Published in final edited form as:

Clin Chim Acta. 2008 August ; 394(1-2): 94–98.

Phosphoethanolamine-complexed C-reactive protein: A pharmacological-like macromolecule that binds to native low-density lipoprotein in human serum

Sanjay K. Singh^a, Madathilparambil V. Suresh^a, Deborah C. Prayther^b, Jonathan P. Moorman^b, Antonio E. Rusiñol^c, and Alok Agrawal^{a,*}

^a Department of Pharmacology, James H. Quillen College of Medicine, East Tennessee State University, Johnson City, TN, USA

^b Department of Internal Medicine, James H. Quillen College of Medicine, East Tennessee State University, Johnson City, TN, USA

^c Department of Biochemistry and Molecular Biology, James H. Quillen College of Medicine, East Tennessee State University, Johnson City, TN, USA

Abstract

Background—C-reactive protein (CRP) is an acute phase plasma protein. An important binding specificity of CRP is for the modified forms of low-density lipoprotein (LDL) in which the phosphocholine-binding sites of CRP participate. CRP, however, does not bind to native LDL.

Methods—We investigated the interaction of CRP with native LDL using sucrose density gradient ultracentrifugation.

Results—We found that the blocking of the phosphocholine-binding sites of CRP with phosphoethanolamine (PEt) converted CRP into a potent molecule for binding to native LDL. In the presence of PEt, CRP acquired the ability to bind to fluid-phase purified native LDL. Because purified native LDL may undergo subtle modifications, we also used whole human serum as the source of native LDL. In the presence of PEt, CRP bound to native LDL in serum also. The effect of PEt on CRP was selective for LDL because PEt-complexed CRP did not bind to high-density lipoprotein in the serum.

Conclusions—The pharmacologic intervention of endogenous CRP by PEt-based compounds, or the use of exogenously prepared CRP-PEt complexes, may turn out to be an effective approach to capture native LDL cholesterol in vivo to prevent the development of atherosclerosis.

Keywords

C-reactive protein; low-density lipoprotein; cholesterol; phosphocholine; phosphoethanolamine

* Corresponding author: Department of Pharmacology, P.O. Box 70577, East Tennessee State University, Johnson City, TN 37614, USA. Tel.: 1 4234396336; fax: 1 4234398773; E-mail address: agrawal@etsu.edu (A. Agrawal).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

This PDF receipt will only be used as the basis for generating PubMed Central (PMC) documents. PMC documents will be made available for review after conversion (approx. 2–3 weeks time). Any corrections that need to be made will be done at that time. No materials will be released to PMC without the approval of an author. Only the PMC documents will appear on PubMed Central -- this PDF Receipt will not appear on PubMed Central.

1. Introduction

C-reactive protein (CRP) is an acute phase protein whose production by the liver is increased in inflammatory conditions [reviewed in ^{1–3}]. Structurally, CRP is a pentameric protein composed of 5 identical subunits [⁴]. The major binding specificity of CRP is for phosphocholine (PCh)-containing substances, such as pneumococcal C-polysaccharide and damaged cells of the myocardial infarcts [^{5–10}]. There are 5 PCh-binding sites in CRP, one located on each subunit [^{4, 11}]. The PCh-binding sites of CRP are also involved in the binding of CRP to non-PCh ligands such as phosphoethanolamine (PEt) [^{6, 12–14}]. Calcium ions are required for these binding reactions of CRP

In vitro, native pentameric CRP has been shown to bind to atherogenic modified forms of low-density lipoprotein (LDL), such as oxidized LDL and enzymatically-modified LDL (E-LDL), in a Ca^{2+} -dependent and reversible manner. The PCh-binding sites of CRP participate in binding of CRP to modified forms of LDL [^{15–20}]. Consistent with the ability of CRP to interact with modified forms of LDL, CRP has been seen deposited at the human atherosclerotic lesions [^{21–23}].

Although native pentameric CRP binds to modified forms of LDL, it does not bind to native LDL. Aggregated forms of native pentameric CRP, however, have been shown to bind to native LDL as well. Native CRP and native LDL interact with each other only when either one is immobilized or modified [^{24–26}]. We previously reported that the blocking of the PCh-binding sites of CRP with PEt enhanced the interaction between CRP and E-LDL [^{1, 27}]. In the current study, we explored the possibility that blocking of the PCh-binding sites of CRP with PEt might also have an effect on the interaction between CRP and native LDL. Surprisingly, we found that CRP, when complexed with PEt, acquired the ability to capture native LDL in whole human serum.

