CROSS-REACTIVITY BETWEEN C-REACTIVE PROTEIN AND IDIOTYPIC DETERMINANTS ON A PHOSPHOCHOLINE-BINDING MURINE MYELOMA PROTEIN*

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C-reactive protein (CRP)[1] was discovered 50 years ago (1) by its ability to form insoluble precipitates with pneumococcal C polysaccharide (PnC). This precipitation reaction was subsequently found to be dependent on the presence of Ca^{++} (2) and inhibitable by various phosphate monoesters (3). After the demonstration that PnC is a ribitol teichoic acid cell-wall polymer (4) comprising choline phosphate (5) in its repeating structure, it was shown (6) that phosphocholine was a more potent inhibitor of the CRP-PnC precipitation reaction than other phosphate monoesters. Equilibrium dialysis experiments indicated that in the presence of Ca^{++}, CRP could bind phosphocholine with an association constant of 1.6×10^5 M^{-1} at 15°C (7). Each of the five, noncovalent subunits of CRP was found to have one binding site for phosphocholine.

Similarly to CRP, the antigen-binding murine myeloma protein S63 (α,κ) was discovered by Cohn (8) by its ability to precipitate with PnC. A number of additional murine myeloma proteins precipitating with PnC were subsequently described (reviewed in [9]). Inhibition studies (10) indicated that phosphocholine was the main determinant group for this precipitation reaction and direct-binding experiments (11) determined association constants varying between 1.6×10^4 M^{-1} and 2.4×10^5 M^{-1} for the binding of phosphocholine to individual myeloma proteins. X-ray diffraction data on the Fab fragment of one phosphocholine-binding myeloma protein (PCBMP), McPC 603 (12) indicated that the hapten-binding site was formed by the first, second, and third hypervariable regions of the heavy chain (H_1, H_2, and H_3, respectively) and by the first and third hypervariable regions of the light chain (L_1 and L_3, respectively). The main interactions between phosphocholine and the immunoglobulin-combining site are electrostatic and are mediated through residues in H_1 and H_2 (13). In addition, a computer search of all available variable (V) region sequences (14) revealed that the H_1 region sequence Phe_{32}-Tyr_{33}-Met_{34}-Glu_{35}, which contains two contacting amino

[1] *Abbreviations used in this paper:* H_1, H_2, H_3 first, second, and third hypervariable regions of the heavy chain, respectively. BSA, bovine serum albumin; CRP, C-reactive protein; E, sheep erythrocytes; E-PnC, sheep erythrocytes sensitized with pneumococcal C-polysaccharide; HOPC, 7N-hexyloctadecane plasma cell; KLH, keyhole limpet hemocyanin; KLH-PC, keyhole limpet hemocyanin-phosphocholine; MAB, monoclonal anti-idiotypic antibody; PCBMP, phosphocholine-binding myeloma protein; PnC, pneumococcal C polysaccharide; SDS, sodium dodecyl sulfate; TCA, trichloroacetic acid; V_H, variable region of the heavy chain; V_L, variable region of the light chain.

acids (Tyr$_{33}$ and Glu$_{35}$), is unique to PCBMP. It is of further interest that the corresponding tetrapeptide from the H$_1$ of a human IgM PCBMP, Fr, is Phe-Tyr-Met-Asp (15).

In contrast to PCBMP, the structure of the phosphocholine-binding site of CRP has not been determined, even though the primary structure has been elucidated (16). Weak homologies between the NH$_2$-terminal amino acid sequence of CRP and the CH$_3$ domain of IgG were noted (17) but their significance was disputed when the complete amino acid sequence of CRP was used for the comparison (16). However, recently Young and Williams (18) pointed out that despite the lack of strong overall sequence homologies between IgG and CRP, there are regions in the primary sequence of CRP that resemble the H$_1$ and H$_2$ regions of murine PCBMP and that the main contacting amino acids of the PCBMP hapten-binding site appear to be present in CRP. These authors suggested that the phosphocholine-binding sites of CRP and PCBMP may have common structural elements. In this study, such common structural elements were sought by using monoclonal antibodies with specificity for idiotypic determinants on the PCBMP 7N-hexyloctadecane plasma cell (HOPC) HOPC 8.

