Human C4b-binding protein (C4BP), which is a regulator of the classical complement pathway C3 convertase, forms high affinity complexes with anticoagulant protein S and with the pentraxin serum amyloid P component (SAP). SAP is a plasma protein present in all amyloid deposits. Recently, SAP was shown to inhibit the complement regulatory functions of C4BP. In this investigation, we have studied the structural requirements for the C4BP-SAP interaction. C4BP was subjected to chymotryptic digestion, which yielded two major fragments corresponding to the central core (160 kDa) and to the cleaved-off tentacles (48 kDa). SAP-Sepharose specifically bound the 160-kDa fragment, suggesting that the central core of C4BP contains the binding site for SAP. In a quantitative affinity chromatography assay, the dissociation constants for binding of intact C4BP and of the 160-kDa central core fragment to SAP were found to be 30 and 70 nM, respectively. Recombinant C4BP composed of only α-chains bound SAP with similar affinity (K_d = 22 nM), whereas nonglycosylated recombinant α-chain C4BP (synthesized in the presence of tunicamycin) bound SAP with lower affinity (K_d = 126 nM). This suggests that the carbohydrate moeity of the central core of C4BP is important for binding of C4BP to SAP in contrast to the C4BP β-chain, which is not required. EDTA, heparin, and phosphorylethanolamine as well as a peptide comprising amino acids 27–39 of SAP were found to completely displace C4BP from the SAP matrix. Moreover, the immobilized SAP peptide bound C4BP in a reaction that, in contrast to the C4BP-SAP interaction, was not dependent on calcium.

C4b-binding protein (C4BP) is a regulator of the complement system (1). It binds multiple C4b molecules, inhibits the assembly of the classical pathway C3 convertase (C4b2a), dissociates C2a from the convertase, and functions as cofactor to factor I in the degradation of C4b. In human plasma, C4BP also interacts with the anticoagulant vitamin K-dependent protein S (2) and with serum amyloid P component (SAP) (3, 4). C4BP is a high molecular weight (MW = 570,000) plasma glycoprotein composed of seven identical α-chains and one β-chain, the arrangement of which gives C4BP an octopus-like structure (1, 5). The α-chain (MW = 70,000) contains eight tandemly repeated, internally homologous modules, designated short consensus repeats (SCRs), and a C-terminal region that contains two cysteines that are involved in interchain disulfide bridging (6). Each α-chain contains a binding site for C4b, the detailed location of which is not known. The β-chain (45 kDa), which is related structurally to the α-chain, contains three SCR modules and a carboxyl-terminal region with two cysteines (7). The α-chain has three sites for N-linked glycosylation, one in SCR-3 and two in SCR-8, whereas the β-chain contains five sites for N-linked glycosylation, two in each of SCR-1 and SCR-2 and one in SCR-3.

SAP is a serum glycoprotein composed of 10 identical, non-covalently linked 25-kDa subunits, which are arranged in two parallel cyclic pentagonal structures interacting face-to-face (8). SAP is related in structure to C-reactive protein, and both proteins belong to the pentraxin protein family (9, 10). Pentraxins have been highly conserved through evolution and are found in all vertebrate species. There are also pentraxins described in invertebrates (11). Although the physiological function of SAP is largely unknown, SAP has received considerable medical interest as it associates with all types of amyloid deposits, including those of Alzheimer’s disease (9, 12). SAP has a high tendency to undergo calcium-dependent self-aggregation, which may be important for its deposition in amyloid. It has lectin-like properties and binds heparin, heparan and dermatan sulfate, and phosphorylcholine (13–15). Another property of SAP is its ability to bind DNA and chromatin (16). SAP displaces type H1 histones and renders solubility to the otherwise insoluble chromatin (17, 18). SAP has also been reported to interact with fibronectin (19) and hirudin (13). All these interactions are calcium-dependent and appear to involve a unique binding site in the SAP molecule and ligands containing phosphorylated or possibly sulfated residues (20, 21).

