Analysis of the interactions between properdin, the third component of complement (C3), and its physiological activation products

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The interactions of properdin with both surface-bound and fluid-phase C3 (the third component of complement) and its activation products have been investigated by using a purified preparation of the 'native' form. At physiological ionic strength, a weak interaction with cell-bound C3b (the larger activation fragment of C3) could be demonstrated. In the presence of Factor B this interaction was enhanced, and further enhancement was seen when C3bBb sites were formed on the erythrocytes. The avidities of properdin for cell-bound iC3b (the initial product of Factors I and H action on C3b) and C3b were compared at low ionic strength, with that measured for iC3b being less than that for C3b. In contrast, the affinities of properdin for fluid-phase C3b, iC3b and C3c (the larger product of Factors I and H or CR1 (the C3b receptor) action on iC3b) were all very similar, and apparently much weaker than that for cell-bound C3b. No interaction with either native C3 or, more surprisingly, C3i (haemolytically inactive C3) could be detected. Properdin also inhibited Factor I binding to, and action upon, cell-bound C3b, but did not inhibit Factor I action on fluid-phase C3b. These data permit a more detailed description of the roles of properdin in the alternative pathway of complement activation, emphasizing its importance in concentrating activation at the activating surface.

INTRODUCTION

Properdin was first described as a serum protein that participated in an antibody-independent immune defence reaction directed against bacterial and yeast infections (Pillemer *et al.*, 1954). This system is now recognized as the alternative pathway of complement activation, with properdin an important regulatory component of the system (Pangburn & Müller-Eberhard, 1984). Properdin is composed of identical 56 kDa glycoprotein subunits (Pensky *et al.*, 1968; Minta & Lepow, 1974; Reid & Gagnon, 1981). Electron micrographs of the purified protein show a mixture, predominantly dimers, trimers and tetramers, but also higher oligomers, of cyclic polymers (Smith *et al.*, 1984). Its serum concentration is approx. 20 µg/ml.

The critical event in activation of the alternative pathway of complement is the deposition of C3b on to a surface and the subsequent binding, either of Factor B or of the regulatory protein, Factor H. Binding of Factor H to C3b (favoured on 'non-activating' surfaces) is the first step in the Factor I-mediated inactivation of C3b. By contrast, binding of Factor B (promoted on 'activators') results in formation of the C3 convertase, C3bBb, and

hence further C3 activation (Fearon & Austen 1977a,b; Pangburn & Müller-Eberhard, 1978). Properdin, by stabilizing C3bBb against intrinsic decay, amplifies this response (Fearon & Austen, 1975). Under appropriate conditions polyvalent binding of properdin to C3b, C3bBb and C3bBb can occur (Farries *et al.*, 1987; Medicus *et al.*, 1976). In addition, binding of properdin, at low ionic strength, to surface-bound C3b in the absence of Factor B has been reported (Medicus *et al.*, 1976).

Clearly, definition of the interactions of properdin with the various products of C3 activation is important to our understanding of mechanisms involved in alternative-pathway activation. However, previous investigations of these have used preparations of properdin that contain undefined proportions of the 'activated' form (Chapitis & Lepow, 1976; Lambris *et al.*, 1984; Medicus *et al.*, 1976; DiScipio, 1981). We have recently demonstrated that 'activated' properdin, which binds to C3b even at physiological ionic strength (Shreiber *et al.*, 1975; Medicus *et al.*, 1976), is a laboratory artefact, and that the physiologically relevant properties of properdin are those of 'native' properdin (Farries *et al.*, 1987). Separation of the two forms of properdin has enabled a