Materials and Methods

Reagents and Chromatographic Media.   Sephadex G-25 and Sepharose 4B were obtained from Pharmacia Fine Chemicals, Piscataway, N. J.; microgranular DEAE-cellulose (Whatman DE-52) from H. Reeve Angel, Clifton, N. J.; electrophoresis grade acrylamide, *N,N'*-methylene-bis-acrylamide, sodium dodecyl sulfate (SDS), and ammonium persulfate from Bio-Rad Laboratories, Richmond, Calif.; carrier-free Na[^{125}I] from Amersham Corp., Arlington Heights, Ill.; lactoperoxidase from Worthington, Biochemical Corp., Freehold, N. J.; phosphocholine chloride, calcium salt, and bovine serum albumin (BSA) from Sigma Chemical Co., St. Louis, Mo.; dinonylphthalate from ICN K & K Laboratories, Inc., Plainview, N. Y.; and dibutylphthalate from Fisher Scientific Co., Pittsburgh, Pa.

Phosphocholine was coupled to Sepharose as described by Chesebro and Metzger (19). Paradiazonium phosphocholine, a gift from Dr. D. Ewert, University of Alabama in Birmingham, Birmingham, Ala., was used to couple phosphocholine to keyhole limpet hemocyanin (KLH) (Calbiochem-Behring Corp., American Hoechst Corp., San Diego, Calif.).

Purification and Radioiodination of CRP and HOPC 8.   Human CRP was purified from ascitic fluid by a previously described method (20), based on the Ca^{++}-dependent affinity of CRP for phosphocholine. The protein was judged pure by electrophoresis on 5–20% polyacrylamide gradient slab gels (21) containing 0.1% SDS. Purified CRP was labeled with ^{125}I by the lactoperoxidase method of Marchalonis (22). The radioiodination mixture was then applied on a 3-ml phosphocholine-Sepharose 4B column and equilibrated in 0.1 M borate-buffered saline, pH 8.5, containing 2 mM CaCl$_2$. The column was washed with the same buffer until the radioactivity of the effluent was negligible. Bound ^{125}I-CRP was then eluted with the same buffer, containing 2 mM EDTA instead of CaCl$_2$. The specific radioactivity of ^{125}I-CRP varied between 0.6 and 0.9 μCi/μg. More than 98% of the radioactivity was protein bound, as determined by trichloroacetic acid (TCA) precipitation.

The mouse PCBMP HOPC 8 (α,κ) (23) was purified from ascites fluid of mice bearing the myeloma HOPC 8 by methods described by Chesebro and Metzger (19). HOPC 8 was radioiodinated with ^{125}I by the lactoperoxidase method (22) in the presence of a 20-fold molar excess of phosphocholine to protect the binding site. ^{125}I-HOPC 8 was separated from free ^{125}I and phosphocholine by gel filtration on a 0.5- × 50-cm Sephadex G-25 column, followed by extensive dialysis against 0.01 M Tris-HCl, 0.15 M NaCl, pH 7.2. The specific radioactivity of ^{125}I-HOPC 8 varied between 1 and 3 μCi/μg. More than 97% of the radioactivity was TCA-precipitable.

Monoclonal Anti-Idiotypic Antibodies (MAB).   Monoclonal antibodies reactive with idiotypic determinants expressed by HOPC-8 were prepared by immunizing A/J mice with purified HOPC-8 myeloma protein and then fusing lymph node cells from these mice with the myeloma

P3-NS1/1-Ag4-1 (24). The details of preparation, purification, specificity analysis, and biological activities of these MAB have been described (25, 26). Briefly, the MAB AB1-2 and GB4-10 were of γ_1,κ class, and reacted with HOPC 8 and TEPC 15 but no other murine PCBMP, and both reacted with an idiotope that was dependent on light and heavy chain association for expression. Although these antibodies are very similar, detailed analysis has revealed that they differ slightly in their fine specificities.[2] MAB, EB3-7 (γ_1,κ) which reacted with an idiotypic determinant expressed by J558 (α,λ) an $\alpha 1 \rightarrow 3$ dextran-binding mouse myeloma protein was used in experiments as a control antibody.

PnC. PnC was isolated from rough *Streptococcus pneumoniae*, strain R36A (American Type Culture Collection, Rockville, Md.), as described by Liu and Gotschlich (27), except that the final enzymatic digestion step was omitted.

