Proteins. Pure rabbit CRP was isolated by calcium-dependent affinity chromatography on pneumococcal C-polysaccharide coupled to cyanogen bromide (CNBr)-activated Sepharose, followed by gel filtration on Sephacryl S300 (Pharmacia [G.B.] Ltd, Hounslow, Middlesex, England) as described elsewhere for human CRP (19). Rat CRP was isolated as previously described (16). Isolated, pure rabbit apoB was kindly provided by Dr. E. Gherardi (Department of Pathology, University of Cambridge, Cambridge, England).

Lipoproteins. VLDL ($d < 1.006$ g/ml) and LDL ($d = 1.019-1.063$ g/ml) were isolated from fresh serum or plasma by sequential ultracentrifugation in solutions of potassium bromide (KBr) (20). Each lipoprotein fraction was washed once by recentrifugation at the higher density limit, and then dialyzed against 0.01 M Tris-buffered 0.14 M NaCl, pH 8.0 containing 0.002 M CaCl_2 (Tris-saline-Ca) to remove the KBr.

Hypercholesterolemic rabbit sera were fractionated by density gradient ultracentrifugation to yield LDL, VLDL, and intermediate density lipoprotein (IDL) essentially as described by Chung et al. (12, 21). Lipoprotein analysis of eluates from CRP-Sepharose columns and of corresponding starting sera were performed under similar conditions (12, 21). The undenatured isolated rabbit lipoprotein fractions gave a single band in 4–30% gradient PAGE and gave a single line in immunodiffusion against anti-whole rabbit serum. No lipoprotein fraction contained detectable CRP.

Antisera. Monospecific sheep anti-rabbit CRP and anti-rabbit apoB sera were raised by immunization with the pure proteins. Goat anti-whole rabbit serum was from Miles Scientific, Slough, England.

Protein Assays. Proteins were approximately quantitated by A_{280} and solutions were checked for light scattering at 320 nm. Specific proteins eluted from CRP-Sepharose columns were identified by immunoelectrophoresis in 1% wt/vol agarose gel (Sea-Kem ME, FMC Corporation, Rockland, ME) in 0.075 M veronal buffer pH 8.6 containing 0.01 M EDTA. The concentration of rabbit CRP was quantitated by single radial immunodiffusion, isolated, pure CRP being used for calibration. Rabbit apoB concentration was measured by electroimmunoassay, using either samples of the starting material or one hyperlipidemic rabbit serum arbitrarily designated as 100 U/l, as calibration standards. Immunoelectrophoretic analysis of CRP was performed in 1% wt/vol agarose gel (Sea-Kem ME) in 0.075 M veronal buffer, pH 8.6 containing either 0.01 M EDTA or 0.002 M calcium lactate.

Lipid Composition. Triglycerides were determined using the kit from Dow Diagnostics (Uniscience Ltd., Cambridge, England). Total and free cholesterol was assayed as de-

scribed (22).

PAGE. Gradient PAGE analysis of native proteins was performed in 4–30% gradient gels (Pharmacia Fine Chemicals, Milton Keynes, England) run according to the manufacturer's instructions. Standard globular proteins (Pharmacia) used as markers were: thyroglobulin (699,000), ferritin (440,000), catalase (232,000), lactate dehydrogenase (140,000), and albumin (67,000).

Agarose Gel Electrophoresis. Lipoprotein electrophoresis was performed by standard techniques (23).

Immobilized Proteins. Isolated rabbit and rat CRP were coupled to CNBr-activated Sepharose beads (Pharmacia) at 5 mg protein per 1 ml beads according to the manufacturer's instructions. After coupling, the CRP-Sepharose was washed extensively with 0.01 M Tris-buffered 1.14 M NaCl, pH 8.0 containing 10 mM EDTA to remove noncovalently associated material. The recommended alternate washes at pH 4.0 and pH 8.0 were avoided to minimize damage to the coupled CRP. Control columns of bovine serum albumin (BSA)-Sepharose were prepared following the manufacturer's instructions, coupling 10 mg BSA (Sigma Chemical Co. Ltd., Poole, Dorset, England) per 1 ml Sepharose.