Abbreviations used: PBS, phosphate-buffered saline (composition is given in the text); Na/K phosphate, buffers made from KH_2PO_4 solutions of the required phosphate molarity and adjusted to the correct pH with NaOH; CFD, complement-fixation diluent (composition is given in the text); BSA, bovine serum albumin; CR1, complement receptor type 1 (the C3b receptor); PAGE, polyacrylamide-gel electrophoresis; C3, complement component C3; C3_i, 'native' or haemolytically active C3; C3_i, haemolytically inactive C3; C3b and Bb, the larger activation fragments of the complement components C3 and Factor B respectively; C3bBb, alternative-pathway C3 convertase; iC3b, the initial product of Factors I and H action on C3b; C3c and C3dg, the major products of Factors I and H or CR1 action on iC3b; CVF, cobra (*Naja Naja kaouthia*) venom factor CVF·Bb, cobra-venom-factor C3 convertase; SBTI, soybean trypsin inhibitor; EA, sheep erythrocytes coated with a rat monoclonal anti-sheep E antibody (SO16); EAC3b, EA coated with C3b; yeast-C3b, yeast cell walls coated with C3b; TSK HW-55(S), Fractogel TSK HW-55(S); $\text{iPr}_2\text{P-F}$, di-isopropyl fluorophosphate; ACD, acid/citrate/dextrose; C3bBbP, properdin-stabilized C3 convertase; C3bB and C3bH, complexes of C3b with Factors B and H respectively.

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reinvestigation of its properties using the 'native' form only. In the present paper, analysis of the interaction of 'native' properdin with C3, C3-derived fragments and complexes of C3 and its fragments with other plasma proteins are reported. In addition, a detailed analysis of the potentially important inhibition of Factor I-mediated inactivation of EAC3b by properdin (Medicus *et al.*, 1976) has been made.

MATERIALS AND METHODS

Buffers and reagents

PBS is 9.6 mM-K/Na phosphate/0.14 M-NaCl, pH 7.2. CFD is 4 mM-sodium barbitone buffer (pH 7.2)/0.145 M-NaCl/0.83 mM-MgCl₂/0.25 mM-CaCl₂, and is made with 'CFD-tablets' (Oxoid, London E.C.4, U.K.). PBS/dextrose is PBS diluted 1:2 in 5% (w/v) D-glucose. BSA, bovine pancreatic trypsin, iPr₂P-F, SBTI, DEAE-Sephacel and CM-Sephadex C-50 were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Activated-thiol-Sepharose 4B, Sepharose 6B and Sephadexes G-75 (superfine) and G-150 (superfine) were purchased from Pharmacia Fine Chemicals (Uppsala, Sweden). Fractogel TSK HW-55(S) was obtained from Merck (Darmstadt, Germany) and Iodogen was purchased from Pierce Chemicals (Rockford, IL, U.S.A.). Monoclonal antibody OX21 [anti-(Factor I); Hsuing *et al.*, 1982] and OX23 [anti-(Factor H); Sim *et al.*, 1983] ascites fluids were the gift of Dr. A. Williams, MRC Cellular Immunology Unit, Oxford, U.K. Antibody preparations from these ascites and from a polyclonal rabbit anti-CVF antiserum were made by using the octanoic acid procedure of Steinbuch & Audran (1969). Anti-CVF-Sepharose, C3b-Sepharose, anti-(Factor I)-Sepharose and anti-(Factor H)-Sepharose were prepared essentially as described by March *et al.* (1974).

Complement components

The complement components C3, properdin and Factors H, B and D were purified from human plasma as described previously (Harrison & Lachmann, 1986; Farries *et al.*, 1987). Factor I was isolated from the same multicomponent preparation, essentially as described by Harrison & Lachmann (1986), but with the omission of chromatography on hydroxyapatite.

Purified properdin was converted into its 'activated' and 'native' forms by gel filtration on Fractogel TSK HW-55(S) (Farries *et al.*, 1987). Native properdin was stored unfrozen at 4 °C and was used in all experiments except those where unfractionated or 'activated' properdin is specified.