Coupling of PnC to Sheep Erythrocytes. PnC was coupled to sheep erythrocytes (E) by the chromium chloride method of Cosenza et al. (28): equal volumes of E (50% vol:vol), PnC (31–250 μg/ml), and chromium chloride (1 mg/ml) in 0.01 M Tris-HCl, 0.15 M NaCl, pH 7.2, were mixed and incubated at room temperature for 4 min. The sensitized erythrocytes (E-PnC) were washed three times with, and resuspended in the same buffer at a final concentration of 5×10^8 cells/ml. Control E were prepared by omitting PnC from the chromium chloride reaction mixture.

Assays for Binding and Inhibition of Binding of ^{125}I-CRP and ^{125}I-HOPC 8 to E-PnC. Binding and inhibition assays were performed in 0.01 M Tris-HCl, 0.15 M NaCl buffer, pH 7.2, containing either 2.5 mM Ca^{++} or 1 mM EDTA, as indicated. In a typical binding experiment, E-PnC (5 $\times 10^8$/ml) sensitized with increasing amounts of PnC were mixed with 50 μl of ^{125}I-CRP (1.8 μg/ml) or ^{125}I-HOPC 8 (2.6 μg/ml). Controls consisted of mixtures of the radiolabeled proteins and nonsensitized, chromium chloride-treated E. The mixtures were incubated at room temperature for 30 min with occasional shaking. To separate free from cell-bound protein, the cells were centrifuged into an oil phase (29). 30-μl aliquots from each reaction mixture were layered on top of 300 μl of a mixture of dibutyl:dinonyl phthalate (2:1; vol:vol) in 400-μl conical polypropylene tubes. Centrifugation was carried out for 90 s at 6,500 g in an Eppendorf microcentrifuge (Brinkmann Instruments, Inc., Westbury, N. Y.). The aqueous phase along with three-fourths of the oil mixture were removed, the tubes were cut just above the cell pellet, and the tips containing the cells were counted in a gamma counter to assess the amount of cell-bound protein.

For inhibition experiments, E-PnC sensitized with 31 μg PnC/5 $\times 10^8$ E were utilized. The reaction mixtures consisted of 50 μl E-PnC (5 $\times 10^8$/ml), 25 μl ^{125}I-CRP (3.6 μg/ml) or 25 μl ^{125}I-HOPC 8 (5.3 μg/ml), and 25 μl of inhibitor at various concentrations. After incubation at room temperature for 30 min, the amount of cell-bound radiolabeled protein was determined as described above. Percent inhibition was calculated from a control mixture lacking the inhibitor.

Binding and Inhibition of Binding of ^{125}I-CRP and ^{125}I-HOPC 8 to MAB. Solid-phase assays were utilized for these studies. Polystyrene microwells (Removawell strips; Dynatech Laboratories, Inc., Dynatech Corp., Alexandria, Va.) were incubated with 300 μl of MAB solution (0.4–26 μg/ml) in 0.01 M Tris-HCl, 0.15 M NaCl buffer, pH 7.2, for 30 min at room temperature. The fluid was aspirated, the microwells washed with buffer, and incubated with 300 μl of a 10 mg/ml solution of BSA in the same buffer to block remaining protein-binding sites. After a 30-min incubation at room temperature, the microwells were washed three times with buffer and used immediately. Control microwells were prepared by omitting the initial incubation step with MAB.

For binding studies, MAB-coated and BSA-coated microwells (controls) were incubated overnight at 4°C with 300 μl of ^{125}I-CRP (0.36 μg/ml) or of ^{125}I-HOPC 8 (0.17 μg/ml) in buffer containing either 2.5 mM Ca^{++} or 1 mM EDTA. The fluid was aspirated, the microwells washed three times with the appropriate buffer, and counted in a gamma counter to assess the amount of bound, radiolabeled protein. Experimental values were corrected for the small amount of radioactivity bound to control BSA-coated tubes.

[2] Kearney, J. F., R. Barletta, Z. S. Quan, and J. Quintans. Monoclonal versus heterogeneous anti-H-8 antibodies in the analysis of the anti-phosphorylcholine response in BALB/c mice. Manuscript submitted for publication.

