A 34-AMINO ACID PEPTIDE OF THE THIRD COMPONENT OF COMPLEMENT MEDIATES PROPERDIN BINDING

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In this study, a peptide of 34 amino acids from the M, 40,000 C terminus 6-chain fragment of C3 was found to mediate properdin (P) binding. The treatment of the M, 40,000 fragment with CNBr generated one major M, 17,000 fragment that was capable of binding P. Amino acid sequence data placed the M, was synthesized. This synthetic peptide bound to P by analyzing this sequence for highly conserved segments within residues 1385 to 1540 of the C3 sequence. After this, we have made some progress by localizing the P-binding domain in the C3 molecule. Using isolated polypeptides of C3, it was found that the P-binding site is localized within the M, 40,000 C-terminal 6-chain fragment of C3.

In this study, using CNBr fragments of the M, 40,000 fragment and synthetic peptides, we were able to localize the P-binding site in a segment spanning amino acids 1402 to 1435 of the C3 sequence. A synthetic peptide corresponding to this region, in addition to binding P, exhibited negative regulatory activity in the activation of the alternative pathway.

MATERIALS AND METHODS

Preparation of CNBr fragments of M, 40,000 fragment. The M, 40,000 C-terminal 6-chain fragment of C3 was purified as previously described (18). The generation and isolation of its CNBr fragments were performed by methods described previously (19).

Peptide synthesis. Peptides representing residues 1402 to 1435 (M34), 1187 to 1205 (P28), and 924 to 966 (K42) (see Fig. 4) of the C3 sequence were synthesized, using an Applied Biosystems 430A synthesizer, employing the standard solid phase method on 4-methylbenzhydrolamine resin (21, 22). The peptides were deprotected and removed from the resin by anhydrous hydrogen fluoride in the presence of anisole (23). Next, the peptides were purified by HPLC on a C18 reversed phase column (Vydac, Hesperia, CA) and their amino acid sequence was confirmed by Edman degradation (24). 

Assays for binding of P to C3b and synthetic peptides. Three different assays were employed: 1) The direct binding of P to C3b and other C3 fragments, as well as the inhibitory effect of synthetic peptides on this binding, was tested by an ELISA described elsewhere (17). Briefly, microtiter wells were coated (overnight, 4°C) with 30 pgiml. Wells were washed and saturated with PBS containing 1% OVA (30 min, 22°C). Next, 50 ul of P or serum, serially diluted, were allowed to react with the C3b or its fragments (30 min 22°C). Bound P was detected with a monoclonal anti-P antibody (Cytopech, San Diego, CA) or a polyclonal anti-P antibody (Atlantic Antibodies, Scarborough, ME) and the corresponding peroxidase-conjugated rabbit anti-mouse Ig or rabbit anti-goat Ig. For the inhibition of P binding to C3b by synthetic peptides or fluid phase C3b, 20 ng P was preincubated (15 min, 22°C) with different amounts of peptides (16 to 1000 ng) or C3b (0.5 to 19 ng) before addition to C3b-coated plates. 2) The direct binding of peptides to P was tested as previously described (for the binding of estradiol) to its R (27). Briefly, 100 nM P or an equal amount of factor H in PBS was incubated (48 h, 22°C) with 200 nM labeled M34. The protein-peptide complexes were separated from free peptide by gel filtration on a Sephadex G-50 (Pharmacia, Uppsala, Sweden) column (50 x 1.8 cm) equilibrated in PBS. 3) The effect of synthetic peptides on alternative pathway activation was measured by quantitating the lysis of RAE by normal human serum (28). The inhibition of RAE lysis by the different synthetic peptides was calculated in relation to that obtained with serum in the absence of any peptide (50% lysis).