Despite earlier reports showing the ability of C4BP to bind immobilized SAP (19), the presence of a C4BP-SAP complex in blood has only recently been recognized (3, 4). The latter studies demonstrate that C4BP interacts with nonaggregated SAP in fluid phase and that the two proteins form a 1:1 stoichiometric complex in plasma. Due to the equimolar concentrations of the two proteins and the high affinity of the interaction, essentially all SAP in blood is bound to C4BP (3), even under acute-phase conditions (22). Sucrose density gradient experiments have shown the complex between SAP, C4BP, and protein S to have the potential to interact with fluid-phase C4b, suggesting that the binding sites for the different molecules on C4BP are distinct (3). However, recent results have shown SAP binding to C4BP to be associated with inhibition of the factor I cofactor activity of C4BP and with inhibition of C4BP binding to C4b-coated surfaces (23).

In this study, we have localized the SAP-binding site to the 160-kDa chymotryptic fragment constituting the central core of C4BP and demonstrate that the binding is influenced by the presence of N-linked carbohydrates on C4BP. A SAP peptide
previously reported to support cell attachment (24) is shown to be involved in the interaction with C4BP. The results provide basis for further understanding of the physiological role of SAP.

**EXPERIMENTAL PROCEDURES**

Materials—Chymotrypsin (64 units/mg) was obtained from Cooper Biomedical. Na$_{125}$I was from DuPont NEN. CNBr-Sepharose was from Pharmacia Biotech Inc. Neuraminidase from Flavobacterium meningosepticum was a kind gift of Dr. W. J. Stenflo (Department of Clinical Chemistry, Lund University). Heparin (mean M$_{r}$ = 15,000, 10,000 IU/ml) was purchased from Pharmacia Biotech Inc.

Proteins—SAP was purified as described previously (4). Fifty ml of SAP (0.77 mg/ml) in 100 mM NaHCO$_3$, 500 mM NaCl was coupled to 5 g of CNBr-Sepharose for 2 h; blocked with 200 mM glycine, pH 8.0, and washed with 100 mM sodium acetate, 1 mM NaCl, pH 4, and finally with TBS (50 mM Tris-HCl, 150 mM NaCl, pH 7.5). The final concentration of SAP in the Sepharose was calculated to be 0.5 mg/ml of gel matrix. The SAP-Sepharose was stored in TBS containing 1 mM CaCl$_2$ and 0.02% NaN$_3$. C4BP was purified as described previously (25). Chymotryptic digestion of C4BP, to remove partially cleaved molecules (26), was performed as described (27). Cells cultured in the presence of tunicamycin yielded recombinant C4BP devoid of N-linked carbohydrate side chains (referred to as N-non-glycosylated rC4BP) (for a detailed characterization of this protein, see Ref. 27). The recombinant C4BP and chymotryptic fragments of C4BP are generically referred to as C4BP derivatives (4). Proteins were radiolabeled with Na$_{125}$I using IODOBeads$^{TM}$ as described (26). Protein concentrations were determined by absorbance at 280 nm using the following extinction coefficients: SAP, 18.2 (24); C4BP, 14.1 (28); and tentacles, 14.8, and core fragments, 12.5 (calculated as described by Perkins et al. (28)). The molecular masses used in the calculations were 230 kDa for SAP, 570 kDa for C4BP, 48 kDa for the cleaved-off tentacle fragments (26). Recombinant C4BP containing only $\alpha$-chains (rC4BP) was expressed in a hamster kidney epithelial-derived cell line (AV-12-664) and purified as described (27). Cells cultured in the presence of tunicamycin yielded recombinant C4BP devoid of N-linked carbohydrate side chains (referred to as N-non-glycosylated rC4BP) (for a detailed characterization of this protein, see Ref. 27). The recombinant C4BP and chymotryptic fragments of C4BP are generically referred to as C4BP derivatives (4). Proteins were radiolabeled with Na$_{125}$I using IODOBeads$^{TM}$ as described (26). Protein concentrations were determined by absorbance at 280 nm using the following extinction coefficients: SAP, 18.2 (24); C4BP, 14.1 (28); and tentacles, 14.8, and core fragments, 12.5 (calculated as described by Perkins et al. (28)). The molecular masses used in the calculations were 230 kDa for SAP, 570 kDa for C4BP, 48 kDa for the cleaved-off tentacles, and 160 kDa for the core fragment.