For inhibition experiments, microwells coated with an amount MAB calculated to result in ~30% binding of the ^{125}I-CRP or ^{125}I-HOPC 8 offered were used. Increasing concentrations of the inhibitor tested dissolved in 0.01 M Tris-HCl, 0.15 M NaCl, pH 7.2, containing 2.5 mM Ca^{++} or 1 mM EDTA were added to the microwells, followed by 25 μl of ^{125}I-CRP (4.4 μg/ml) or 25 μl ^{125}I-HOPC 8 (2 μg/ml). After overnight incubation at 4°C, the fluid was aspirated, the microwells washed three times with buffer and the amount of bound radioactivity determined. Percent inhibition was calculated from a control lacking inhibitor after correcting all experimental values for the small amount of radioactivity bound nonspecifically to control, BSA-coated microwells.

Protein Determinations. Concentrations of purified proteins were determined spectrophotometrically at 280 nm using an extinction coefficient ($E_{1\,cm}^{1\%}$) of 19.5 CRP (30) and 15.0 for HOPC 8. For calculating molar concentrations, the molecular weight of the phosphocholine-binding subunit of each protein was utilized—21,000 for the noncovalent subunit of CRP (16) and 75,000 for the heavy-light chain monomer of HOPC 8 (31).

Results

As expected, both ^{125}I-CRP and ^{125}I-HOPC 8 bound equally well to E-PnC in buffer containing 2.5 mM Ca^{++} (Fig. 1). On a mole per mole basis, equimolar amounts of pentameric CRP and 7S monomer of HOPC 8 bound to the E-PnC throughout the linear portion of the dose-response curves. As shown, under the experimental conditions used, neither protein bound to control, chromium chloride-treated E. When this experiment was repeated using buffer containing 1 mM EDTA instead of Ca^{++}, the binding curve for ^{125}I-HOPC 8 was identical to that shown in Fig. 1, whereas the binding of ^{125}I-CRP was completely abolished.

Binding of either radiolabeled protein to E-PnC could be inhibited by micromolar concentrations of phosphocholine (Fig. 2). This is in agreement with previous data demonstrating that phosphocholine is a potent inhibitor of the precipitation reactions between either CRP (6) or HOPC 8 (10, 23) and PnC. Finally, the results of cross-
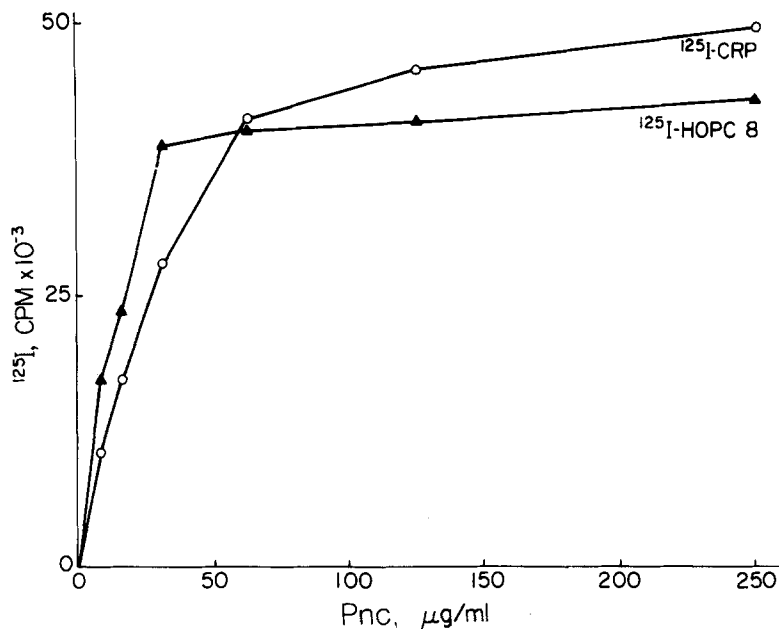

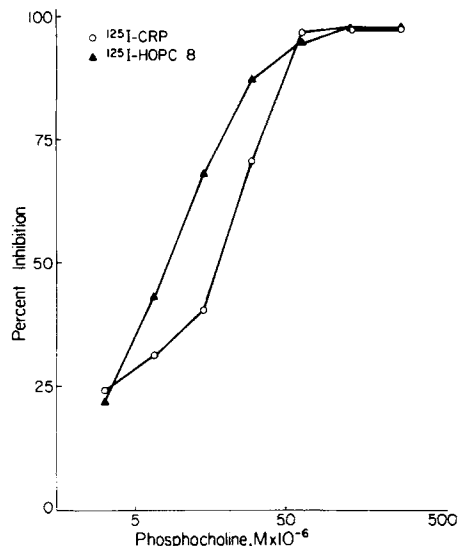FIG. 1. Binding of ^{125}I-CRP and ^{125}I-HOPC 8 to E-PnC prepared with variable amounts of PnC.

