
Brief Definitive Report

MHC CLASS III PRODUCTS: An Electron Microscopic Study of the C3 Convertases of Human Complement

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The genes encoding C2, C4, and Factor B of human complement map between the B locus of class I products and the D locus of class II products in the MHC on chromosome 6 (1). These proteins, together with C3, are the precursors of the C3 convertases of the classical and the alternative pathway of complement (2, 3). Formation of C3 convertase is the critical event in both pathways: it eventuates in opsonization of particles, release of inflammatory peptides, C5 convertase formation, and cell lysis. The two enzymes, which have the structural formulas C4b,2a and C3b,Bb¹ are serine proteases (2). The catalytic sites reside in the atypically large C2a (80 kd) and Bb (63 kd) subunits, whose N-terminal amino acid sequences show no homology with those of other serine proteases (4–6). These C3 convertases are also unusual in terms of their kinetic and thermodynamic instability. The catalytic subunits, once dissociated from their cofactors, cannot rebind to form an active enzyme.

We have recently shown that subunit Bb displays a two-domain structure that is unusual among serine proteases (7). We now wish to report that C2a also displays a two-domain structure, that the zymogens C2 and Factor B possess three domains, and that the morphologies of the enzyme complexes, their analogous subunits and precursors are very similar.

Materials and Methods

Generation of Fluid Phase C4b,2a or C3b,Bb. C2, C3, C3b, C4, C4b, Factor B, C1s, Factor D were isolated as described (2, 8, 9). 80 µg C4, 40 and µg C2, and 1.2 µg C1s (molar ratios 100:100:3) or 80 µg C3b, 40 µg Factor B, and 0.5 µg Factor D (molar ratios 100:100:5) were incubated in the presence of 0.5 mM MgCl₂ and 0.15 mM CaCl₂ at 37°C

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¹ *Abbreviations used in this paper:* C2a, Bb, C4b, C3b, major cleavage fragments of C2, Factor B, C4 and C3; VBS, buffer containing 3.5 mM 5,5'-diethylbarbituric acid, 143 mM NaCl, pH 7.4; VBE, VBS containing 10 mM EDTA; kd, kilodaltons; SDS, sodium dodecyl sulfate; DFP, diisopropylfluorophosphate.

in a total volume of 277 μ l VBS. At timed intervals, three aliquots were withdrawn: 1) 5 μ l was diluted into ice cold volatile buffer (0.1 M ammonium acetate, 0.05 M ammonium bicarbonate, 0.1 mM EDTA, pH 7.2) and immediately mounted for electron microscopy. 2) 10 μ l was transferred to 90 μ l of ice cold VBE containing C3 for determination of C3 convertase activity (10). 3) 15 μ l was subjected to SDS polyacrylamide gel (8%) electrophoresis (11).

Electron Microscopy. Samples of 5 μ g/ml in the above volatile buffer were adsorbed to thin carbon films and negatively stained with 1% uranyl formate (Eastman, Rochester, NY) using the pleated sheet technique (12). Images similar in character but inferior in quality were obtained when nonvolatile buffers used. Pleated regions of the grid surface were photographed at a primary magnification of 64,000 in a Hitachi 12 A transmission electron microscope operating at 75 kV with a 200- μ m C₂ aperture and a 50- μ m objective aperture. Magnification calibration of the instrument was performed by standard methods (13).

Results

The respective reaction mixtures for enzyme formation were prepared at 0°C, transferred to 37°C, and aliquots were then removed at timed intervals for immediate assay of C3 convertase activity, processing for electron microscopy, and SDS polyacrylamide gel electrophoresis. Both C3 convertases were formed very rapidly and although some uncleaved C2 and Factor B remained after 0.5