**Glycosidase Treatment—** C4BP (1.5 mg) was incubated with neuraminidase (0.5 million U/mg of protein) for 2.5 h at 37°C in 50 mM sodium acetate buffer, pH 5.5. In a total volume of 0.5 ml. Neuraminidase-treated C4BP was affinity-purified on a 5-ml monoclonal antibody 104-Sepharose column. After overnight incubation in TBS, C4BP bound to the column was eluted with 3 M guanidine in TBS and dialyzed against TBS. The absence of N-linked glycosylation on rC4BP after tunicamycin treatment of the cells was tested by SDS-polyacrylamide gel electrophoresis. The combined QAE-Sephacel fractions was detected by dot-blot assay and quantified by enzyme-linked immunosorbent assay using biotinylated monoclonal antibody 104 and a polyclonal antibody against C4BP, as described previously (22).

Quantitative SAP-Sepharose Affinity Chromatography—To determine the apparent $K_d$ values for the interactions between SAP and intact C4BP or the different C4BP derivatives, the quantitative affinity chromatography (QAC) method (29) was used essentially as described by Persson et al. (30). A slurry of the SAP-Sepharose matrix (300 $\mu$l) was incubated with a trace amount of $^{125}$I-labeled C4BP in TBS containing 1 mM CaCl$_2$ and 0.1% bovine serum albumin for 20 min at room temperature under gentle mixing. The gel was allowed to settle, and two aliquots of the supernatant (2.5 $\mu$l each) were removed and counted on a γ-counter. Free C4BP, C4BP derivatives, or SAP was added to the incubation mixture (added volumes of 5 $\mu$l), and the mixing and settling procedure was repeated. A maximum of six determinations were performed in each experiment in order to maintain the concentration of tracer at the end of the experiment at least 90% of the initial concentration. A control without the SAP matrix was run in parallel in which the same volumes were withdrawn for analysis and used as blank. As the concentrations of tracer removed from test and control tubes were not identical, a difference in the specific activity of C4BP was produced in each determination even though the difference never exceeded 5% of the total radioactivity (within the assay error). A correction was included in the calculations considering this difference. The distribution of C4BP between the fluid and matrix phases was used to estimate its affinity for immobilized or free SAP. The fraction of C4BP bound to immobilized SAP was determined as $1 - [[C4BP]_{\text{matrix}}/[C4BP]_{\text{control}}]$, representing the concentrations of C4BP in solution in the presence or absence of the SAP matrix, respectively. To characterize the interaction between C4BP and the SAP matrix, increasing concentrations of C4BP were incubated with a fixed amount of SAP matrix, or alternatively, a fixed concentration of C4BP was incubated in the presence of increasing concentrations of SAP matrix. The binding parameters were estimated from the fit of the data by nonlinear regression analysis to the single rectangular hyperbola of Equation 1.

$$[C4BP]_T = [C4BP]_T^0 + [C4BP]_T^0 - [C4BP]_{T,\text{bind}}$$  (Eq. 1)

$$K_d = \frac{[C4BP]_T^0}{[C4BP]_{T,\text{bind}}}$$  (Eq. 2)

where the subscripts B, F, and T denote bound, free, and total concentrations, respectively, and $K_{C4BP-SAP}$ and $K_{C4BP-M}$ are the dissociation constants of C4BP to the SAP matrix and SAP in solution, respectively.

Equations 1 and 2 assume a 1:1 stoichiometry of the C4BP-SAP interaction (3) and $[M]_T = [C4BP]_T$. Under the experimental conditions, [SAP]$_T >> [C4BP]_T$, and therefore, [SAP]$_T$ was a good approximation of [SAP]$_T$.

The $K_d$ for the interaction between the SAP matrix and the different C4BP fragments or recombinant C4BP had the same form as the previous equation (29). This assumes a 1:1 stoichiometry of the interaction and an excess of matrix over the C4BP tracer. The
results are expressed as fraction of C4BP bound as a function of the concentration of C4BP<sub>m</sub> (C4BP fragments or recombinant C4BP). As [C4BP<sub>der</sub>] < [C4BP<sub>m</sub>] or [M]<sub>r</sub>, the total concentration of C4BP<sub>der</sub> is a good approximation of its free concentration. Dissociation constants were calculated by nonlinear regression with the STATISTICA program using mean values of at least five determinations.