C3b was generated from purified C3 by using a CVF·Bb C3 convertase (Nicol & Lachmann, 1973). Some residual C3i, not cleaved by CVF·Bb, was converted into C3b with trypsin, and the reaction was terminated by the addition of excess SBTI. C3a was removed by gel filtration in 20 mM-Na/K phosphate/1 M-NaCl, pH 6.0, on Sephadex G-75 (superfine). The C3b pool was treated with 1.5 mM-iPr₂P-F for 2 h at 4 °C, and CVF and CVF·Bb removed by passage through anti-CVF-Sepharose. iC3b was generated from C3b (200 mg) by incubation with Factors I (1.8 mg) and H (2 mg) for 3 h at 37 °C and isolated by gel filtration on Sephadex G-75 (superfine) followed by immuno-adsorption with OX21 [anti-(Factor I)]- and OX23 [anti-(Factor H)]-Sepharoses. SDS/PAGE analysis indicated

that tryptic activity had not been fully inhibited and that some further proteolysis (about 30%) of the 68 kDa polypeptide of iC3b, generating C3c- and C3d-like fragments, had occurred. C3i was made by treatment of purified C3 with 0.125 M-methylamine for 90 min at 37 °C in 12.5 mM-sodium borate/0.15 M-NaCl, pH 8.5. Native C3 (C3n) was freshly prepared before use by adsorbing C3 with activated-thiol-Sepharose to remove C3i (Tack *et al.*, 1981). C3c was prepared as described by Davis *et al.* (1984) and CVF by the method of Harrison & Lachmann (1986).

Proteins were radiolabelled with ¹²⁵I by using the solid-phase Iodogen procedure of Fraker & Speck (1978).

Cellular intermediates and binding studies

Sheep erythrocytes coated with antibody (EA) or antibody plus C3b (EAC3b) were prepared as previously described (Harrison & Lachmann, 1986; Farries *et al.*, 1987). EAiC3b were prepared by incubating EAC3b (2.5 × 10⁹/ml) in PBS and at 37 °C for 60 min with Factor H (2 mg/ml) and Factor I (84 µg/ml). The cells were then washed thoroughly in PBS. Uptake of C3b and iC3b was assessed using ¹²⁵I-radiolabelled clones 3, 4 and 9 (Lachmann *et al.*, 1980, 1981; Ross *et al.*, 1983).

Binding of radiolabelled proteins to cellular intermediates was measured as described previously (Cuatrecasas & Hollenberg, 1976; Iida *et al.*, 1982; Farries *et al.*, 1987). Because of the heterogeneity in subunit composition of the properdin oligomer, functional avidities for properdin binding were calculated by using the subunit molecular mass of 56 kDa.

Factor I assay on EAC3b

The effect of properdin on Factor I activity against cell-bound C3b was assessed by incubating EAC3b (2 × 10⁸ cells/ml; 50000 C3b molecules/cell), Factor H (0.12 mg/ml) and serial dilutions of Factor I in 0.10 ml of 33% (v/v) CFD containing 3.3% (w/v) D-glucose and BSA (2 mg/ml), in the presence or absence of properdin (21 µg/ml). After incubation (37 °C for 5 min), all samples were washed twice in PBS and once in CFD (4 °C, 1 ml/wash). The cells were then resuspended in 0.1 ml CFD containing BSA (2 mg/ml), properdin (unfractionated) (20 µg/ml), Factor B (40 µg/ml) and Factor D (1 µg/ml). After 5 min incubation at 37 °C, 1 ml of 1% (v/v) guinea-pig serum in CFD/10 mM-EDTA was added to each sample and the incubation continued a further 15 min. Haemolysis was then measured by pelleting the cells and reading the absorption of the supernatant at 415 nm. (Factor I-mediated cleavage of bound C3b abolishes its haemolytic activity.)

Assay of Factor I activity on fluid-phase C3b

Factor I-mediated cleavage of ¹²⁵I-C3b (50 µg/ml, 20 Ci/mg) was assayed in 0.1 ml volumes of PBS/dextrose/5 mM-NaN₃ with Factor I (0.5 µg/ml) and Factor H (0.1 mg/ml). These were incubated at 37 °C in the presence of various concentrations of 'native' properdin. After 1 and 2 min incubation, 40 µl portions were taken and added to 50 µl of 1% (w/v) SDS/1% (v/v) 2-mercaptoethanol. These samples were analysed by SDS/PAGE, using the buffer system of Laemmli (1970) and an 8% (w/v) polyacrylamide gel. After autoradiography of the dried gel, C3b cleavage was determined by excision and counting of the C3b α'-chain-derived polypeptides from each track.