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1 To whom reprint requests should be addressed.
2 Abbreviations used in this paper: C3b, major cleavage fragment of C3; P, properdin; RAE, rabbit erythrocytes.
RESULTS

Localization of the P-binding site to a M, 17,000 fragment of C3. To localize further the P-binding site, CNBr fragments of the M, 40,000 C-terminal a-chain fragment were generated. The CNBr fragments were isolated by reversed-phase HPLC (Fig. 1). Analysis of the different HPLC fractions by ELISA for binding to P revealed that only the fractions 25 and 26 bound P. SDS/PAGE analysis showed that these fractions contained a M, 17,000 fragment (data not shown). Edman degradation of this fragment found the 10 amino-terminal residues to be Thr-Gly-Phe-Ala-Pro-Asp-Thr-Asp-Asp-Leu. Comparing this sequence with the amino acid sequence predicted from the C3 cDNA, and considering the location of the CNBr cleavage sites within the M, 40,000 fragment, the M, 17,000 P-binding fragment was placed within amino acid 1387 to 1540 of the C3 sequence.

Localization of the P-binding site to a 34-amino acid sequence by use of synthetic peptides. The sequence of the M, 17,000 fragment was analyzed for segments of high sequence conservation with mouse C3 (29) and rabbit C3 (30), which bind P, and low sequence conservation with the homologous proteins C4 (31), mouse C5 (32), and \( \alpha_2 \)-macroglobulin (33), which do not bind P (Fig. 2). The sequence of this fragment is highly conserved within the C3 from different species, but is poorly conserved in the corresponding segment of human C4, mouse C5, and \( \alpha_2 \)-macroglobulin (see Discussion). Most striking were the gaps in the segment 1404 to 1432. Consequently, the segment 1402 to 1435 was synthesized and tested for its ability to inhibit the binding of P to C3b. Figure 3 shows a representative experiment of the inhibition of P binding to C3b by this peptide (M34) or by fluid phase C3b. Fluid phase C3b at a concentration of 2.25 pM inhibited the binding of P to microtiter plate-bound C3b by 50%. Inhibition of P binding to C3b by the peptide M34 (120 nM, 50% inhibition) suggested an affinity of M34 for P 55-fold lower than that of fluid phase C3b. Control synthetic peptides (maximum concentration, 1 mM) of similar length representing other areas of C3 had no effect on this interaction. The sequence of these peptides is shown in Figure 4. The direct binding of M34 to P was tested by incubation of labeled P with factor H for 48 h at room temperature. The peptide-protein mixtures were then gel filtered through a G-50 Sephadex column equilibrated in PBS. Figure 5 shows that when the P-M34 complex was analyzed by gel filtration a distinct peak of radioactivity was eluted in the void volume, followed by a large peak that corresponded to the free peptide. In contrast, when gel filtration of the factor H-M34 mixture was performed, the void volume peak was not de-
The aim of this study was to localize further the P-binding site of C3, presumably by competing for binding. The complete inhibition of P binding to C3b by M34 suggests that C3b interacts with the use of a synthetic peptide covering this region. The peptide M34 bound to P and inhibited its interaction with P, as well as the Ba fragment of B, bind C3b (36–38); 5) the conglutinin-binding site in C3 was localized to a conformational site spanning residues 1187 to 1249 of the C3 sequence (40); and 7) a M, 30,000 N-terminal factor H fragment binds to C3 and serves as cofactor for its cleavage by factor I (41).

Previous studies of ours have localized the P-binding site in C3 within a M, 40,000 C-terminal α-chain fragment (17). The aim of this study was to localize further the P-binding site within this M, 40,000 fragment. After showing that a M, 17,000 fragment binds P, its primary sequence (residues 1387 to 1540) was compared with the sequences of C3 from other species, which bind P, and to other homologous proteins. Because the M34 peptide binds P and inhibits its binding to C3b, we tested the ability of M34 to inhibit the alternative pathway. Figure 6 illustrates the inhibitory effect of the M34 peptide on the lysis of RaE by human serum. A 60-fold molar excess of M34 over the C3 concentration in serum blocked, by 50%, the lysis of RaE (34). The control peptides in the filtration. 1251-labeled M34 (1197–1224) KFLTTAKDKNRWEDPCLOLYWATSYA had no inhibitory effect.

Inhibition of the alternative pathway by the M34 synthetic peptide. Because the M34 peptide binds P and inhibits its binding to C3b, we tested the ability of M34 to inhibit the alternative pathway. Figure 6 illustrates the inhibitory effect of the M34 peptide on the lysis of RaE by human serum. A 60-fold molar excess of M34 over the C3 concentration in serum blocked, by 50%, the lysis of RaE via the alternative pathway. The control peptides in the same concentration had no inhibitory effect.

