Mapping of the properdin-binding site in the third component of complement

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The properdin-binding site in the human third complement component (C3) was mapped by using isolated C3b, C3c, α- and β-chains of C3 and C3 polypeptide fragments and an enzyme-linked-immunosorbent-assay procedure. The C3 chains and the polypeptide fragments were purified to homogeneity by preparative sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. The α-chain polypeptides included a 68 kDa and a 43 kDa polypeptide, which were generated by cleavage of C3b with factors I and H, and a 40 kDa, 33 kDa (C3d) and 27 kDa polypeptide, which were generated by cleavage of C3b with porcine elastase. It was shown that properdin binds to C3b, C3c, α-chain, and to the 43 kDa (factor-I + H-derived), as well as to 40 kDa (elastase-derived) α-chain fragment, but not to the β-chain 68 kDa, 33 kDa (C3d) and 27 kDa α-chain fragments. Thus the binding site for properdin resides on the 40–43 kDa *C*-terminal α-chain fragment of C3.

The third complement component (C3) plays a central role within the complement system by participating in both classical and alternative pathways of complement activation (Muller-Eberhard, 1975; Reid & Porter, 1981) and also by binding to cell receptors and triggering various cellular functions (Dierich *et al.*, 1982; Fearon & Wong, 1983). The versatility of C3 depends on multiple binding sites for several complement components such as factors H, B, I, properdin, and C5. Apart from these binding sites, different cell membrane receptors can be distinguished on the basis of their specificity for the C3b (C3bR), iC3b (iC3bR), C3d (C3dR), C3e (C3eR) and C3a (C3aR) fragments of C3 (Dierich *et al.*, 1982; Fearon & Wong, 1983.

Properdin, a basic serum glycoprotein, expresses

its principal function in the alternative pathway by interacting with, and stabilizing, the complex proteinases C3bBb or C3bnBb. This leads to the enhancement of the convertase activity and to some extent to protection against inactivation by factors I and H (Medicus *et al.*, 1976). Properdin exists in two forms, the native properdin found in fresh serum, and the active properdin obtained usually on purification (Götze *et al.*, 1977). Previous studies using sucrose gradients have shown that properdin forms complexes with purified C3, C3b and C3c and that the affinity of properdin for C3c is higher than that for C3 or Ceb (Chapitis & Lepow, 1976). Despite this knowledge, the exact site within the C3 molecule that interacts with properdin has not as yet been defined. In the present study, by using isolated polypeptides of the C3 molecule, we have identified a 40–43 kDa polypeptide at the *C*-terminus of the α-chain as the site of C3 in which properdin binds.

Materials and methods

Complement components

Human C3, factors H and I were purified from fresh plasma as previously described (Lambris *et al.*, 1980). C3 was first passed through an anti-(factor H)–agarose column to remove contaminating factor H (Lambris *et al.*, 1980) and then through an AT–Sepharose column (Pharmacia

Fine Chemicals, Uppsala, Sweden) to remove inactive C3 (Lambris & Ross, 1982). Purified factors H and I were treated with 2mM-(final concn.) di-isopropyl fluorophosphate (Sigma Chemical Co., Munich, Germany) for 30min at 37°C so as to inhibit other contaminating enzymic activities. Properdin was purified from human serum as described previously (Reid, 1982).

Preparation of C3 fragments

C3b bound to AT–Sepharose (AT–Sepharose–C3b) (Lambris & Ross, 1982) was cleaved by either porcine elastase (Tack et al., 1982) or factor I and H (Ross et al., 1982). Briefly, 25 ml of pelleted AT–Sepharose–C3b (4 mg/ml) were resuspended at 50% packed volume with 10mM-phosphate/140mM-NaCl, pH 7.5, and mixed with 1 mg of factor H and 100 μg of factor I or 1.2 mg of elastase for 3 h at 37°C. The AT–Sepharose–C3b gel was then pelleted by centrifugation and both the washed pellet as well as the supernatant were subjected to a preparative SDS/polyacrylamide-gel electrophoresis with 5–20% (w/v) polyacrylamide and 2% 2-mercaptoethanol as a reducing agent, as described by Laemmli (1970). Before electrophoresis, the samples were labelled with fluorescamine (Udenfriend et al., 1972) to detect the protein bands under u.v. light. The protein bands were cut out and minced into small pieces, then the proteins were eluted by overnight incubation at 25°C in 50mM-Tris/HCl (pH 7.9)/0.1 mM-EDTA/150mM-NaCl. The eluate was mixed with 4 vol. of cold acetone (30 min, −25°C) to precipitate the proteins. The pelleted proteins were dissolved in 50 μl of 6M-guanidinium chloride and after 30 min at room temperature the solution was diluted with 2.5 ml of phosphate-buffered saline containing 20% (v/v) glycerol and used in subsequent studies. The C3c and C3d fragments of C3 generated with elastase were purified as previously described (Tack et al., 1982).