Fig. 1. Analysis of the binding of properdin to EA, EAC3b, EAC3bB and EAC3bBb

Dilutions of a solution of ^{125}I -radiolabelled properdin ($80 \mu\text{Ci}/\text{mg}$), ranging from 75 to 1200 ng/ml, were mixed with (6.25×10^7 cells/ml of) EA ($\square$), EAC3b ($\blacksquare$), EAC3b plus Factor B ($0.1 \text{ mg}/\text{ml}$) (EAC3bB; $\blacktriangle$) or EAC3b plus Factor B ($0.1 \text{ mg}/\text{ml}$) and Factor D ($3.3 \mu\text{g}/\text{ml}$) (EAC3bBb; $\triangle$) in 0.2 ml of PBS/BSA ($2 \text{ mg}/\text{ml}$)/ 2 mM - MgCl_2 . These were incubated at 37°C for 20 min before duplicate 0.1 ml samples were assayed for binding. The results were analysed by the Scatchard method and expressed as binding of 56 kDa monomers. The EAC3b used carried approx. 5800 C3b molecules/cell.

RESULTS

Binding of properdin to EA, EAC3b and EAC3bBb

The avidities of 'native' properdin for the cell-bound complexes EAC3b, EAC3bB and EAC3bBb were determined by using ^{125}I -labelled properdin. The results are shown in Fig. 1. Factors B and D were used in moderate excess to enhance formation of the unstable complexes of B and Bb with C3b (Pangburn, 1986). At physiological ionic strength the avidity of properdin for EAC3b was too low to be measured. Nevertheless, there was significantly more binding to EAC3b than to EA. The apparent avidity (K_{app}) of properdin for the cellular

Fig. 2. Analysis of the binding of properdin to EAC3b and EAiC3b at low ionic strength

EAiC3b were prepared from EAC3b and titrated as described in the Materials and methods section. Conversion into iC3b was between 80 and 100% complete and the cells used carried approx. 5000 molecules of C3b or iC3b/cell. Dilutions of a solution of ^{125}I -radiolabelled properdin ($0.1 \text{ mCi}/\text{mg}$), ranging from 75 to 1200 ng/ml, were mixed with EAC3b ($\triangle$) or EAiC3b ($\blacktriangle$) (1.25×10^8 cells/ml) in 0.2 ml of PBS/dextrose/ 5 mM - NaN_3 /BSA ($4 \text{ mg}/\text{ml}$). These were incubated at 37°C for 30 min before duplicate 0.1 ml samples were assayed for binding. The data are expressed as binding of 56 kDa monomers.

intermediate was considerably enhanced, to about $4 \times 10^8 \text{ M}^{-1}$, in the presence of Factor B. Even though measurement of binding to EAC3bBb must be performed under non-equilibrium conditions, the estimated avidity for EAC3bBb (about $2 \times 10^9 \text{ M}^{-1}$) was 5-fold higher than that for EAC3bB. In addition, more properdin-binding sites were measured on EAC3bBb ($21\,900$ 56 kDa subunits/cell) than on EAC3bB ($15\,200$ /cell); these values correspond to three to four monomeric units per C3b.

Binding of properdin to EAiC3b

The binding of properdin, at low ionic strength, to EAC3b and to EAiC3b was also analysed (Fig. 2). Conversion into EAiC3b significantly reduced the apparent avidity of properdin for the cells (from $3.1 \times 10^7 \text{ M}^{-1}$ to $2.3 \times 10^7 \text{ M}^{-1}$). As the conversion of C3b into iC3b was not complete (but $> 80\%$), residual C3b may be responsible for much of the apparent binding to EAiC3b. The EAiC3b cells were derived from the C3b-bearing cells, and the total number of C3-derived ligands will therefore be the same on both cell types.