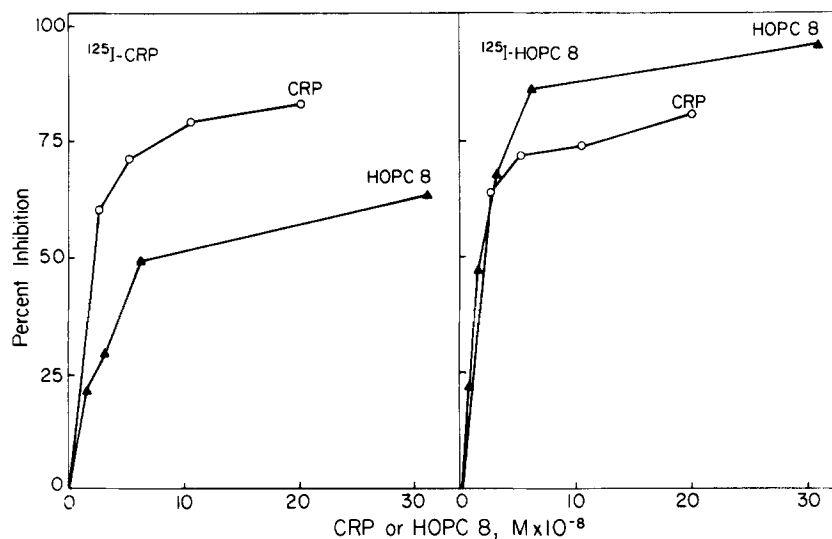Fig. 2.   Inhibition of binding of ^{125}I-CRP and ^{125}I-HOPC 8 to E-PnC by phosphocholine.

Fig. 3.   Inhibition of binding of ^{125}I-CRP (A) and ^{125}I-HOPC 8 (B) to E-PnC by cold CRP and HOPC 8.

inhibition experiments between HOPC 8 and CRP are shown in Fig. 3. 50% inhibition of binding of ^{125}I-HOPC 8 to E-PnC required equimolar concentrations of cold HOPC 8 or CRP. On the other hand, 50% inhibition of ^{125}I-CRP binding required an eightfold greater concentration of HOPC 8 than of CRP.

The findings described above and the observation by Young and Williams (18) that certain regions of the CRP amino acid sequence are homologous to the first two hypervariable regions of the heavy chain of PCBMP, led us to examine the possible interaction between CRP and GB4-10, a monoclonal antibody with specificity for idiotypic determinants on HOPC 8. Initial experiments indicated that GB4-10 at

micromolar concentrations could inhibit the binding of ^{125}I-HOPC 8 to E-PnC, and that at 30- to 50-fold higher concentrations, it could also partially inhibit the binding of ^{125}I-CRP to the sensitized cells. This apparent interaction between CRP and GB4-10 was further explored by direct-binding experiments using a solid-phase assay system. Fig. 4 illustrates a representative binding experiment. Inspection of the curves indicates that in the presence of Ca^{++}, binding of ^{125}I-HOPC 8 was observed when as little as 0.8 μg of GB4-10/1 ml were used to coat the microwells. Binding of ~50% of the ^{125}I-HOPC 8 offered required 1.6 μg of GB4-10/1 ml. On the other hand, binding of ^{125}I-CRP was not observed until 6 μg GB4-10/ml were used and 50% binding required 12 μg/ml. In the presence of 1 mM EDTA, the binding of ^{125}I-CRP to GB4-10 was completely inhibited, whereas the binding of ^{125}I-HOPC 8 was not affected (Fig. 4). Similar results were obtained with a second independent anti-HOPC 8 MAB, AB1-2. AB1-2 was prepared similarly to GB4-10, but it differs from the latter in fine specificity. Again, binding of ^{125}I-CRP was Ca^{++}-dependent, whereas no Ca^{++} requirement was evident for ^{125}I-HOPC 8. Finally, as a control a third unrelated MAB, EB3-7, was used in a similar experiment. EB3-7 has specificity for idiotypic determinants on J558, an α1→3 dextran-binding murine myeloma protein (α,λ). Neither ^{125}I-HOPC 8 nor ^{125}I-CRP bound to microwells coated with ≤30 μg/ml of EB3-7 either in the presence or absence of Ca^{++}.