Affinity Chromatography Experiments. CRP-Sepharose columns of 1 ml volume were equilibrated in Tris-saline-Ca. 10 ml fresh serum were passed slowly over the columns at 21°C and were followed with Tris-saline-Ca until no further material absorbing at 280 nm was eluted. Bound protein was then eluted with 0.01 M Tris-buffered 0.14 M, NaCl pH 8.0 containing 0.01 M EDTA (Tris-saline-EDTA). The capacity of immobilized rabbit CRP to bind apoB-containing lipoproteins was determined as follows: 200- μ l aliquots of rabbit CRP-Sepharose beads (bearing 250 μ g of CRP) suspended 1:4 in Tris-saline-Ca were rotated for 2 h at 21°C with different quantities of hypercholesterolemic rabbit serum. Tris-saline-Ca was added as necessary to make a total volume of 1.7 ml. The beads were then washed with Tris-saline-Ca until there was no further material absorbing at 280 nm in the supernatant. 100 μ l Tris-saline-EDTA was added to each aliquot of centrifuged beads and the eluates were assayed for apoB.

Ultracentrifugation Experiments. Ultracentrifugation in linear 10–40% wt/vol sucrose gradients was performed as previously described (12).

Gel Filtration Experiments. Samples (0.7 ml) of acute phase rabbit serum were loaded on a 1 \times 58 cm column of Sephacryl S300, equilibrated and eluted at 10 ml/h with either Tris-saline-Ca or Tris-saline-EDTA. The column was calibrated with standard globular proteins all known molecular weight (Pharmacia): bovine thyroglobulin, 669,000; bovine liver catalase, 232,000; rabbit muscle aldolase, 158,000; bovine serum albumin, 67,000; bovine pancreatic chymotrypsinogen A, 25,000; and bovine pancreatic ribonuclease A, 13,700; and also with isolated rabbit CRP in Tris-saline-Ca and Tris-saline-EDTA.

Results

Calcium-dependent Binding of Serum Proteins by CRP-Sepharose. Fresh rabbit serum was passed over columns of rabbit CRP-Sepharose. After washing with Tris-saline-Ca the columns were eluted with Tris-saline-EDTA. Control experiments were performed by passing the same quantity of serum over BSA-Sepharose columns under identical conditions. Significant amounts of material absorbing at 280 nm and containing cholesterol were always eluted from the CRP columns, the yield being greater after offering hypercholesterolemic serum (Table I). Similar experiments were performed offering fresh rat serum to rat CRP-Sepharose and control columns, and normal human serum to rabbit CRP-Sepharose, rat CRP-Sepharose, and control columns. In all cases greater quantities of protein were eluted from CRP-Sepharose columns compared with control columns.

Characterization by Gradient PAGE of the Serum Proteins Bound by CRP-Sepharose. The material eluted with EDTA after passing serum over CRP-Sepharose

TABLE I
Calcium-dependent Binding of Rabbit Serum Proteins by Rabbit CRP-Sepharose

Serum offered	Lipid and apoB content of serum offered			Absorbance and cholesterol content of EDTA eluate:				
	Cholesterol mg/ml	Triglyceride mg/ml	ApoB U/l	CRP-Sepharose		Cholesterol free/total mg/ml	BSA-Sepharose	
				A ₂₈₀	A ₃₂₀		A ₂₈₀	A ₃₂₀
Normal serum	0.18	0.64	1.8	0.18	0.01	—/0.12	0.04	0.00
Hypercholesterolemic serum (nonfasted)	6.75	1.67	115	1.00	0.33	2.91/8.20	0.05	0.01
Hypercholesterolemic serum (fasted)	6.80	3.36	100	0.80	0.28	3.02/4.28	0.04	0.01

10 ml of each serum was offered to 1 ml of Sepharose bearing either 5 mg rabbit CRP or 10 mg BSA. After extensive washing with Tris-saline-Ca, bound protein was eluted with Tris-saline-EDTA. Measurements above were made on the second 1 ml fraction eluted which contained the peak of eluted protein.

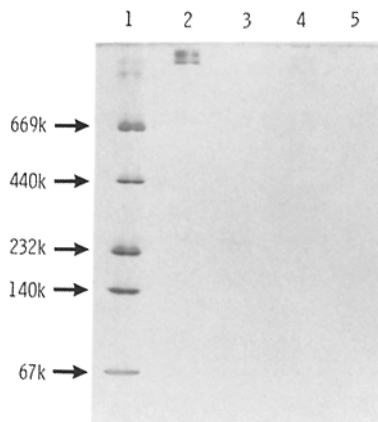


FIGURE 1. 4–30% gradient PAGE of undenatured proteins. Marker proteins of known molecular weight, track 1; eluate from rabbit CRP-Sepharose after passage of hypercholesterolemic rabbit serum (HRS), track 2, or normal rabbit serum (NRS), track 4; eluate from BSA-Sepharose after passage of HRS, track 3, or NRS, track 5. *k*, $\times 10^3$.