Interaction of properdin with fluid-phase C3 derivatives

The relative affinities of soluble C3 products for properdin were assessed by titrating their ability to inhibit, at low ionic strength, ^{125}I -properdin binding to EAC3b (Fig. 3). Properdin bound to C3b, iC3b and C3c with very similar affinities, with 50% inhibition occurring at about $5 \times 10^{-7} \text{ M}$ ($0.1 \text{ mg}/\text{ml}$). This implies an approxi-

Fig. 3. Analysis of properdin binding to fluid-phase C3 derivatives

C3 derivatives were prepared as described in the Materials and methods section and dialysed into PBS/dextrose. Mixtures containing 600 ng of ^{125}I -labelled properdin/ml (75 $\mu\text{Ci}/\text{ml}$), dilutions of C3 derivatives, BSA (4 mg/ml) and 5 mM- NaN_3 in 0.2 ml of PBS/dextrose were incubated at 37 °C with EAC3b (2.5×10^8 cells/ml). Duplicate 0.1 ml samples from each incubation were assayed for binding after 30 min. $\square$, C3n; $\circ$, C3i; $\triangle$, C3b; $\blacktriangle$, iC3b; $\bullet$, C3c. The EAC3b used carried approx. 5000 C3b molecules/cell.

mate affinity of $2 \times 10^6 \text{ M}^{-1}$, which is only about 7% of the avidity observed on erythrocyte-bound C3b ($3.1 \times 10^7 \text{ M}^{-1}$; Fig. 2) in the same low-ionic-strength buffer. There was no interaction between native C3 and properdin and, surprisingly, C3i, a functional analogue of C3b, also failed to inhibit properdin binding to EAC3b.

Effect of properdin on Factor I activity on EAC3b

If the inhibition of Factor I action on C3b by properdin reported by Medicus *et al.* (1976) occurred under physiological conditions, it would provide a potentially important feature in the control of alternative-pathway activation. Our observations on properdin binding to cell-bound and fluid-phase C3 derivatives also suggest that a comparison of the effect of properdin on fluid-phase and cell-bound substrates of Factor I would be valuable.

The inactivation of cell-bound C3b, by serial dilutions of Factor I, was therefore assessed as described in the Materials and methods section. After incubation with Factor I, stabilized C3 convertases (C3bBbP) were assembled on residual C3b sites, and cells bearing these lysed by the addition of guinea-pig serum in EDTA. Cells carrying only iC3b and/or C3dg are not lysed. The results (Fig. 4) show that properdin at 21 $\mu\text{g}/\text{ml}$ significantly inhibited the activity of Factor I at concentrations of less than 4 $\mu\text{g}/\text{ml}$.

Effect of properdin on Factor I binding to EAC3b

As Factor I requires a cofactor, Factor H, for its action on C3b, the above effect could have been caused by inhibition of either one of the components. However, binding studies have shown that properdin does not compete with Factor H for binding to EAC3b (Pangburn & Müller-Eberhard, 1978; Farries, 1986) or zymosan-C3b (DiScipio, 1981). The inhibition of Factor I activity is likely, therefore, to be a direct effect of properdin on Factor I, and suggests that the two

Fig. 4. Inhibition by properdin of Factor I activity on EAC3b

The effect of properdin on the inactivation of cell-bound C3b by serial dilutions of Factor I was assessed as described in the Materials and methods section. Haemolysis (adsorption at 415 nm) is shown as a function of Factor I concentration in the presence ($\triangle$) or absence ($\blacktriangle$) of properdin (21 $\mu\text{g}/\text{ml}$).