Binding of either ^{125}I-CRP or ^{125}I-HOPC 8 to GB4-10 could be inhibited by similar concentrations of KLH-linked phosphocholine (KLH-PC) (Fig. 5). However, free phosphocholine inhibited only the binding of ^{125}I-CRP, although it failed to inhibit that of ^{125}I-HOPC 8 at concentrations up to 0.45 mM. Finally, in the presence of Ca^{++}, the binding of ^{125}I-HOPC 8 to GB4-10 could be inhibited by cold HOPC 8 as well as by a 50-fold higher molar concentration of cold CRP (Fig. 6). Substitution of
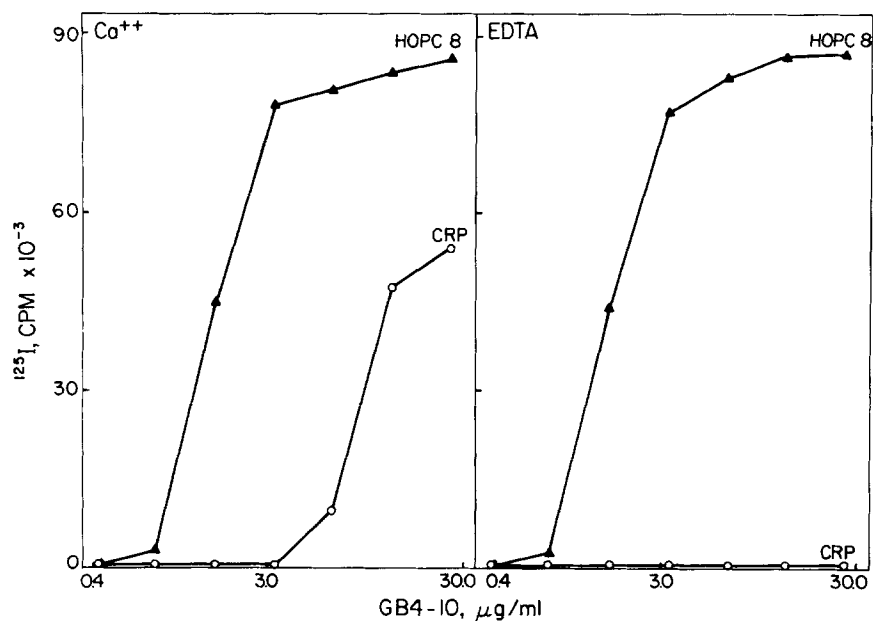

Fig. 4. Binding of ^{125}I-CRP and ^{125}I-HOPC 8 to immobilized GB4-10 in the presence of 2.5 mM Ca^{++} (A) or 1 mM EDTA (B).
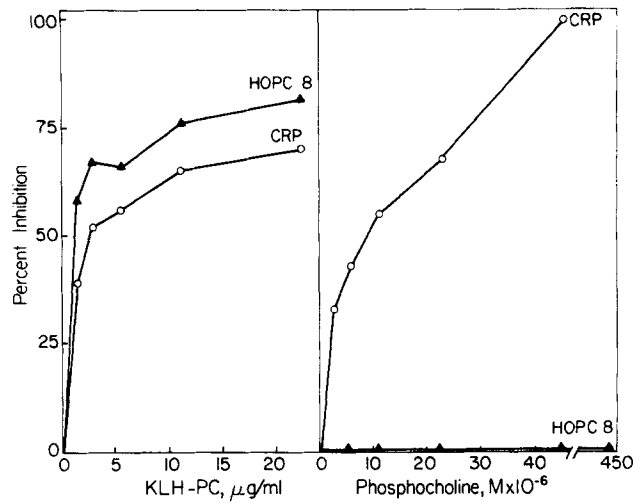
Fig. 5.   Inhibition of binding of ^{125}I-CRP and ^{125}I-HOPC 8 to immobilized GB4-10 by KLH-PC (A) or phosphocholine (B).
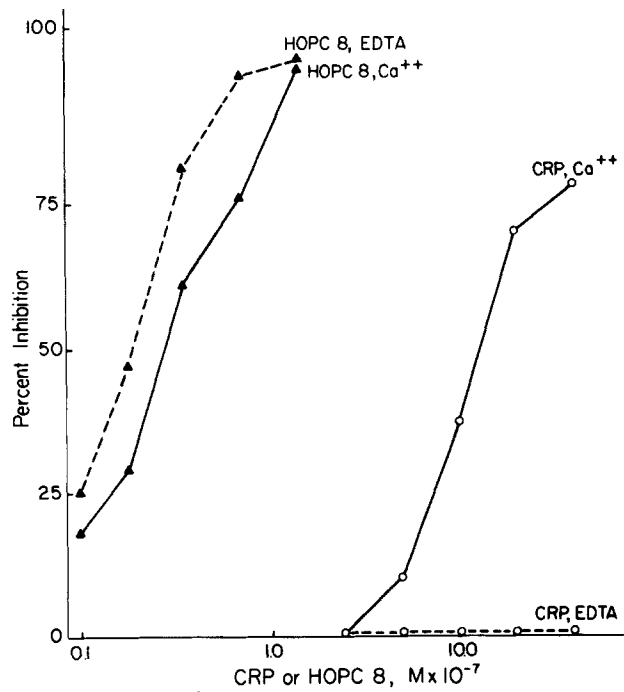