or control columns was analyzed in gradient PAGE, without denaturation. The eluate from rabbit CRP columns after passing normal or hypercholesterolemic rabbit serum yielded a protein band that trailed from the origin and corresponded to an apparent molecular weight of 2×10^6 or more. This band appeared to be almost identical in eluates from CRP-Sepharose columns following the passage of fasted or nonfasted hypercholesterolemic serum, but was much fainter after passing normal rabbit serum over the columns. No protein bands could be detected in the eluates from control columns (Fig. 1). The EDTA eluate from rat CRP-Sepharose after passage of fresh rat serum and the eluates from rat CRP-Sepharose and rabbit CRP-Sepharose after passage of fresh normal human serum yielded a similar band of high apparent molecular weight.

Identification of Material Selectively Bound by CRP-Sepharose as Lipoprotein. When

proteins eluted from rabbit CRP-Sepharose after passage of normal rabbit serum were subjected to electrophoresis in agarose gel and then stained with Sudan Black, a faint band corresponding in mobility to normal serum β -lipoprotein was revealed (Fig. 2). Proteins eluted from rabbit CRP-Sepharose after passage of hypercholesterolemic serum from either fasted or nonfasted animals migrated in a broader band of β -mobility corresponding to the β -lipoproteins in the starting sera. Traces of material with pre- β mobility were also present (Fig. 2). Immuno-electrophoretic analysis of these same eluates using either anti-whole rabbit serum or anti-apoB serum gave a single arc of precipitation. This was in the β -region and corresponded in position to the arc produced by apoB-containing material in normal rabbit serum and to the faster portion of the arc produced by hypercholesterolemic serum (Fig. 3).

Characterization of the Material Selectively Bound by CRP-Sepharose as LDL and VLDL. The material selectively bound by rabbit CRP-Sepharose was subjected to density gradient ultracentrifugation and was compared with the behavior of lipoprotein in the starting sera. Insufficient material was recovered from normal rabbit serum to be analyzed in this way. The eluates after passage of hypercholesterolemic serum from either fasted or nonfasted rabbits gave two peaks of material, absorbing at 280 nm and containing cholesterol and apoB, which corresponded to the densities of normal VLDL and LDL. There was also some

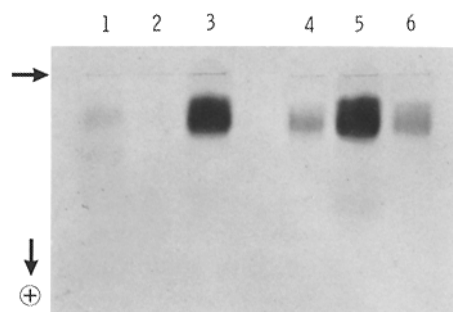


FIGURE 2. Lipoprotein electrophoresis of eluates from rabbit CRP-Sepharose, Sudan Black stain. Track 1, NRS; track 3, HRS (nonfasted); track 5, HRS (fasted); track 2, eluate after passage of NRS; track 4, eluate after passage of HRS (nonfasted); track 6, eluate after passage of HRS (fasted).

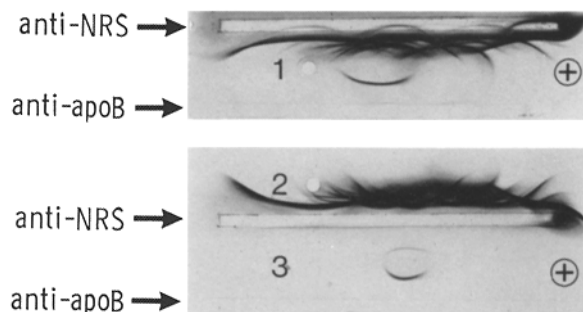


FIGURE 3. Immuno-electrophoresis of eluate from rabbit CRP-Sepharose. Wells 1, 2, HRS (nonfasted); well 3, eluate after passage of HRS (nonfasted).

material between these peaks floating at intermediate density (Fig. 4).