Fig. 5. Inhibition by properdin of Factor I binding to EAC3b

Serial dilutions of properdin were mixed with 125 ng of ^{125}I -labelled Factor I/ml (1.8 mCi/mg) and EAC3b (2.5×10^8 cells/ml). These mixtures were incubated at 4 °C for 5 min in the presence ($\triangle$) or absence ($\blacktriangle$) of Factor H (0.1 mg/ml). Triplicate 55 μl samples from each, still at 4 °C, were then assayed for Factor I binding. The EAC3b used contained approx. 5800 C3b molecules/cell.

components have competing binding sites on C3b. Non-equilibrium association of ^{125}I -Factor I with EAC3b, in the presence of Factor H, can be detected at 4 °C in low-ionic-strength buffer (Pangburn & Müller-Eberhard, 1978). This binding was therefore assayed in the presence

Fig. 6. Effect of properdin on Factor I activity against fluid phase C3b

The time course of cleavage of ^{125}I -labelled C3b by Factors I ($0.5 \mu\text{g/ml}$) and H ($100 \mu\text{g/ml}$), at 37°C and in low-ionic strength buffer, was measured in the presence of various concentrations of properdin, as described in the Materials and methods section. Each data point shown represents the average of two measurements from duplicate incubations. $\square$, $0 \mu\text{g}$ of properdin/ml in the absence of Factors I and H; $\bullet$, $\blacktriangle$, $\blacksquare$ and $\triangle$, Factors I and H and 0 , 0.9 , 9 , and $90 \mu\text{g}$ of properdin/ml respectively.

of serial dilutions of properdin (Fig. 5). In the presence of Factor H, properdin potently blocked Factor I binding. Inhibition was half-maximal at approx. 600 ng of properdin/ml (or 10^{-8} M properdin monomers). As properdin has already been shown to inhibit Factor I action, the reduced binding cannot be explained by accelerated conversion of C3b into iC3b. One explanation for these data is that properdin and Factor I have competing binding sites on C3b. In contrast with the findings of Pangburn & Müller-Eberhard (1978), no detectable Factor I binding was found in the absence of Factor H, even in the absence of properdin.

Effect of properdin on Factor I activity on fluid-phase C3b

The cleavage of ^{125}I -C3b by Factor I in the presence of Factor H was measured at low ionic strength in the presence of various concentrations of properdin (Fig. 6). In contrast with the effect observed on cell-bound C3b, a large excess of properdin did not significantly inhibit the initial rate of the cleavage reaction. Similarly, properdin did not inhibit Factor-I-mediated cleavage of C3i (results not shown).

DISCUSSION

A number of studies of the interaction of properdin with C3, its complexes and its derivatives have been published (Schreiber *et al.*, 1975; Medicus *et al.*, 1976; Chapitis & Lepow, 1976; DiScipio, 1981; Lambris *et al.*, 1984). However, in all of these there has been no attempt to separate the contributions of 'native' and 'activated' properdin, and the preparations used will have contained

variable amounts of each species. We have recently shown that 'activated' properdin is an artefact of isolation with aberrant C3-binding properties (Farries *et al.*, 1987), and we present here a detailed analysis of the interactions of 'native' properdin with both surface-bound and fluid-phase C3 derivatives.

Interpretation of the data obtained from Scatchard analysis of the binding of properdin to surface-bound intermediates is complex for two reasons: the structure of the protein and the nature of its ligands. Properdin is an oligomeric protein of identical subunits, but with variable subunit composition (Reid & Gagnon, 1981; Smith *et al.*, 1984). Thus heterogeneous multivalent interactions are possible, with higher oligomers, containing a greater number of potential binding sites, having a higher avidity for surface-bound intermediates (Farries *et al.*, 1987). Complexities also arise because the ligands EAC3bB and EAC3bBb are themselves unstable. The observed avidities will therefore be less than the true values. In the analysis of the data presented in the present paper, apparent avidities were calculated by using the only unambiguous value available, the properdin subunit molecular mass of 56 kDa . Although the measured avidities of properdin for each ligand cannot be regarded as absolute values, the relative avidities will represent true differences.