Synthetic Peptides—A 13-mer peptide, Pep-1 (EKPLONFTLCFRA), corresponding to residues 27–39 of SAP, and a scrambled peptide, Pep-2 (LNRCKLEQPTFRA), were synthesized using an Applied Biosystems 431A peptide synthesizer. After purification by high pressure liquid chromatography and lyophilization, peptides were dissolved in a 50 mM acetic/acetate solution, pH 4, at a 3 mM concentration. Before use, the peptides were diluted in 0.25 mM Tris-HCl, pH 7.5, to a concentration of 0.2 mM and then further diluted in TBS. Peptides were carboxymethylated as described previously (31).

Microwell Binding Assays—For studies of binding of 125I-C4BP to immobilized Pep-1, 2.5% glutaraldehyde-treated polyvinyl chloride plates (Linbro/Titertek) were used. The plates were incubated with 10 μg/ml Pep-1 diluted in 150 mM NaCl at 4°C overnight and then quenched with 1% bovine serum albumin in TBS. A 125I-C4BP tracer (1.0–1.5 × 10⁶ cpm/well) was added together with increasing concentrations of peptides and incubated for 4 h at room temperature. After three washes with ice-cold washing buffer (TBS containing 1 mM CaCl₂ and 0.1% Tween 20), the bound radioactivity was measured in a γ-counter.

RESULTS

SAP-Sepharose Affinity Chromatography of C4BP and Its Fragments—C4BP digested with chymotrypsin was applied to a SAP-Sepharose column. The 160-kDa central core of C4BP, comprising the eight SCRs and the carboxyl-terminal non-repeat region of the α-chains, was specifically retained, while the 48-kDa α-chain fragment containing the six N-terminal SCRs passed through the column (data not shown). Incubation of C4BP with chymotrypsin for a prolonged time (16 h), to ensure complete digestion of the α-chains, did not modify the ability of the 160-kDa central core fragment to bind to SAP-Sepharose. In a similar experiment, plasma C4BP treated with neuraminidase was tested for SAP-Sepharose binding, and it was found to be retained on the column to the same extent as the intact control protein.

Quantitative Affinity Chromatography of C4BP Binding to SAP Matrix—The C4BP-SAP interaction was further characterized with a quantitative affinity chromatography method that used SAP-Sepharose as the matrix (Fig. 2). Increasing concentrations of C4BP were added to a fixed amount of SAP matrix, and the distribution of C4BP between the free and bound states was measured. Under these experimental conditions, an apparent K<sub>d</sub> of 30 nM was calculated for the interaction between C4BP and the SAP matrix (K<sub>C4BP-M</sub>) (Fig. 2A). The concentration of binding sites for C4BP was 60 nM, suggesting that ~20% of the SAP that was coupled to the matrix is accessible as C4BP ligand. In a second experimental approach, the C4BP concentration was kept constant, and the amount of SAP matrix was varied. The measured K<sub>d</sub> of 38 nM (K<sub>C4BP-M</sub>) (Fig. 2B) agreed well with the K<sub>d</sub> of 30 nM calculated under the first experimental conditions. In a third approach, increasing concentrations of SAP were added, which displaced the C4BP that was bound to the matrix (Fig. 2C). This allowed us to calculate the K<sub>d</sub> for the C4BP-SAP interaction in fluid phase (K<sub>C4BP-SAP</sub>). The estimated K<sub>d</sub> of 15 nM should be interpreted with caution because the result of the experiment may be somewhat affected by the tendency of SAP to self-aggregate. Moreover, SAP may also interact directly with the agarose, which could bias the measurements. However, self-aggregation of SAP mainly occurs at calcium concentrations above 1.5 mM (1 mM used in our experiments), and in addition, the affinity of SAP for agarose is much lower than that for C4BP (3).