Quantitation of Lipoprotein Binding by Rabbit CRP-Sepharose. The capacity of the rabbit CRP-Sepharose beads to bind lipoprotein was quantitated by offering increasing quantities of hyperlipidemic serum to a known volume of beads (Table II), and measuring the apoB content of the material bound and subsequently eluted with Tris-saline-EDTA. The binding capacity of 250 μ g CRP could be saturated by the apoB-containing lipoproteins in 100 μ l of hypercholesterolemic serum (cholesterol 6.4 mg/ml, triglyceride 3.15 mg/ml).

Gel Filtration of Acute Phase Rabbit Serum. When acute phase rabbit sera were subjected to gel filtration chromatography on columns of Sephacryl S300 eluted with either Tris-saline-EDTA or Tris-saline-Ca containing 10 mM phosphoryl choline, the CRP always eluted in the same volume as did isolated rabbit CRP run on the same columns. However, when the same sera were chromatographed with Tris-saline-Ca as the eluant a proportion of the CRP in some cases eluted with a much greater apparent molecular weight in the void volume of the column, peaking in the same fractions that contained the peak of apoB. The presence and quantity of these "heavier" species of CRP were directly related to the apoB content of the starting serum; thus they were either absent or detected in only trace amounts in most sera from animals fed on a normal diet (Fig. 5).

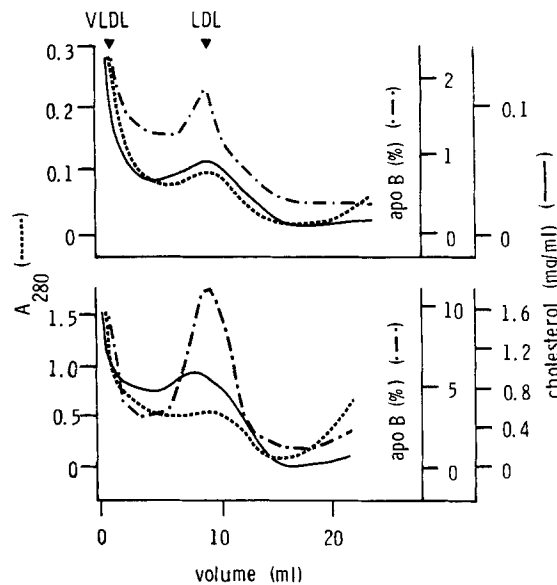


FIGURE 4. Density gradient ultracentrifugation of eluate from rabbit CRP-Sepharose after passage of HRS (nonfasted) (above), and whole HRS (nonfasted) (below).

TABLE II
Quantitation of Lipoprotein Binding by Rabbit CRP-Sepharose

Volume of serum* (μ l)	0	5	10	20	50	100	200	400
ApoB bound (% of starting serum)	0	0.6	1.3	2.6	4.0	5.9	5.8	5.6

* Hypercholesterolemic rabbit serum, cholesterol 6.4 mg/ml; triglyceride 3.15 mg/ml.

All sera from animals fed on a cholesterol-rich diet contained raised apoB levels and in all cases the CRP eluted entirely in the void volume (Fig. 5).

Isolated rabbit CRP eluted from gel filtration columns in the same fractions in the presence of calcium or EDTA indicating that the different behavior of CRP in acute phase serum eluted with these different buffer systems was not due to alteration in the behavior of the CRP per se. Addition to normolipemic acute phase rabbit serum, before gel filtration, of isolated rabbit VLDL from normal rabbit serum caused all the CRP to elute in the void volume. This suggests that fluid phase interaction between CRP and plasma lipoproteins particles may be responsible for the altered chromatographic behavior of CRP in hyperlipidemic acute phase serum.

It was notable that the recovery of CRP after gel filtration in the presence of calcium ions was always less than that after elution in EDTA. ~50% of the CRP