2. Materials and Methods

2.1. Purification of CRP

CRP was purified from pleural fluid as described earlier [⁷]. Purified CRP was stored in 10 mmol/l Tris-HCl, 150 mmol/l NaCl, pH 7.2 (TBS) at -80°C . After thawing, the stored CRP was not used as such in the experiments. Instead, CRP was centrifuged and subjected to gel filtration on a Superose 12 column (10/300 GL; GE Healthcare, Piscataway, NJ) using the Biologic Duo Flow Protein Purification System (Bio-Rad, Hercules, CA) to remove fragmented, denatured, monomeric, and aggregated CRP from the purified, stored, native pentameric CRP preparations. The column was eluted with TBS and the fractions containing pentameric CRP were collected, diluted in appropriate Ca^{2+} -containing buffer as described below, and then used in the assays the same day. After the re-purification step, we labeled our CRP as freshly-purified CRP.

2.2. Purification of native LDL

Native LDL was isolated from normal human plasma donated by healthy laboratory volunteers by sequential sedimentation on discontinuous density gradient centrifuged at 52,000 rpm ($184,000 \times g$) at 12°C in a Beckman T_i70 rotor, adjusting the density with solid potassium bromide, according to a published method [²⁸]. Floating LDL ($1.05 > d > 1.02 \text{ g/ml}$) was collected and washed by a second centrifugation under the same conditions at a density of 1.063 g/ml to ensure a preparation free of high-density lipoprotein (HDL). Next, LDL was dialyzed against $\text{Ca}^{2+}/\text{Mg}^{2+}$ -free phosphate buffer saline (Invitrogen, Carlsbad, CA) containing 1 mmol/l EDTA and was purified by gel filtration on a Superose 12 column. In a pilot experiment, we subjected this preparation of native LDL to sucrose density gradient

ultracentrifugation. We expected that all LDL would float on the top of the sucrose gradient. However, this was not the case. Much LDL settled at the bottom of the gradient, suggesting that native LDL was unstable and a major portion of it was damaged and, therefore, unsuitable for CRP-binding experiments. We, therefore, subjected all native LDL to sucrose density gradient ultracentrifugation, collected the floating LDL from the top, and then mixed with CRP immediately to perform CRP-binding sucrose flotation experiments as described below.

The additional purification of native LDL by sucrose density gradient ultracentrifugation was performed as follows [16]: 550 μ l of 80% sucrose in TBS containing 1.25 mg of gel filtration-purified native LDL was transferred to Beckman centrifuge tubes which were then overlaid with 2.2 ml of 25% sucrose and 165 μ l of 4% sucrose. The tubes were then centrifuged for 4 h at 90,000 rpm at 10°C in a Beckman TL100 ultracentrifuge, fixed angle rotor TLA100.3. After centrifugation the particles floating on the top of the tube were discarded. Then, the top 500 μ l of the clear solution was collected which contained native LDL. This preparation of native LDL was used immediately for CRP-binding sucrose flotation experiments. The concentration of protein in the native LDL preparations was measured using a protein assay kit (Bio-Rad).

2.3. CRP-binding sucrose flotation experiments

The binding of CRP to fluid-phase purified native LDL was assessed by sucrose flotation experiments [16]. Four reactions were set up: 1) 550 μ l of 80% sucrose in TBS containing 100 μ g LDL. 2) 550 μ l of 80% sucrose in TBS containing 400 μ g CRP. 3) 550 μ l of 80% sucrose in TBS containing 100 μ g LDL and 400 μ g CRP. 4) 550 μ l of 80% sucrose in TBS containing 100 μ g LDL, 400 μ g CRP, 50 mmol/l PEt (P0503, Sigma-Aldrich, St Louis, MO). In all reactions, the final concentration of CaCl_2 was 5 mmol/l. CRP, LDL, PEt and calcium were allowed to react at room temperature for 15 min, before addition of sucrose. These mixtures were then transferred to Beckman centrifuge tubes and overlaid with 2.2 ml of 25% sucrose and 165 μ l 4% sucrose. The tubes were centrifuged for 4 h at 90,000 rpm at 10°C in the Beckman ultracentrifuge. After centrifugation, the tubes were photographed using Sony Cyber-shot (DSC-P73) camera. Then, 6 fractions were collected starting from the top, and the absorbance at 280 nm was measured. Some fractions were subjected to 10% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions, Coomassie blue staining, and Western blot to visualize CRP and LDL. For Western blots, polyclonal anti-CRP (Sigma-Aldrich, St Louis, MO) and polyclonal anti-apolipoprotein B (Calbiochem, San Diego, CA) antibodies were used.