The purified 160-kDa central core fragment of C4BP competitively inhibited binding of C4BP to SAP-Sepharose, whereas the 48-kDa fragment was without effect (Fig. 3A). The calculated K<sub>d</sub> for the interaction between the 160-kDa central core fragment and the SAP matrix (K<sub>core-M</sub>) was ~2-fold greater than the K<sub>d</sub> for the interaction between the 160-kDa central core fragment and the SAP matrix. Therefore, the interaction of C4BP with the SAP matrix is dependent on the presence of the core region of C4BP.
SAP Binds to the Central Core of C4BP

![Image](82x389 to 274x733)

**Fig. 3. Binding of C4BP chymotryptic fragments to SAP immobilized on matrix.** A, measurement of C4BP bound to the SAP matrix as a function of varying concentrations of C4BP fragments. The solid lines represent the best fit of data to Equation 2. $[M]K_{C4BP-M}$ was fixed to 1.82, and the $K_{core-M}$ value was fixed to 70 nM for the C4BP core fraction (●; $r^2 = 0.94$) and 2970 nM for the C4BP tentacle fraction (○; $r^2 = 0.26$). Data are the means ± S.E. of five experiments. **B**, measurement of 160-kDa C4BP core binding to a fixed concentration of SAP matrix as a function of core concentration (▲). The experiment was performed as described for plasma-purified C4BP (see Fig. 2 and “Results”) in a total volume of 300 µl. The solid line was drawn using $K_{core-M}$ = 58 nM and a maximal concentration of C4BP binding sites of 105 nM. The variance of the curve was $r^2 = 0.96$. Data are the means ± S.E. of three experiments.

A peptide comprising amino acids 27–39 of SAP (Pep-1) has been shown to sustain cell binding when coupled to surfaces (24) and to displace SAP from heparin and other ligands (21). The effect of Pep-1 on C4BP binding to the SAP matrix was tested in the QAC system (Fig. 5B). The presence of 20 µM Pep-1 completely displaced C4BP from the SAP matrix. Carboxymethylated Pep-1 was even more efficient in displacing C4BP from SAP-Sepharose. A peptide with a scrambled sequence (Pep-2) gave only a 20% displacement at 120 µM. Binding of C4BP to Pep-1-coated Wells—Possible binding of C4BP to immobilized Pep-1 was investigated in order to elucidate whether this SAP sequence could sustain binding of C4BP by itself. Almost 30% of the added C4BP tracer was retained by the immobilized peptide, and binding of labeled C4BP was inhibited by Pep-1 (Fig. 6) and unlabeled C4BP (data not shown). In contrast to the interaction between C4BP and SAP, binding of C4BP to immobilized Pep-1 was found not to be dependent on the presence of calcium (Fig. 6, inset).

**DISCUSSION**

The interaction between SAP and C4BP that occurs in the blood stream (3, 4) affects the functions of both proteins. Thus, C4BP bound to SAP has reduced capacity to regulate the classical C3 convertase of complement (23). Moreover, calcium-dependent self-aggregation of SAP is inhibited by binding of C4BP to SAP (3), which may be important to keep SAP soluble.
The $\beta$-chain of C4BP, which is imperative for the interaction between C4BP and protein S, was found to be dispensable for binding of C4BP to SAP. Thus, the multiple carbohydrate side chains of the $\beta$-chain do not affect binding of C4BP to SAP. We have recently shown that C4BP lacking N-linked carbohydrate side chains is able to bind SAP-Sepharose (27), which is surprising because SAP is known to recognize carbohydrate chains (21). However, the carbohydrate side chains still appear to affect the C4BP-SAP interaction because N-monglycosylated C4BP bound SAP with 4-fold lower affinity than intact C4BP. Treatment of C4BP with neuraminidase did not impede binding of C4BP to SAP-Sepharose, indicating that terminal sialic acids are not involved in the interaction.

Digestion of C4BP with chymotrypsin results in two major fragments, the 160-kDa central core and the 48-kDa cleaved-off tentacles (32). From a chymotryptic digest, the core fraction was retained on a SAP-Sepharose column, suggesting that this portion of the molecule contains the SAP-binding site. This was further shown by the specific displacement of C4BP from SAP-Sepharose by the central core fragment. However, it cannot be

excluded that the cleaved-off tentacle fragments, to some extent, affect the C4BP-SAP interaction because the affinity of the binding of the central core to SAP was consistently found to be 2-fold lower than that of the binding of intact C4BP to SAP. It is noteworthy that the eight SCRs and part of the C-terminal non-repeat region of the C4BP $\alpha$-chain belong to the best preserved regions of the molecule in different species (33, 34). This part of C4BP is presumably involved in the polymerization of the chains that occurs during its biosynthesis. It might also be speculated that a SAP-binding motif is preserved in this part of the molecule.