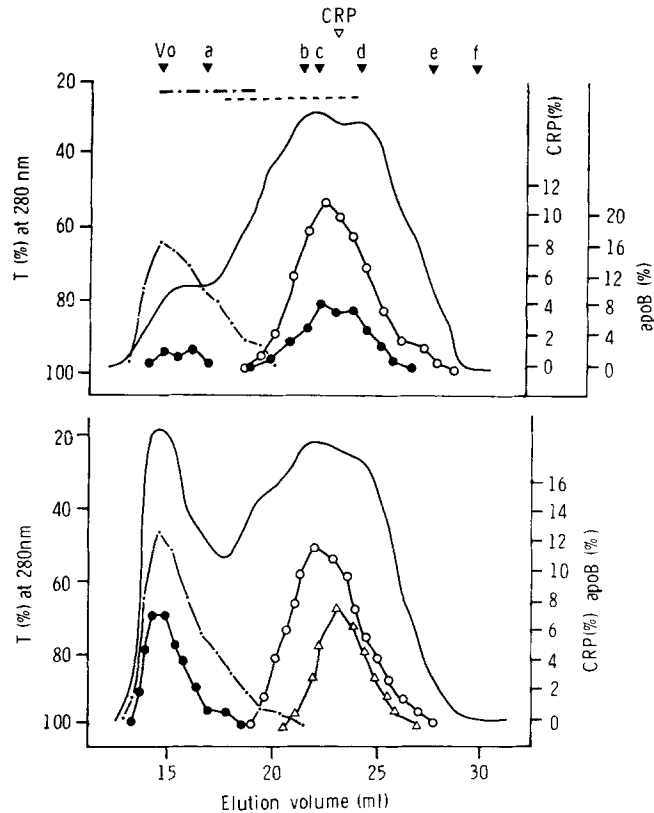


FIGURE 5. Gel filtration of acute phase rabbit serum. Above, sample from rabbit on normal diet (CRP 230 mg/l; cholesterol 0.26 mg/ml; triglyceride 0.46 mg/ml; apoB 1.3 U/l); below, sample from same animal after induction of hypercholesterolemia (CRP 136 mg/l; cholesterol 6.4 mg/ml; triglyceride 2.93 mg/ml; apoB 90 U/l). —, transmittance (%) of the effluent at 280 nm; elution volumes for marker proteins: a, 669,000; b, 232,000; c, 158,000; d, 67,000; e, 25,000; f, 13,700, and isolated pure CRP were the same in all eluants. - - - -, fractions containing IgM, and ——— IgG; ●, elution profile of CRP in Tris-saline-Ca; ○ in Tris-saline-EDTA; and △ in Tris-saline-Ca containing 10 mM phosphoryl choline; ····· elution profile of apoB was identical in all eluants. CRP and apoB concentrations expressed as percentage of values in starting serum.

bound to the chromatographic medium in a calcium-dependent interaction, and was quantitatively recovered by subsequent washing with EDTA.

Ultracentrifugation of Acute Phase Rabbit Serum. When hyperlipidemic acute phase rabbit serum was ultracentrifuged in sucrose density gradients in the presence of EDTA, the CRP migrated to the same place as isolated CRP and was not detected in any of the fractions at the top of the gradient that contained all the apoB (Fig. 6). Two hyperlipidemic acute phase sera were centrifuged in the presence of calcium. In one, almost all the CRP floated at the top of the gradient in the same fractions as the apoB (Fig. 6). In the other, however, the CRP still sedimented to the position of free CRP and this was a reproducible observation with that particular serum, despite the fact that it yielded "heavy" CRP on gel filtration.

Electrophoretic Mobility of CRP in Acute Phase Rabbit Serum. Isolated rabbit CRP showed slow γ -mobility when electrophoresed in agarose in the presence of calcium, whereas in EDTA it had fast γ -mobility (Fig. 7). This difference probably reflects the known binding of calcium by CRP and the weak calcium-dependent binding of CRP to agarose. CRP in most samples of normolipidemic acute phase rabbit serum had the same mobility in the presence and absence of calcium as did isolated CRP (Fig. 7) and this was quite distinct from the β -mobility of apoB-containing lipoproteins. Furthermore, the immunoelectrophoretic precipitation arc with anti-CRP serum did not stain with Sudan Black. However, in some samples of apparently normolipidemic acute phase serum there was an anodal extension of the CRP precipitation arc and this extension stained with Sudan Black (Fig. 8). In all samples of hyperlipidemic acute phase serum from cholesterol-fed rabbits, some or all of the CRP migrated in the presence of calcium to a β -position and this fast-migrating material stained with Sudan Black (Fig. 8). Identical results were obtained using fresh heparinized acute phase plasma from either normolipemic or hyperlipemic rabbits. These results indicate that in the presence of increased amounts of apoB-containing lipoproteins some or all of

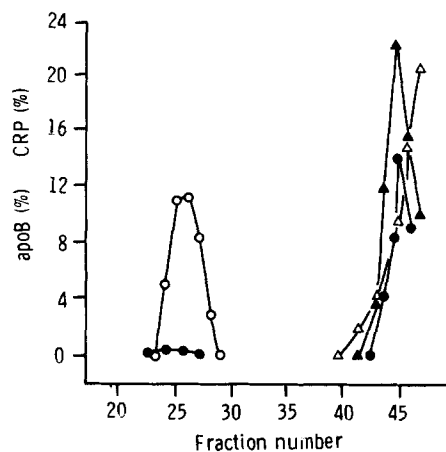


FIGURE 6. Sucrose density gradient ultracentrifugation of acute phase hyperlipidemic rabbit serum. CRP (●) and apoB (▲) in calcium; CRP (○) and apoB (△) in EDTA-containing gradients, as percentage of value in starting serum.