Antibodies

Rabbits were immunized with C3c and C3d fragments prepared by elastase cleavage of purified C3b. In e.l.i.s.a. tests, anti-C3c reacted with C3, C3b and C3c but was unreactive with C3d. The anti-C3d reacted with C3, C3b and C3d but not with C3c. Goat antibody to human properdin was purchased from Atlantic Antibodies (Scarborough, ME, U.S.A.). All the antibodies were used as IgG fractions isolated by column chromatography on DEAE-Sephacel (Pharmacia) in 10mM-phosphate buffer, pH 8.0. Peroxidase-labelled antibodies to rabbit and goat IgG were purchased from DAKO (Boehringer Ingelheim Diagnostica, Ingelheim, Germany).

Assay of properdin binding to C3 fragments

The binding of properdin to C3b and to its fragments was measured by an e.l.i.s.a. Briefly, microtitre wells were first coated (overnight, 4°C) with 25 μl of the above proteins (20 μg/ml of phosphate-buffered saline/20% glycerol, pH 7.5). Wells were washed, saturated with phosphate-buffered saline containing 20 μg of gelatin/ml and then allowed to react (30min at 22°C) with serially diluted properdin (0.3–40 μg/ml of phosphate-buffered saline/gelatin). Bound properdin was detected with goat anti-properdin antibody and peroxidase-conjugated rabbit anti-goat Ig.

Results and discussion

Our direct binding assay confirms that properdin binds to C3c but not to C3d (Fig. 1), as demonstrated in previous association studies done with sucrose density gradients (Chapitis & Lepow, 1976). In fact, we found that properdin binds to C3c as well as it does to C3b. Preincubation of solid-phase-bound C3b with anti-C3c antibodies completely abolished the binding of properdin to C3b, but preincubating with anti-C3d antibodies was ineffective (results not shown). To localize the binding site further, we tested the binding of properdin to the isolated α- and β-chains of C3. As Fig. 1 shows, properdin bound to the α- but not to
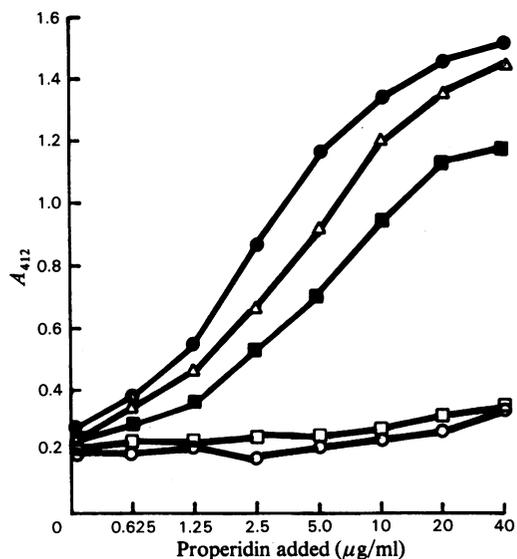

Fig. 1. *Dose-dependence of properdin binding to C3 fragments*

Various dilutions of properdin (μl each) were applied to e.l.i.s.a. plates precoated with C3b (●), C3c (△), C3d (○), α-chain (■) or β-chain (□). The binding of properdin to C3 fragments was assayed with a goat anti-properdin antibody, followed by a peroxidase-labelled rabbit anti-goat antibody.
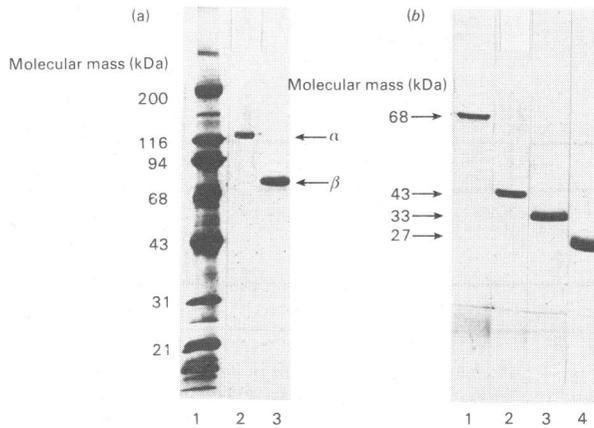
Fig. 2. (a) SDS/polyacrylamide-gel electrophoresis on a 5–20% polyacrylamide gel of α-chain (gel a2) and β-chain (a3) of C3 after reduction with 2-mercaptoethanol, and (b) SDS/polyacrylamide-gel electrophoresis of 68 kDa (gel b1), 43 kDa (b2), 33 kDa (b3), and 27 kDa (b4) α-chain fragments of C3