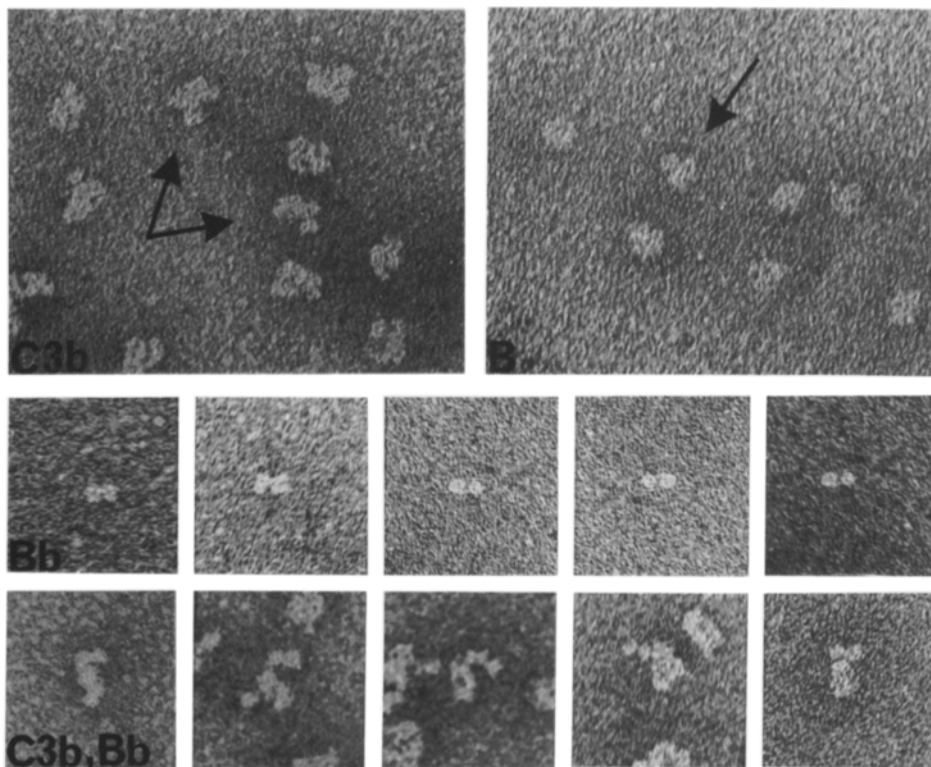


FIGURE 1. Electron micrographs of the human alternative pathway C3 convertase, C3b, Bb and precursors. C3b displays a highly irregular substructure (arrows). Factor B appears globular with the suggestion (arrow) of a three domain substructure. Bb possesses two discrete globular domains, only one of which makes contact with C3b within the enzyme complex. All images magnified \times 480,000.

min, further incubation did not result in increased C3 convertase activity due to the rapid decay-dissociation of both enzymes. Field view electron micrographs (not shown) also showed that C3b,Bb complexes and C4b,2a complexes were pronounced at the early time points and that they gradually diminished until no complexes were seen after 10 min.

Comparison of the electron micrographs depicted in Figs. 1 and 2 shows C3b (~176 kd) and C4b (~185 kd) have a similar globular appearance, consistent with hydrodynamic evidence (2, 14) and to have an irregular, apparently multiple-domain substructure. This is especially pronounced in C3b images (arrows, Fig. 1). Both molecules would be contained within a parallelepiped measuring approximately $125 \text{ \AA} \times 75 \text{ \AA} \times 65 \text{ \AA}$. The most conspicuous projection in both cases (arrows) is that of a larger head region connected to a smaller tail-like structure. Micrographs of the precursors C3 and C4 appeared indistinguishable from those of C3b and C4b.

The catalytic site-bearing subunits Bb (Fig. 1) and C2a (Fig. 2) appear very similar, both consisting of two globular domains connected by a thin linker segment $\sim 10 \text{ \AA}$ long corresponding to $\sim 5 \text{ kd}$ in molecular weight. Although both domains of Bb appear nearly identical, one domain of C2a often appears larger. Measurement of center-to-center distances gave $50 \pm 2 \text{ \AA}$ ($\pm \text{SD}$) for Bb

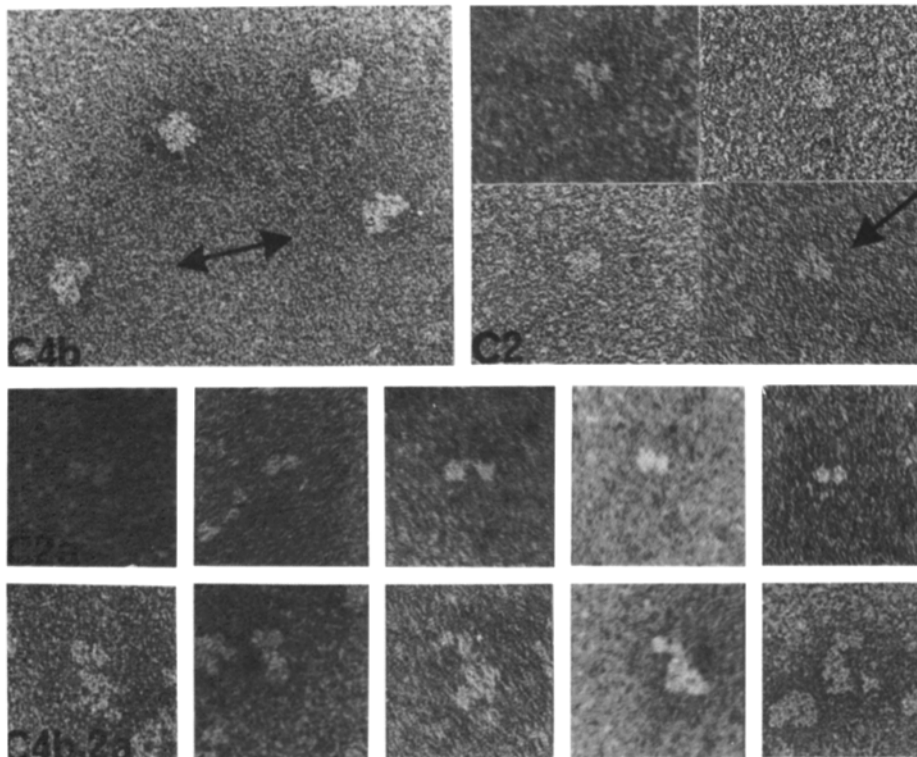


FIGURE 2. Electron micrographs of human classical pathway C3 convertase, C4b,2a and precursors. C4b appears very similar to C3b (Fig. 1); both proteins share a distinctive structure noted by arrows. C2 is globular with the suggestion of domain substructure (arrow). C2a possesses two discrete globular domains of unequal size. Only one domain attaches to C4b in the bimolecular complex C4b,2a. All images magnified $\times 480,000$.