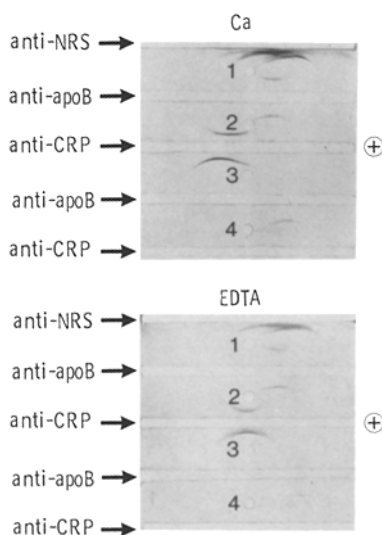


FIGURE 7. Immunoelectrophoresis of NRS (well 1), normolipemic acute phase rabbit serum (well 2), pure rabbit CRP (well 3) and pure rabbit VLDL (well 4) in calcium and EDTA-containing gels.

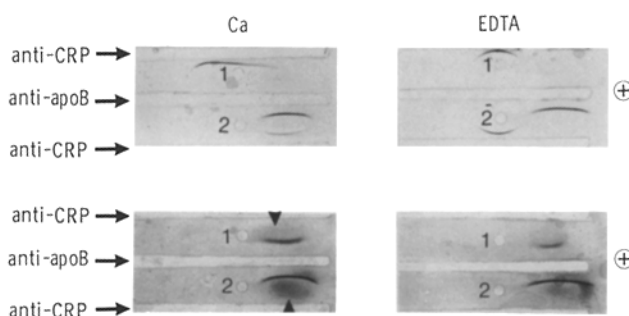


FIGURE 8. Immunoelectrophoresis of acute phase NRS (well 1) and acute phase HRS (well 2) in calcium and EDTA. Coomassie Blue stains (above), Sudan Black (below). Arrows, anti-CRP precipitation arcs staining with Sudan Black.

the CRP exists in a complex with lipoprotein. Addition to normolipemic acute phase serum of pure, isolated VLDL or LDL from normal or acute phase serum, or VLDL, LDL, or IDL from hypercholesterolemic serum, caused an anodal shift in the electrophoretic mobility of some or all of the CRP in calcium-but not in EDTA-containing gels. The magnitude of this effect was clearly dependent on the quantity of VLDL that was added (Fig. 9). LDL had a similar effect. Addition of isolated VLDL in Tris-saline-Ca to pure isolated CRP in the same buffer also caused an anodal shift in the mobility of CRP.

Discussion

We show here that aggregated rabbit CRP and rat CRP both selectively bind apoB-containing lipoproteins *in vitro* from autologous serum and also from human serum. Their behavior thus corresponds exactly with that of human CRP

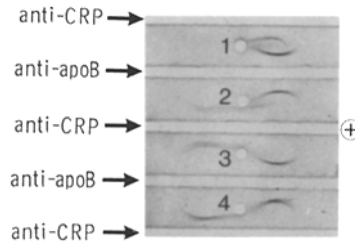


FIGURE 9. Effect of VLDL on electrophoretic mobility of rabbit CRP. Acute phase normo-lipemic rabbit serum mixed before electrophoresis with isolated VLDL containing apoB 15 U/l (well 1), 3.7 U/l (well 2), 0.9 U/l (well 3); Tris-saline-Ca control (well 4).

(12), suggesting that the capacity of CRP molecules, under appropriate circumstances, to recognize and interact with particular classes of lipoprotein particles may be a conserved and possibly fundamental function of CRP in all species. We also find that fluid phase rabbit CRP forms complexes with apoB-containing lipoproteins without the need for prior aggregation and the complexing depends on the concentration of these lipoproteins. The serum lipoprotein concentration in animals on a normal diet is usually too low to permit formation of detectable amounts of complexed CRP, but sera from hypercholesterolemic rabbits contain most or all of the CRP in a complexed form. Furthermore, such complexes can be generated in serum by *in vitro* addition of isolated apoB-containing lipoproteins of low, intermediate, or very low density, or merely by mixing pure CRP with isolated lipoprotein preparations.