The α- and β-chains of C3 were purified from an SDS-containing preparative gel after reduction of C3 with 2-mercaptoethanol. The 68 kDa and 43 kDa α-chain fragments were purified by preparative SDS/polyacrylamide-gel electrophoresis after cleavage of C3b with factors I and H, and the 33 kDa and 27 kDa fragments after cleavage of C3b with porcine elastase. Gel (a)1 contained the following molecular-mass standards: myosin, 200 kDa; β-galactosidase, 116 kDa; phosphorylase b, 94 kDa; bovine serum albumin, 68 kDa; ovalbumin, 43 kDa; carbonic anhydrase, 31 kDa; and soya-bean trypsin inhibitor, 21 kDa.
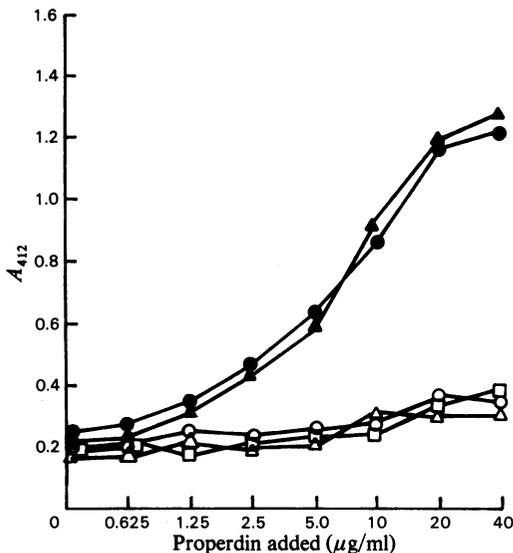

Fig. 3. Binding of properdin to α-chain fragments: 43 kDa (▲); 40 kDa (●); 27 kDa (○); 68 kDa (△); and 33 kDa (□)

the β-chain. Since properdin did not bind to C3d or β-chain, one would seek the active binding sites either to the N- or to C-terminal part of the α-chain.

To determine which of these two possibilities is correct, we tested binding of properdin to elastase or factor I- and H-derived C3b peptides. Under these conditions, the β-chain remains uncleaved and five different peptides are generated from the α-chain (Fig. 2). Limited digestion of C3b with factors I and H gives rise to two α-chain polypeptides: (a) a 68 kDa polypeptide that includes the C3d and the N-terminus of the chain, and (b) a 43 kDa peptide that contains the C-terminus (Davis et al., 1983). In contrast, digestion of the α-chain of C3b with elastase generated three main SDS / polyacrylamide - gel - electrophoresis - identifiable polypeptides: (a) a 33 and 27 kDa polypeptide, which are parts of the 68 kDa polypeptide and (b) a 40 kDa polypeptide, which is part of the factor I- and H-generated 43 kDa polypeptide (Tack et al., 1982). As Fig. 3 shows, properdin binds to 43 and 40 kDa peptides but not to 68 and 27 kDa peptides. Thus properdin binding to C3b can be placed at an area towards the C-terminus that is included within the 40–43 kDa polypeptide (Fig. 4).

Of particular interest is the indication in our experiments that interactions between two molecules of the complement system can occur without the need for a configurationally intact molecule. Further studies of ours have shown that enzymic peptides derived from factor H can interact with C3 (Alsenz et al., 1983). These findings strongly suggest that enzymic or synthetic peptides derived from the sequence of the C3 or other components
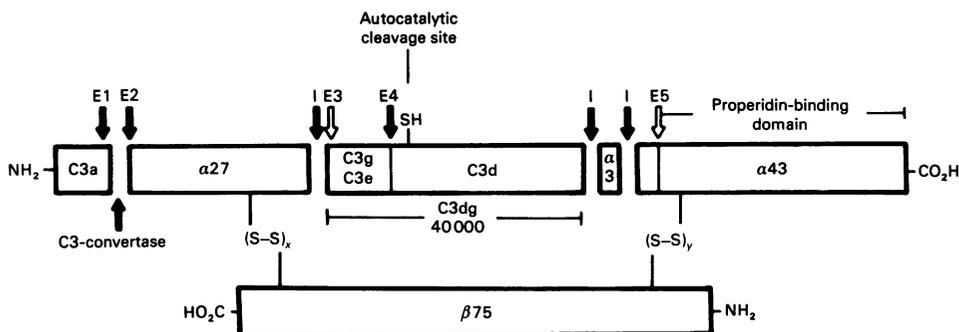
Fig. 4. *A schematic representation of the C3 molecule*
Sites of autocatalytic cleavage and of cleavage by C3 convertase, elastase (E) and factor I (I) are indicated. Positioning of C3d, α27 and α43 within the α-chain of C3 is based on primary structural data of C3 fragments generated with factor I (Davis *et al.*, 1983). The positioning of elastase cleavage sites E3 and E5 is based on the size of the fragments generated by cleavage of C3b with elastase. All other assignments are based on direct structural analysis (Tack *et al.*, 1982; Davis *et al.*, 1983).

that might contain the binding sites will be able to interact with their appropriate ligands. It is not known whether such interactions of peptides with ligand molecules will (a) merely inhibit interactions of the respective native molecule with the ligand, or (b) induce functional and molecular modifications on the ligand similar to those brought about by the native molecule. At any rate, it may not be unlikely that once the binding sites are identified and peptides containing these sites have been isolated or synthesized, one might use them for positive or negative modifications of the complement system.

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