The binding of CRP to native LDL in normal whole human serum was determined by sucrose flotation experiments performed as follows, unless otherwise mentioned. Five reactions were set up: 1) 550 μ l of 80% sucrose in TBS containing 40 μ l serum, 200 μ g CRP and 5 mmol/l CaCl_2 . 2) 550 μ l of 80% sucrose in TBS containing 40 μ l serum, 200 μ g CRP, 50 mmol/l PEt and 5 mmol/l CaCl_2 . 3) 550 μ l of 80% sucrose in TBS containing 40 μ l serum, 200 μ g CRP, 50 mmol/l PEt and 10 mmol/l EDTA. 4) 550 μ l of 80% sucrose in TBS containing 40 μ l serum and 5 mmol/l CaCl_2 . 5) 550 μ l of 80% sucrose in TBS containing 200 μ g CRP and 5 mmol/l CaCl_2 . These reactions were subjected to centrifugation as described above. After centrifugation, the tubes were photographed, and then six fractions were collected starting from the top. The fractions were analyzed for cholesterol and CRP. The total cholesterol and HDL/LDL levels were determined by using cholesterol/cholesteryl ester quantification kit (Biovision, Mountain View, CA) and HDL and LDL/VLDL cholesterol quantification kit (Biovision), respectively. In these flotation experiments, CRP was used at high concentrations to generate visible and enough quantity of CRP-LDL complexes.

3. Results

3.1. In the presence of PEt, CRP acquired the ability to bind to purified native LDL in the fluid-phase

We used sucrose density gradient ultracentrifugation to investigate fluid-phase interaction between CRP and purified native LDL (Fig. 1). When native LDL alone was centrifuged (gradient A), some of the native LDL, as expected, remained floating at the top of the gradient while, for reasons unknown, some of the native LDL settled at the bottom of the gradient. When CRP alone was centrifuged (gradient B), CRP remained at the bottom of the gradient covering fractions 5 and 6. When mixed together, CRP did not bind to native LDL (compare the gradient in C with A and B). CRP remained in fractions 5 and 6 at the bottom of the gradient while LDL remained at the top of the gradient. Strikingly, in the presence of PEt (gradient in D), a protein-containing white band (indicated by arrow) was observed in fraction 3, reflecting the formation of a new complex whose density was different than that of both CRP and native LDL.

We analyzed fraction 3 collected from gradient D to investigate the composition of the new complex formed in the presence of PEt (Fig. 1E). Coomassie blue staining of the SDS-PAGE gel showed the presence of both CRP and LDL in this complex (lane 4). The presence of LDL in this complex was further confirmed by Western blot (lane 7). Thus, in the presence of PEt, fluid-phase CRP acquired the ability to bind to fluid-phase native LDL. Two different preparations of native LDL gave similar results.

3.2. In the presence of PEt, CRP acquired the ability to bind to native LDL in whole human serum

Because purified native LDL may be susceptible to subtle oxidation even during short-term storage, we used whole human serum as the source of native LDL and performed sucrose density gradient ultracentrifugation to demonstrate the binding of PEt-complexed CRP to native LDL (Fig. 2A). In the presence of PEt (reaction 2), a white band (indicated by arrow) was visible in fraction 5 indicating the formation of a new complex. This complex was not formed in the absence of PEt (reaction 1) or when CRP and serum were centrifuged separately (reactions 4 and 5). The formation of the complex in reaction 2 was Ca^{2+} -dependent because the complex was not formed in the presence of EDTA (reaction 3).