These observations with rabbit CRP are significantly different from our experience with human CRP (12), complexes of which with lipoprotein are not demonstrable in normal serum or acute phase serum, despite a normal concentration in man of apoB-containing lipoproteins that is well into the range that promotes formation of complexes of CRP with lipoproteins in rabbit serum. This different behavior may reflect known minor differences in the fine binding specificity of rabbit and human CRP (9) and/or differences in the quality or quantity of the presumed phospholipid ligand(s) in the apoB-containing lipoproteins of the two species. Experiments to elucidate this point are in progress.

Although it is possible to generate fluid phase complexes of rabbit CRP *in vitro* by addition to acute phase serum of rabbit VLDL, LDL, or IDL, the great bulk of the plasma lipoprotein in hypercholesterolemic animals is an abnormal lipoprotein known as β -VLDL (24). The CRP-lipoprotein complexes that form *in vivo* in the circulation therefore, presumably contain mainly this lipoprotein class. However, further work is required to isolate the rabbit CRP-lipoprotein complexes formed in the circulation *in vivo*, to separate them from noncomplexed lipoproteins, and then to characterize precisely the complexed lipoproteins. In this respect it is worth noting that the apparent preponderance of LDL binding by both rabbit and human CRP when aggregated and immobilized on Sepharose 4B beads may well reflect the fact that the size of LDL particles permits them to gain access to most of the bead volume. In contrast, the much bigger VLDL particles are largely excluded from Sepharose 4B and are therefore not available to be bound by the aggregated CRP in this *in vitro* model system.

There is great variation between species in both the normal levels and behavior

of CRP as an acute phase reactant. For example, human and rabbit CRP are normally trace proteins (<1 mg/l) and may rise to a peak of 400 mg/l (3), while the serum of healthy rats contains 300–500 mg/l, rising to 1–2 g/l in an acute phase response (16). These differences may be related in part to the fact that rat CRP is glycosylated (16), while human (25) and rabbit CRP are not. Notwithstanding this fact, the very stable phylogenetic conservation of amino acid sequence, subunit composition, and particularly binding specificity among these proteins (5) indicate that the role of CRP is likely to be similar in all animals. Indeed, identical functions of a plasma protein may be subserved by either high or low circulating levels, provided the synthesis rate of the protein is sufficient to encompass its functional consumption.

The present results suggest that the function of CRP in all species may be related to its capacity to interact with lipoproteins and this may have important implications both for its normal physiology and for its pathophysiology in relation particularly to atherosclerosis.

Summary

Immobilized rabbit and rat C-reactive protein (CRP) were found to selectively bind apolipoprotein B (apoB)-containing lipoproteins (low density lipoprotein, LDL and very low density lipoprotein, VLDL) from whole serum in a manner similar to that previously reported with human CRP. In acute phase human serum the CRP is in a free form, not complexed with lipoprotein or any other macromolecular ligand, and in acute phase serum from most rabbits fed on a normal diet the rabbit CRP was also free. However, in acute phase serum or heparinized plasma from hypercholesterolemic rabbits part or all of the CRP was found by gel filtration and immunoelectrophoretic techniques to be complexed with β -VLDL, an abnormal apoB-containing plasma lipoprotein present in these animals. The presence of extent in different serum samples of CRP complexed with lipoprotein correlated closely with the serum apoB concentration. The formation of complexes between native, unaggregated rabbit CRP in solution and apoB-containing lipoproteins was readily demonstrable experimentally both with the isolated proteins and in whole serum. In all cases these interactions were calcium-dependent and inhibitable by free phosphoryl choline.

The present findings extend earlier work in man and the rabbit and indicate that among the C-reactive proteins from different species, which are structurally highly conserved, the capacity for selective binding to apoB-containing plasma lipoproteins is also a constant feature. These interactions may therefore be related to the *in vivo* function of CRP in all species and this function may in turn be relevant to pathological conditions, such as atherosclerosis, in which lipoproteins are important.

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616 LIPOPROTEINS BOUND BY RABBIT AND RAT C-REACTIVE PROTEIN

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