and $55 \pm 2 \text{ \AA}$ (\pm SD) for C2a. The diameter of each Bb domain is therefore estimated to be 42 \AA and using a \bar{V} of 0.72 (15), the estimated molecular weight of one domain is ~ 28 kd. Assuming the smaller domain of C2a to be of the same size as the domains of Bb, the larger domain of C2a has a diameter of 47 \AA , which corresponds to ~ 39 kd. The catalytic subunit of both enzymes is oriented approximately orthogonal to the long axis of the cofactor, and bound through only one of the two domains.

The zymogens Factor B (Fig. 1) and C2 (Fig. 2) both appear globular with diameters of $80 \pm 8 \text{ \AA}$ (\pm SD) and $85 \pm 8 \text{ \AA}$ (\pm SD), respectively. However, pronounced substructure is evident. Bipartite images of Factor B and C2 are consistent with their cleavage pattern by proteases (4, 6, 14, 15): two regions, one approximately twice as large as the other. Often, three-lobed structures are seen, each lobe measuring $\sim 40 \text{ \AA}$ in diameter. Consistent with these images is the similarity in molecular weight of the activation fragments Ba and C2b (2, 3) and of the estimated molecular weights of the domains of Bb and C2a.

Discussion

The apparent presence of multiple domains in C3b and C4b is consistent with the fact that both cofactors possess multiple protein binding sites. These sites include the Bb or C2a binding site, the metastable binding site or reactive thioester (16–18) through which these molecules covalently attach to cell surfaces, sites for regulatory proteins which either abrogate or enhance the activity of the C3 convertases (2), and sites specific for cell surface receptors (19).

It is probable that C3 and C4 arose by gene duplication because: (a) the anaphylatoxins C3a and C4a, which are derived from the α -chain of these proteins, show considerable amino acid sequence homology (20); (b) the regions surrounding the reactive thioester also indicate homology (21); (c) both proteins are synthesized as single chain polypeptides and subsequently processed to multiple chain structures (3); and (d) they are functional analogues. The morphological similarity between C3b and C4b deduced by electron microscopy further suggests a common evolutionary origin, even though C4b is 15 kd greater in molecular mass than C3b, possesses a three-chain rather than a two-chain structure and the genes of human C3 and C4 map to different chromosomes (22, 23).

We have shown that subunits Bb and C2a both consist of two discrete globular domains, although previous attempts to cleave these proteins with proteases failed to reveal the two-domain substructures (4, 6, 15). The finding that C2a and C2a and Bb bind to their cofactors through only one domain indicates that this domain is the binding domain and suggests but does not prove that the other may be the catalytic domain. In the case of C2a, the smaller of the two domains (~ 28 kd), which is comparable in size to the Bb domains, may be postulated to contain the enzymatic site. Such intramolecular arrangement would localize the catalytic site to a domain similar in size and shape to other serine proteases (24). If the freely projecting domain of the catalytic subunits in both C3 convertases contains the catalytic site, then this domain would represent the COOH-terminal half of C2a and Bb, because the reactive serine residue of serine proteases resides in that portion of the molecule (5). That both enzymes are serine proteases has

been deduced from their diisopropyl-fluorophosphate (DFP) inhibitability (2) and from sequence analyses of Bb (4, 5). Although it is clear that in both proteases the catalytic site resides in a discrete 28-kd domain and that cofactor attachment occurs through one domain only, it is not known whether both sites reside in the same domain. Crystal structure comparisons (24, 25) of chymotrypsinogen and α -chymotrypsin have elucidated in detail the critical role of the conserved NH₂-terminal residues in the canonical refolding events. Because these amino acid residues are not present in Bb (4, 5), the activation process must be different from that of other serine proteases.

The suggestion that C2 arose from Factor B by gene duplication is consistent with their functional and physicochemical similarities, their structural similarity deduced by electron microscopy, and the fact that both genes map in close proximity to each other (1). However, the multiple domain substructures of these proteins suggest a more intricate evolutionary process that remains to be defined.

Summary

We have reported a transmission electron microscopic study of the two C3 convertases of human complement and their precursors. The corresponding proteins and complexes of the classical and alternative pathway appear very similar. Cofactors C3b and C4b are nearly indistinguishable and display a characteristic but highly irregular substructure. C2 and Factor B are globular with diameters of $85 \pm 8 \text{ \AA}$ and $80 \pm 8 \text{ \AA}$ and both consist of three discrete globular domains each $\sim 40 \text{ \AA}$ in diameter. Bb and C2a each contain two domains connected by a short linker segment. Both domains of Bb and one domain of C2a are 42 \AA in diameter (28 kd), while the second domain of C2 is 47 \AA in diameter (39 kd). Attachment of the enzymatic subunits to cofactors occurs through one domain only.

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