To determine whether the amount of CRP in fraction 5 was different than in fraction 6 in the presence of PEt (reaction 2) compared to that in the absence of PEt (reaction 1), we performed Western blot using anti-CRP antibodies on fractions 5 and 6 collected from all 5 types of reactions (Fig. 2B). As shown, CRP was present in both fractions 5 and 6 in all reactions. However, some CRP shifted from fraction 6 to fraction 5 in the presence of PEt, indicating the formation of a CRP-containing complex in fraction 5 of reaction 2. Since native LDL was supposed to float in fraction 1, we also performed Western blot on fraction 1 from all 5 reactions. It was not obvious, but CRP was also present in fraction 1 of reaction 2, indicating the formation of another species of CRP-containing complex. Thus, PEt facilitated the formation of two types of CRP-containing complexes: one that floated on the top of the gradient and another in fraction 5.

We estimated the total cholesterol content in fraction 5 collected from all 5 types of reactions (Fig. 2C). As shown, the concentration of total cholesterol in fraction 5 containing the CRP-containing complex (reaction 2) was 4-fold more than in fraction 5 from other reactions, indicating that, in the presence of PEt, CRP captured cholesterol from the serum.

Next, we investigated the effects of increasing amounts of CRP on the capture of cholesterol from the serum (Fig. 3). The intensity of the white band formed in reaction 2 increased with the increasing concentrations of CRP, indicating that the formation of the complex (white band

shown by arrows) containing both CRP and cholesterol was CRP-dose-dependent (Fig. 3A). The intensity of the white bands increased with the increasing amounts of serum also. In these complexes, the concentration of total cholesterol also increased with the increasing amounts of CRP and serum (Fig. 3B).

3.3. PEt-complexed CRP bound to LDL, but not to HDL, in the serum

To determine whether, in the presence of PEt, CRP was binding to LDL only, we measured the concentration of both LDL and HDL cholesterol in the complex present in fraction 5. There was no HDL in the complex indicating that the PEt-complexed CRP selectively captured native LDL cholesterol, but not HDL cholesterol, in the serum (Fig. 4). These results obtained from the experiments using whole serum also indicated that the binding of PEt-complexed CRP to purified native LDL, shown in Fig. 1, was not due to possible presence of any modified LDL in the native LDL preparations.

4. Discussion

In this study, we investigated the binding of CRP to native LDL. The storage of purified pentameric CRP causes dissociation of pentameric CRP into monomeric CRP [18]. Therefore, in all experiments, we used freshly-purified CRP, and not the stored CRP, to avoid contamination with monomeric, degraded, or aggregated CRP. Thus, the results reported in this paper apply to native pentameric CRP only. Our major findings were: 1. In the presence of PEt, CRP acquired the ability to bind to purified fluid-phase native LDL. 2. PEt-complexed CRP captured native LDL from whole human serum also. 3. In serum, the PEt-mediated binding of CRP to native LDL was selective for LDL because PEt-complexed CRP did not bind to HDL.

Our data indicate that modification of native LDL to expose its PCh groups is not required for binding of CRP to native LDL and that PEt-complexed CRP binds to native LDL through groups that are common in native LDL and modified forms of LDL, such as cholesterol [19]. It has been shown previously that only immobilized or aggregated CRP can bind to native LDL [24–26], suggesting that CRP needs to be stimulated to form aggregates to be able to bind to native LDL. We observed that the CRP-LDL complexes, formed in the presence of PEt, floated as a separate band in the middle of the sucrose gradients, indicating that PEt may be acting through stimulating aggregation of CRP. This interpretation is supported by the experiments in which PEt did not enhance the binding of immobilized, already aggregated, CRP to native LDL [26]. Regardless of the mechanism of action of PEt in the binding of CRP to serum LDL, our current data provide a possible explanation for a previously reported observation that lipids rich in phosphatidylethanolamine isolated from natural gas-utilizing bacteria reduced plasma cholesterol levels [29].