Although weak, the binding of properdin at physiological ionic strength to EAC3b was demonstrably greater than that to EA. Its avidity for EAC3bB was considerably increased over that for EAC3b, and approx. 5-fold greater still for EAC3bBb. The estimated binding capacities for properdin of EAC3bBb ($21\,900$ monomers/cell) and EAC3bB ($15\,200$ monomers/cell) were similar,

and correspond to three or four monomers of properdin/C3b ligand. This binding should be selective for higher oligomers of properdin, as these have the higher avidities for EAC3bB and EAC3bBb. These values are similar to those published by DiScipio (1981), who found an avidity of properdin for zymosan-C3bB of $1.1 \times 10^8 \text{ M}^{-1}$, and a properdin/C3b ratio of 4.2 monomers/C3b ligand. (In that study the properdin was unfractionated and the avidity calculated by assuming the molecule to be trimeric.)

Although the avidities of properdin for EAC3b and EAiC3b are too low to be measured under physiological conditions, they can be compared at low ionic strength. Properdin bound significantly better to EAC3b than to EAiC3b. [This is consistent with the release of 'activated' properdin from EAC3b treated with Factor I reported by Medicus *et al.* (1976).] Although the avidity for EAC3b was less than 10% of that found for EAC3bB at normal ionic strength, it was still 15 times greater than that estimated for fluid-phase C3b at the same low ionic strength. (The avidity of properdin for fluid-phase C3b, again at low ionic strength, was also indistinguishable from that for iC3b and C3c.)

Although the lack of any demonstrable interaction between C3n and properdin was as anticipated, a surprising finding was that 'native' properdin bound C3b but not C3i. These C3 derivatives have similar structural properties and express similar interactions with Factors B, H and I (Isenman *et al.*, 1981; Pangburn *et al.*, 1981; von Zabern *et al.*, 1981). The novel distinction described here suggests that C3b and C3i are not as similar as is generally assumed. However, an interaction between properdin and C3iB has been demonstrated by chemical cross-linking of properdin to the Factor B component of the complex (Farries *et al.*, 1988) and an interaction between C3i and 'activated' properdin has also been observed (results not shown; Schreiber *et al.*, 1975).

From these binding data it can be deduced that, within C3, there exists a properdin-binding site. Clearly this can contribute only a part of the site existing on EAC3bB or EAC3bBb, as the affinity of properdin for these complexes is far higher than for EAC3b. The site appears to be more favourably expressed on surface-bound C3b than on the fluid-phase ligand. Analysis of the interaction of properdin with fluid-phase derivatives has indicated that the site is hidden in native C3, but expressed on the major activation products C3b, iC3b and C3c. It may well also be protected, at least in part, in C3i. These data are in accord with the findings of Lambris *et al.* (1984), who reported similar affinities of properdin for C3b and C3c. In that study interactions were defined using C3-derivative-coated microtitre plates, and further analysis with isolated C3 fragments identified a site of interaction between properdin and C3 on the 43 kDa C-terminal portion of the C3 α -chain. This portion of the polypeptide is present in C3b, iC3b and C3c, supporting the deductions made from binding studies with the intact ligands. Although these data are broadly in agreement with the density-gradient investigations of Chapitis & Lepow (1976), we find no evidence for a stronger association of properdin with C3c than that with C3b. Both Lambris *et al.* (1984) and Chapitis & Lepow (1976) studied interactions at physiological ionic strength and so their findings presumably relate to 'activated' properdin.

From both our data (the present paper; Farries *et al.*, 1987, 1988) and from the work of others it is possible to put forward a detailed model for the participation of properdin in the alternative-pathway-dependent amplification of C3 activation. This is illustrated in Scheme 1 and outline below.

(i) The interactions between properdin and fluid-phase C3 derivatives are either very weak or undetectable. Moreover, there is no difference in affinity between C3b, C3c or iC3b and properdin. As the latter fragments play no part in the amplification pathway, properdin can be deduced to have negligible influence on 'tick-over' of the alternative pathway (hydrolysis of C3 to C3i and random low-level fluid-phase proteolysis of C3 to C3b). This is supported by the lack of inhibition by properdin of Factor-I-mediated cleavage of C3b or C3i and by the inability of exogenous 'native' properdin to accelerate C3 consumption in serum significantly in the absence of an activator (Götze *et al.*, 1977).