Both the interaction between C4BP and immobilized C4b and the factor I cofactor activity of C4BP are modified by binding of SAP to C4BP (23). SAP probably affects C4b binding by an allosteric mechanism rather than by direct steric hindrance because the binding site on C4BP for C4b appears to be located within the three most N-terminal SCRs of the C4BP $\alpha$-chain. Thus, these SCRs of mouse C4BP were found to support binding of C4b (35). Moreover, results of electron microscopy of the C4BP-C4b complex (36) and comparisons of C4BP sequences of different species (34) suggested that the C4b-binding site is located within the first three SCRs. SAP may bind to multiple $\alpha$-chains (to SCR-8) of the same C4BP molecule, thus acting as a cross-linker. This may lock the C4BP molecule in a "closed" conformation similar to that suggested by Perkins et al. (28) (Fig. 7). This conformation is quite different from the "open" conformation observed in electron microscopy (36), and this difference suggests that the C4BP molecule is highly flexible. The locked closed conformation may reduce the ability of C4BP to interact with multiple C4b molecules on a surface and impede the factor I cofactor activity of C4BP (23). This model is compatible with the observed ability of C4BP to form a ternary complex with SAP and C4b in fluid phase and the ability of this complex to bind protein S (4). The model may also explain how a monoclonal antibody reacting with SCR-6 is able to inhibit the interaction between C4b and C4BP (37). The monoclonal antibody may act as SAP acts, locking C4BP in a closed conformation.

Several compounds known to interfere with C4BP-SAP complex formation were tested in the QAC method, and the obtained results were in accordance with those on record (4, 15,
Phosphorylethanolamine efficiently disrupted the C4BP-SAP interaction; phosphorylcholine had only a minor effect; phosphorylserine was without effect. A recent report demonstrated that both the amino acid residues that bind phosphorylethanolamine as each of the SAP monomers (Fig. 7). If so, the sequence of Pep-1 might contribute to binding of SAP to C4BP even though it may not be the whole binding site.

Several reports suggest that SAP is involved in the opsonization process (17). Thus, SAP interacts with immune complexes (41), macrophage receptors (42), and neutrophils (43). The C4BP-SAP complex may represent inert forms of both C4BP and SAP in blood that are converted to their active states when dissociating from the complex.