Although the experiments involving LDL-deficient serum and purified HDL remain to be performed, the ability of the CRP-PEt complexes to bind to fluid-phase native LDL is important for several reasons. First, statins, the cholesterol-lowering agents, reduce the risk of atherosclerosis; still many individuals receiving statins and with normal cholesterol levels develop atherosclerosis [30–33]. We speculate that the CRP-PEt complexes, after binding to native LDL, may cause lowering of the LDL levels because CRP-bound LDL would likely be catabolized. Second, the binding of CRP to native LDL trapped in the artery walls may prevent modification of LDL because, in the case of oxidized LDL, it has been shown that the binding of CRP to oxidized LDL prevents its further oxidation [34]. Finally, CRP may bind to native LDL trapped in the artery walls to prevent formation of macrophage foam cells because it has been shown that native LDL can also cause formation of foam cells [35]. In summary, it is likely that LDL cholesterol may not act as bad cholesterol if the LDL is present as CRP-PEt-LDL complexes in the circulation.

Recently, in *ApoB*^{100/100}*Ldlr*^{-/-} murine model of human-like atherosclerosis, CRP was found to be atheroprotective and the importance of CRP-LDL interaction in this protection was noted [36]. We have not yet started *in vivo* experiments using injections of CRP-PEt complexes into murine models of atherosclerosis but we hypothesize that the pharmacologic intervention of endogenous CRP by PEt-based compounds, or using the properties of exogenously prepared CRP-PEt complexes to capture native LDL, may turn out to be an effective approach to accelerate CRP-mediated prevention of the development of atherosclerosis.

Acknowledgements

This work was supported by a grant from the National Institutes of Health (R01HL071233 to A.A.).

Abbreviations

CRP	C-reactive protein
E-LDL	enzymatically-modified LDL
PCh	phosphocholine
PEt	phosphoethanolamine
TBS	10 mmol/l Tris-HCl, 150 mmol/l NaCl, pH 7.2

References

1. Singh SK, Suresh MV, Voleti B, Agrawal A. The connection between C-reactive protein and atherosclerosis. *Ann Med* 2008;40:110–20. [PubMed: 18293141]
2. Sjowall C, Wettero J. Pathogenic implications for autoantibodies against C-reactive protein and other acute phase proteins. *Clin Chim Acta* 2007;378:13–23. [PubMed: 17239838]
3. De Ferranti S, Rifai N. C-reactive protein and cardiovascular disease: a review of risk prediction and interventions. *Clin Chim Acta* 2002;317:1–15. [PubMed: 11814453]
4. Shrive AK, Cheetham GMT, Holden D, et al. Three dimensional structure of human C-reactive protein. *Nature Struct Biol* 1996;3:346–54. [PubMed: 8599761]
5. Volanakis JE, Kaplan MH. Specificity of C-reactive protein for choline phosphate residues of pneumococcal C-polysaccharide. *Proc Soc Exp Biol Med* 1971;136:612–4. [PubMed: 4395924]
6. Agrawal A, Simpson MJ, Black S, Carey MP, Samols D. A C-reactive protein mutant that does not bind to phosphocholine and pneumococcal C-polysaccharide. *J Immunol* 2002;169:3217–22. [PubMed: 12218140]
7. Suresh MV, Singh SK, Ferguson DA Jr, Agrawal A. Human C-reactive protein protects mice from *Streptococcus pneumoniae* infection without binding to pneumococcal C-polysaccharide. *J Immunol* 2007;178:1158–63. [PubMed: 17202380]
8. Kushner I, Rakita L, Kaplan MH. Studies of acute-phase protein. II localization of Cx-reactive protein in heart in induced myocardial infarction in rabbits. *J Clin Invest* 1963;42:286–92. [PubMed: 13927613]
9. Lagrand WK, Niessen HWM, Wolbink GJ, et al. C-reactive protein colocalizes with complement in human hearts during acute myocardial infarction. *Circulation* 1997;95:97–103. [PubMed: 8994423]
10. Pepys MB, Hirschfield GM, Tennent GA, et al. Targeting CRP for the treatment of cardiovascular disease. *Nature* 2006;440:1217–21. [PubMed: 16642000]