(ii) The alternative pathway is activated when C3b binds to surfaces that reduce its interaction with Factor H relative to that with Factor B (protected surfaces; Fearon & Austen, 1977a,b; Pangburn & Müller-Eberhard, 1978). As incubation of rabbit erythrocytes or measles-virus-infected cells with mixtures of purified C3 and Factors B, D, H and I results, in the absence of properdin, in amplified C3 activation (Schreiber *et al.*, 1978; Sissons *et al.*, 1980), it is clear that properdin is not necessarily involved in the initial recognition of activating surfaces. However, once a surface-bound C3 convertase, C3bBb, is formed, it will be stabilized by a univalent interaction with properdin, resulting in amplified C3b generation.

(iii) As the activated acyl group of C3b has a short half-life (60 μs ; Sim *et al.*, 1981), foci of C3b will occur around the initial site of C3b deposition. Such foci will favour multivalent attachment of properdin polymers to C3b, C3bB and C3bBb ligands, resulting in a far higher avidity of interaction, enhanced Factor B binding and enhanced stabilization of active C3bBb complexes. Properdin involvement will therefore provide a further mechanism whereby amplification of C3b generation and deposition is focused to the activating surface. This scheme is supported by the observation that properdin is depleted from the fluid-phase by activating surfaces [e.g. zymosan (Pillemer *et al.*, 1954); rabbit erythrocytes (Pangburn *et al.*, 1983)].

An additional mechanism whereby properdin might cause amplified C3b deposition on to a surface is indicated by the observation that it inhibits Factor I binding to, and action on, C3b. This implies either that the binding sites on C3b for these molecules are close or overlapping, or possibly that properdin interferes sterically with an interaction between Factors I and H (properdin does not interfere with Factor H binding to C3b). In addition, we have shown that, in the presence of physiological concentrations of properdin, Factor I action on EAC3b is inhibited. Significant inhibition of Factor I action on EAC3b was only seen at Factor I concentrations less than 4 $\mu\text{g}/\text{ml}$ (plasma concentration 30–50 $\mu\text{g}/\text{ml}$), and both of these experiments were performed at low ionic strength. As properdin interacts only weakly with bound C3b, a univalent properdin-bound-C3b interaction would not therefore, under physiological conditions, significantly alter the rate of Factor I action. Nevertheless, inhibition might occur if

Scheme 1. A model for the involvement of properdin in the alternative pathway of complement activation

The phases illustrated are: (i) fluid-phase 'tick-over' and initial C3b deposition on to surfaces; (ii) 'recognition' of surfaces which activate the alternative pathway; (iii) amplification of C3b generation and deposition; (iv) decay and inactivation of C3 convertases.

the properdin interaction with C3b was stabilized by multivalent attachment to additional C3b, C3bB or C3bBb sites (as would be expected at foci of deposition). Multivalently bound properdin could therefore directly inhibit Factor-I-mediated inactivation of surface-fixed C3b, protecting potential convertase sites and contributing to amplification of C3 conversion.

(iv) Throughout this process, loss of C3bBb C3 convertase enzymes will occur as Bb dissociates spontaneously from the surface-bound C3bBbP complex. This release is accelerated by Factor H (Weiler *et al.*, 1976; Whaley & Ruddy, 1976). As properdin has a far lower affinity for C3b and for C3bH than for C3bBb it too will dissociate from the site as Bb is displaced unless interaction is stabilized by secondary interactions with other C3bB and C3bBb complexes. Unprotected C3bH complexes will be converted into iC3b by Factor I, and thus the 'active' C3b-coated surface will be steadily converted into an 'inactive' iC3b-coated surface, with the release of properdin still in its 'native' state (Farries *et al.*, 1987).

It has long been apparent that properdin plays a vital role in the amplification of C3b generation and deposition. Our data limit this role to amplification at the activating surface with no role, either at 'tick-over' or amplification, in the fluid-phase.

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