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REFERENCES

1. Dahlbäck, B. (1991) Thromb. Haemostasis 66, 49–61
2. Dahlbäck, B., and Sterfio, J. (1981) Proc. Natl. Acad. Sci. U.S.A. 78, 2512–2516
3. Schwabé, R., Dahlbäck, B., and Næsestuen, G. L. (1990) J. Biol. Chem. 265, 21749–21762
4. Schwabé, R., Dahlbäck, B., and Næsestuen, G. L. (1991) J. Biol. Chem. 266, 12996–12990
5. Hillarp, A., and Dahlbäck, B. (1988) J. Biol. Chem. 263, 12759–12764
6. Chung, L. P., Bentley, D. R., and Reid, K. B. (1985) Biochem. J. 230, 133–141
7. Hillarp, A., and Dahlbäck, B. (1990) Proc. Natl. Acad. Sci. U.S.A. 87, 1183–1187
8. Painter, R. H., De Escallón, I., Masey, A., Pinteric, L., and Stern, S. B. (1982) Ann. N.Y. Acad. Sci. 389, 199–213
9. Pepys, M. B., and Baltz, M. L. (1983) Adv. Immunol. 34, 141–221
10. Rubio, N., Sharp, P. M., Rits, M., Zahedi, K., and Whitehead, A. S. (1993) J. Biochem. (Tokyo) 113, 277–284
11. Robey, F. A., and Liu, T. Y. (1981) J. Biol. Chem. 256, 969–975
12. Pepys, M. B., Baltz, M. L., de Beer, F. C., Holford, S., Breathnach, S. M., Black, M. M., Tribe, C. R., Evans, D. J., and Feinstein, A. (1982) Ann. N.Y. Acad. Sci. 389, 286–298
13. Hamazaki, H. (1987) J. Biol. Chem. 262, 1456–1460
14. Hamazaki, H. (1989) Biochem. Biophys. Acta 996, 231–239
15. Schwabé, R. A., Dahlbäck, B., and Næsestuen, G. (1993) Biochemistry 32, 4907–4915
16. Hoffs, M. B., and Butler, P. J. G. (1987) Biochem. Biophys. Res. Commun. 148, 308–313
17. Breathnach, S. M., Koffer, H., Sepp, N., Ashworth, J., Woodrow, D., Pepys, M. B., and Hintner, H. (1989) J. Exp. Med. 170, 1433–1438
18. Hills, P. S., Sauero-Nava, L. D., Duclos, T. W., and Mold, C. (1992) J. Immunol. 149, 3689–3694
19. de Beer, F. C., Baltz, M. L., Holford, S., Feinstein, A., and Pepys, M. B. (1981) J. Exp. Med. 154, 1134–1149
20. Kimishita, C. M., Gewurz, A. T., Siegel, J. N., Ying, S. C., Hugli, T. E., Coe, J. E., Gupta, R. K., Huckman, R., and Gewurz, H. (1992) Protein Sci. 1, 700–709
21. Loveless, R. W., Floyd-O’Sullivan, G., Raynes, J. Y., Yuen, C. T., and Feizi, T. (1992) EMBO J. 11, 813–819
22. García de Frutos, P., Mohamed Aliin, R. I., Härdfeldt, Y., Zäiler, B., Dahlbäck, B. (1994) Biochim. Biophys. Acta 1217, 815–822
23. García de Frutos, P., and Dahlbäck, B. (1994) J. Immunol. 152, 2430–2437
24. Dhawan, S., Fields, R. C., and Robey, F. A. (1990) Biochim. Biophys. Acta 1027, 1284–1290
25. Hillarp, A., Hoffs, M. B., and Dahlbäck, B. (1989) FEBS Lett. 259, 53–56
26. Hillarp, A., and Dahlbäck, B. (1987) J. Biol. Chem. 262, 11300–11307
27. Härdfeldt, Y., García de Frutos, P., and Dahlbäck, B. (1995) Biochem. J. 308, 795–800
28. Perkins, S. J., Chung, L. P., and Reid, K. B. (1986) Biochem. J. 233, 797–807
29. Hoffs, M. B., and Winzor, D. J. (1987) Anal. Biochem. 163, 331–338
30. Persson, E., Hoffs, M. B., and Sterfio, J. (1993) Biochim. Biophys. Acta 1130, 2253–2259
31. Sterfio, J. (1976) J. Biol. Chem. 251, 355–363
32. Naoi, S., Mizuguchi, K., Ishiura, C., and Koyama, J. (1982) J. Biochem. (Tokyo) 125, 578–582
33. Hillarp, A., Thern, A., and Dahlbäck, B. (1994) J. Immunol. 153, 4190–4199
34. García de Frutos, P., and Dahlbäck, B. (1995) Biochim. Biophys. Acta 1261, 285–299
35. Ogata, R. T., Mathias, P., Bradt, B. M., and Cooper, N. R. (1993) J. Immunol. 150, 2273–2280
36. Dahlbäck, B., Smith, C. A., and Müller-Eberhard, H. J. (1983) Proc. Natl. Acad. Sci. U.S.A. 80, 3461–3465
37. Hoffs, M. B., and Sterfio, J. (1983) Biochim. Biophys. Acta 782, 337–343
38. Emsley, J., White, H. E., O’Hara, P. B., Oliva, G., Srinivasan, N., Tickle, I. J., Blundell, T. L., Pepys, M. B., and Wood, S. (1994) Nature 367, 338–345
39. Christner, R. B., and Mortensen, R. F. (1994) Biochim. Biophys. Acta 1249, 228–232
40. Perkin, R. N., Steinkeasserer, A., Norman, D. G., Kieffer, B., Wiles, A. C., Sims, R. B., and Campbell, I. D. (1993) Mol. Biol. 232, 268–284