Fig. 6.   Inhibition of binding of ^{125}I-HOPC 8 to immobilized GB4-10 by cold HOPC 8 and CRP in the presence of 2.5 mM Ca^{++} or 1 mM EDTA.

EDTA for Ca^{++} abolished the inhibitory ability of CRP without affecting that of HOPC 8. The binding of ^{125}I-CRP to GB4-10 could be inhibited by cold CRP but not by HOPC 8 at the highest concentration tested. However, it should be noted that this last experiment was technically difficult because the concentration of GB4-10

used to coat the tubes was ~10-fold higher than that used for binding of ^{125}I-HOPC 8.

Discussion

The human acute-phase protein CRP and the murine PCBMP HOPC 8 (α,κ) share a common binding specificity for phosphocholine residues of PnC (6, 10). In addition, CRP and HOPC 8 exhibit similar association constants for the free hapten phosphocholine: 1.6×10^5 M^{-1} and 2.4×10^5 M^{-1}, respectively (7, 11). Although no direct comparison has been made, the two proteins should not be expected to have identical fine-binding specificities. Binding of CRP but not of PCBMP to PnC can be inhibited by phosphate monoesters, whereas choline inhibits the binding of PCBMP but not of CRP. A further essential difference between the binding specificities of the two proteins is the requirement of Ca^{++} for CRP. Despite these differences, under the experimental conditions used in this study, the two proteins were found to bind equally well to E-PnC and to require equimolar amounts of phosphocholine for 50% inhibition (Figs. 1 and 2). In addition, cross-inhibition experiments (Fig. 3) indicated that equimolar concentrations of HOPC 8 and CRP were required for 50% inhibition of binding of ^{125}I-HOPC 8 to E-PnC, further emphasizing the similarities between the two proteins in terms of binding specificity and affinity. On the other hand, in the converse experiment, eightfold more HOPC 8 than CRP was required for 50% inhibition of binding of ^{125}I-CRP. This finding could not be readily attributed to differences in affinity or fine specificity of the two proteins. It could be explained though by previous data (32) indicating that CRP may have a secondary binding specificity for N-acetyl-galactosamine residues of PnC.

The overall similarity in binding specificity and affinity between HOPC 8 and CRP raised the possibility of structural similarities in their phosphocholine-binding sites. Such structural homology was previously suggested by Young and Williams (18) who pointed out that certain regions in the primary structure of CRP bear resemblance to H_1 and H_2 of PCBMP. Monoclonal antibodies with specificity for idiotypic determinants associated with the variable regions of PCBMP should be sensitive probes for such structural homologies because idiotypic determinants are found exclusively on the FV fragment of immunoglobulins (33) and may be associated with antigen-binding sites (34, 35). In most strains of mice, the anti-phosphocholine response is dominated by a restricted set of antibodies defined by the TEPC 15 idiotype (36), and anti-idiotypic sera to TEPC 15 have been used extensively in genetic and cellular studies of the immune response to phosphocholine. An idiotypic cross-reactivity between a human phosphocholine-binding IgM protein and a murine PCBMP, MOPC 167 has also been reported (37). Both MAB used in this study were raised against HOPC 8 and recognized an idiotope dependent on H and L chain association for expression. This idiotope was not present in other PCBMP tested, MOPC 167, M511, and W 3207, which have amino acid sequence differences from HOPC 8 in the V_H and V_L regions and are negative for the TEPC 15 idiotype (9).