11. Thompson D, Pepys MB, Wood SP. The physiological structure of human C-reactive protein and its complex with phosphocholine. *Structure* 1999;7:169–77. [PubMed: 10368284]
12. Coll JM. Immunochemical recognition of the binding of C-reactive protein to solid-phase phosphorylethanolamine. *Rev Espanol Fisiol* 1988;44:169–78.
13. Oliveira EB, Gotschlich EC, Liu TY. Comparative studies on the binding properties of human and rabbit C-reactive proteins. *J Immunol* 1980;124:1396–402. [PubMed: 6766973]
14. Schwalbe RA, Dahlbäck B, Coe JE, Nelsestuen GL. Pentraxin family of proteins interacts specifically with phosphorylcholine and/or phosphorylethanolamine. *Biochemistry* 1992;31:4907–15. [PubMed: 1375509]
15. Chang MK, Binder CJ, Torzewski M, Witztum JL. C-reactive protein binds to both oxidized LDL and apoptotic cells through recognition of a common ligand: phosphorylcholine of oxidized phospholipids. *Proc Natl Acad Sci USA* 2002;99:13043–8. [PubMed: 12244213]
16. Bhakdi S, Torzewski M, Klouche M, Hemmes M. Complement and atherogenesis: binding of CRP to degraded, nonoxidized LDL enhances complement activation. *Arterioscler Thromb Vasc Biol* 1999;19:2348–54. [PubMed: 10521363]
17. Biró A, Thielens NM, Cervenák L, Prohászka Z, Füst G, Arlaud GJ. Modified low density lipoproteins differentially bind and activate the C1 complex of complement. *Mol Immunol* 2006;44:1169–77. [PubMed: 16938346]
18. Ji SR, Wu Y, Potempa LA, Qiu Q, Zhao J. Interactions of C-reactive protein with low-density lipoproteins: implications for an active role of modified C-reactive protein in atherosclerosis. *Int J Biochem Cell Biol* 2006;38:648–61. [PubMed: 16376133]
19. Taskinen S, Kovanen PT, Jarva H, Meri S, Pentikainen MO. Binding of C-reactive protein to modified low-density-lipoprotein particles: identification of cholesterol as a novel ligand for C-reactive protein. *Biochem J* 2002;367:403–12. [PubMed: 12102655]
20. Van Tits L, De Graff J, Toenhake H, Van Heerde W, Stalenhoef A. C-reactive protein and annexin A5 bind to distinct sites of negatively charged phospholipids present in oxidized low-density lipoprotein. *Arterioscler Thromb Vasc Biol* 2005;25:717–22. [PubMed: 15692104]
21. Reynolds GD, Vance RP. C-reactive protein immunohistochemical localization in normal and atherosclerotic human aortas. *Arch Pathol Lab Med* 1987;111:265–9. [PubMed: 2435257]
22. Sun H, Koike T, Ichikawa T, et al. C-reactive protein in atherosclerotic lesions: its origin and pathophysiological significance. *Am J Pathol* 2005;167:1139–48. [PubMed: 16192648]
23. Torzewski M, Rist C, Mortensen RF, et al. C-reactive protein in the arterial intima: role of C-reactive protein receptor-dependent monocyte recruitment in atherogenesis. *Arterioscler Thromb Vasc Biol* 2000;20:2094–9. [PubMed: 10978254]
24. De Beer FC, Soutar AK, Baltz ML, Trayner IM, Feinstein A, Pepys MB. Low density lipoprotein and very low density lipoprotein are selectively bound by aggregated C-reactive protein. *J Exp Med* 1982;156:230–42. [PubMed: 7086355]
25. Fu T, Borensztajn J. Macrophage uptake of low-density lipoprotein bound to aggregated C-reactive protein: possible mechanism of foam-cell formation in atherosclerotic lesions. *Biochem J* 2002;366:195–201. [PubMed: 12033985]
26. Nunomura W, Hatakeyama M. Binding of low density lipoprotein (LDL) to C-reactive protein (CRP): a possible binding through apolipoprotein B in LDL at phosphorylcholine-binding site of CRP. *Hokkaido J Med Sci* 1990;65:474–80. [PubMed: 2227796]
27. Singh SK, Suresh MV, Prayther DC, Moorman JP, Rusinol AE, Agrawal A. C-reactive protein-bound enzymatically modified low-density lipoprotein does not transform macrophages into foam cells. *J Immunol* 2008;180:4316–22. [PubMed: 18322245]
28. Brown AJ, Leong SL, Dean RT, Jessup W. 7-Hydroperoxycholesterol and its products in oxidized low density lipoprotein and human atherosclerotic plaque. *J Lipid Res* 1997;38:1730–45. [PubMed: 9323583]
29. Muller H, Hellgren LI, Olsen E, Skrede A. Lipids rich in phosphatidylethanolamine from natural gas-utilizing bacteria reduce plasma cholesterol and classes of phospholipids: a comparison with soybean oil. *Lipids* 2004;39:833–41. [PubMed: 15669758]
30. Brown MS, Goldstein JL. Lowering LDL: not only how low, but how long? *Science* 2006;311:1721–3. [PubMed: 16556829]