### DISCUSSION

There is considerable interest in the multifunctional role of C3 because of its interactions with several serum proteins, cell surface receptors, and proteins from foreign particles. The elucidation of the molecular features related to the different C3 functions requires structural analysis of the C3-ligand interactions. Nine binding sites involving the C3 molecule have been studied: 1) a 6-amino acid C3 peptide (residues 72 to 77) has been found to bind to the C3α-R (34); 2) a 10-amino acid C3 peptide (residues 1227 to 1236) binds to the C3d-R (CR2) (19) and mimics the effect of C3d in the growth of B cells (F. Melchers and J. D. Lambris, unpublished observations); 3) a 21-amino acid C3 peptide (residues 1383 to 1403) binds to the iC3b-R (CR3) (35); 4) a M, 33,000 C-terminal factor B fragment, as well as the Ba fragment of B, bind C3b (36–38); 5) the conglutinin-binding site in C3 was localized to a conformational site spanning residues 1187 to 1249 of the C3 sequence (40); and 7) a M, 30,000 N-terminal factor H fragment binds to C3 and serves as cofactor for its cleavage by factor I (41).

The binding of M34 to P was inhibited by excess cold M34 (data not shown). In addition, when 125I-labeled P28 (19) was incubated with P the elution profile was similar to that of M34-factor H mixture.

Figure 4. Amino acid sequence of the three synthetic peptides used in this study.

Figure 5. The binding of radiolabeled peptides to P as determined by gel filtration. 125I-labeled M34 (○) or P28 (▲) incubated with P or 125I-M34 (●) with factor H, for 48 h at 25°C and subsequently gel filtered on Sephadex G-50 column. Fractions (2 ml) were collected and counted for radioactivity.
earlier studies on the interaction of P with zymosan-bound C3b in which the stoichiometry was determined to be 1 (42). The appKa of fluid phase C3b for P is 55-fold higher than that of the M34 peptide and 64-fold lower than the Ka of zymosan-bound C3b (42). The affinity differences (if the appKa approximates the Ka) between the zymosan-bound C3b vs fluid phase C3b was also observed in the interaction of factor H with C3b (42, 43). The inhibition of the alternative pathway by this peptide suggests that it may be used to study both the role of P in the alternative pathway during infection and the mechanism of C3 convertase stabilization.

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REFERENCES


ERRATUM

In the March 1, 1988 issue of The Journal, Volume 140, No. 5, page 1579 in the article by Maria E. Daoudaki, J. David Becherer, and John D. Lambris entitled "A 34-Amino Acid Peptide of the Third Component of Complement Mediates Properdin Binding" mistakes were made in Figure 4 and in the "Materials and Methods" section due to discrepancies in the numbering of amino acid residues of C3. This resulted from the inadvertent inclusion or omission of the signal peptide sequence. The authors have submitted a new figure and legend, which are presented here to clarify this matter.

Figure 4. Amino acid sequence of the three peptides used in this study. The numbering of the amino acid residues is based on the deduced amino acid sequence of C3 (Ref. 20) after subtracting the signal peptide sequence.

ANNOUNCEMENTS

Immunology Faculty Positions. The Department of Microbiology invites applications for 2 full-time tenure-track positions at any faculty level. Applicants must have a Ph.D. or M.D. and at least 2 years of postdoctoral experience. Successful candidates must establish competitive, funded research programs and help teach medical and graduate students. The Medical College and its Cancer Center are expanding in areas of molecular biology, neurosciences, bone marrow and organ transplantation, gastroenterology, and ophthalmology. Applicants with interests that may complement these areas are encouraged to apply. Send resume, summary of research interests, and names of three references to: Immunology Search Committee, Department of Microbiology, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226. Affirmative action/equal opportunity employer.

Research Associate (M.D. or Ph.D.) to work on the molecular biology of inherited humoral immunodeficiencies in humans. Three years experience in molecular immunology required, especially genomic cloning, DNA and RNA sequencing, tissue culture and immunochemical assays. Position for 2 years beginning immediately. Salary negotiable. Send CV and names of two references to: Personnel Department, Bowman Gray School of Medicine of Wake Forest University, 300 South Hawthorne Road, Winston-Salem, NC 27103.

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