Preliminary experiments indicated that GB4-10 could inhibit the binding of ^{125}I-CRP to E-PnC. However, compared with ^{125}I-HOPC 8, much higher concentrations of GB4-10 were required and only partial inhibition was observed. This finding could perhaps be explained by the previously mentioned secondary-binding specificity of CRP for sugar residues of PnC (32). It nevertheless raised questions concerning the

specificity of the interaction between CRP and GB4-10. The direct-binding experiments (Fig. 4) clearly demonstrated the specificity of the interaction between ^{125}I-CRP and the anti-HOPC 8 MAB. First, no binding of ^{125}I-CRP to EB3-7 an unrelated MAB, was observed. Second, at the highest concentration of GB4-10 used, 66% of the ^{125}I-CRP offered bound to the immobilized MAB. This makes it highly unlikely that contaminants in the CRP preparation, such as anti-phosphocholine antibodies, were responsible for the observed binding. Third, EDTA completely inhibited the binding of ^{125}I-CRP to either GB4-10 or AB1-2 without affecting the binding of ^{125}I-HOPC 8.

A Ca^{++} requirement was observed not only for the binding of ^{125}I-CRP to GB4-10 and AB1-2, but also for the inhibition of binding of ^{125}I-HOPC 8 to GB4-10 by CRP. The Ca^{++} dependence of these interactions might be relevant to the role of this anion in the binding of CRP to phosphocholine. CRP has been shown to bind Ca^{++} with an association constant of 1.3×10^4 M^{-1} at 25°C and a valence of one to two per subunit (3). The exact role of Ca^{++} in the CRP-phosphocholine interaction is not known, but it has been suggested (3) that it might directly participate in binding the phosphate group. Our present data show that the expression of antigenic determinants of CRP, cross-reacting with idiotypic determinants on HOPC 8, depends on Ca^{++}. Furthermore, these determinants are apparently intimately associated with the phosphocholine-binding site, as indicated by the hapten-inhibition experiments (Fig. 5). It therefore appears, that Ca^{++} acts as an allosteric effector of CRP, essential for the proper conformation of its phosphocholine-binding site. Evidence for Ca^{++}-induced conformational changes of CRP has been previously provided by circular dichroism studies (18).

The finding that antigenic determinants on CRP, a nonimmunoglobulin molecule, cross-react with idiotypic determinants might be important in understanding the evolutionary origin of antibody-combining sites.

Summary

Binding of human ^{125}I-C-reactive protein (CRP) to sheep erythrocytes sensitized with pneumococcal C polysaccharide (E-PnC) was found to be Ca^{++}-dependent and inhibitable by phosphocholine, CRP, and HOPC 8. Binding of ^{125}I-HOPC 8 to E-PnC was Ca^{++}-independent but could also be inhibited by phosphocholine, CRP, and HOPC 8. Thus, CRP and HOPC 8, despite a differential Ca^{++} requirement, share a common binding specificity for phosphocholine. A monoclonal anti-idiotypic antibody (MAB), GB4-10, prepared in A/J mice immunized with BALB/c HOPC 8 inhibited the binding of both ^{125}I-CRP and ^{125}I-HOPC 8 to E-PnC. In addition, both proteins bound to GB4-10 immobilized on polysterene tubes. Interestingly, binding of ^{125}I-CRP to GB4-10 required Ca^{++}. Similar results were also obtained with another MAB (AB1-2) prepared similarly to GB4-10, whereas neither protein bound to a control MAB (EB3-7) against an $\alpha 1 \rightarrow 3$ dextran-binding myeloma protein, J558. Binding of ^{125}I-HOPC 8 to GB4-10 could be inhibited by HOPC 8, keyhole limpet hemocyanin-phosphocholine but not phosphocholine, and in the presence of Ca^{++} by CRP. These data indicate that CRP bears antigenic determinants cross-reacting with certain idiotypic determinants on HOPC 8. They also suggest that Ca^{++} acts as an allosteric effector, perhaps stabilizing the phosphocholine-binding site of CRP.

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