31. Devaraj S, Rogers J, Jialal I. Statins and biomarkers of inflammation. *Curr Atheroscler Rep* 2007;9:33–41. [PubMed: 17169243]
32. Voleti B, Agrawal A. Statins and nitric oxide reduce C-reactive protein production while inflammatory conditions persist. *Mol Immunol* 2006;43:891–6. [PubMed: 16054696]
33. Wang HR, Li JJ, Huang CX, Jiang H. Fluvastatin inhibits the expression of tumor necrosis factor- α and activation of nuclear factor- κ B in human endothelial cells stimulated by C-reactive protein. *Clin Chim Acta* 2005;353:53–60. [PubMed: 15698590]
34. Rufail ML, Ramage SC, Van Antwerpen R. C-reactive protein inhibits in vitro oxidation of low-density lipoprotein. *FEBS Lett* 2006;580:5155–60. [PubMed: 16962105]
35. Kruth HS, Huang W, Ishii I, Zhang WY. Macrophage foam cell formation with native low density lipoprotein. *J Biol Chem* 2002;277:34573–80. [PubMed: 12118008]
36. Kovacs A, Tornvall P, Nilsson R, Tegnér J, Hamsten A, Björkegren J. Human C-reactive protein slows atherosclerosis development in a mouse model with human-like hypercholesterolemia. *Proc Natl Acad Sci USA* 2007;104:13768–73. [PubMed: 17702862]

Fig. 1.

In the presence of phosphoethanolamine (PEt), CRP acquired the capability to bind to purified native LDL in the fluid-phase. A representative of 2 sucrose flotation experiments is shown. Four different reactions (A–D) were subjected to sucrose density gradient ultracentrifugation. From each tube, six fractions (1–6 on the y-axis) were collected and A_{280} was measured as shown on the x-axis. The arrow indicates the location of a protein-containing band which formed in the presence of PEt and floated in fraction 3. (E) Fraction 3 from reaction D, whose A_{280} is shown by hatched bar, was subjected to SDS-PAGE, Coomassie blue staining (lanes 1–4), and Western blot (lanes 5–7). A representative gel is shown. Lane 1, molecular weight

marker; Lanes 2 and 5, purified native LDL; Lanes 3 and 6, purified CRP; Lanes 4 and 7, fraction 3 from reaction D.

Fig. 2.

Capture of serum LDL by PEt-complexed CRP. (A) A representative of 2 sucrose flotation experiments is shown. Five reaction mixtures (1–5) were subjected to sucrose density gradient ultracentrifugation. The arrow points to the white band formed in reaction 2 which contained CRP, serum, PEt and Ca²⁺. From each tube, 6 fractions were collected. (B) A representative Western blot, using anti-CRP antibodies, on fractions 1, 5 and 6 from all 5 reactions, as described in A, is shown. (C) The concentration of total cholesterol in fraction 5 from all 5 reactions is shown.

Fig. 3.

The interaction between PEt-complexed CRP and serum LDL is a CRP dose-dependent reaction. (A) A representative of two sucrose flotation experiments is shown. Reaction 2 (mixture of CRP, serum, PEt, and Ca^{2+}) was subjected to sucrose density gradient ultracentrifugation as described in Fig. 2, except that increasing amounts of CRP and serum were used. The arrows indicate the position of the white band formed in each tube. From each tube, 6 fractions were collected as described in Fig. 2. (B) Concentration of total cholesterol in fraction 5 containing the white band collected from all 12 tubes shown in A.

Fig. 4. PEt-complexed CRP binds to LDL, but not to HDL, in whole human serum. The fraction 5 containing the white band and collected from reactions 1, 2 and 4, shown in Fig. 2A, was subjected to measurement of LDL and HDL levels. The results of a representative assay are presented. The concentrations of HDL and LDL are shown on the y-axis.