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POLYMERIZATION OF FACTOR B OF THE ALTERNATIVE COMPLEMENT PATHWAY VIA DISULFIDE BOND(S) IN THE PRESENCE OF Cu^{2+} AND STIMULATION BY C3b, THE MAJOR FRAGMENT OF C3

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Summary: Factor B (B) of the alternative complement pathway has been found to dimerize via disulfide bond(s) in the presence of $CuCl_2$. Poly B has no B activity. The Bb fragment was also dimerized, indicating that one free sulfhydryl group on the Bb portion might be involved in polymerization. The Ba fragment was not dimerized. C3b, the major fragment of C3, has the capacity to stimulate polymerization of B. Incubation of C3b, B and factor D in the presence of Mg^{2+} and Cu^{2+} resulted in the formation of poly B and diminished cleavage of B. These results suggest that polymerization of B due to Cu^{2+} might be partly responsible for the impairment of C3 convertase activity of the alternative pathway.

Factor B (B) is a plasma glycoprotein of the alternative complement pathway, which interacts with C3b, the major fragment of C3, in the presence of Mg^{2+} rendering it susceptible to cleavage by factor D (D). The resulting complex, C3bBb, shows C3 convertase activity, cleaving C3 into C3a and C3b.

Copper ions (Cu^{2+}) catalyze the oxidation of sulfhydryl groups in proteins to generate disulfide bond(s) with concomitant polymerization. Such disulfide-bond polymerization due to Cu^{2+} has been described for fibronectin (1). In this communication, we show that B is polymerized via disulfide bond(s) in the presence of Cu^{2+} and that polymerization is stimulated by C3b. Some di-

Abbreviation: B, factor B of the alternative complement pathway; D, factor D of the alternative complement pathway; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis.

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valent cations, including Cu^{2+}, have been reported by Kaneko et. al. to inhibit C3 convertase activities of both the classical and alternative pathways (2). Our findings partially elucidate the mechanisms of Cu^{2+} impairment of C3 convertase activity of the alternative pathway.

Materials and Methods

Purification of human B, D and C3.
Human B and D were isolated as previously described (3). Human C3 was isolated by a modification of the procedure of Hammer et. al. (4). Briefly, human citrated plasma was chromatographed on DEAE-Sephacel and then on Heparin 5pw.

Preparation of C3b, Ba and Bb.
C3b was prepared by digesting C3 with insoluble trypsin and isolated by the procedure of Pangburn (5). The Ba and Bb fragments were prepared by incubating 100 μg of C3b, 200 μg of B and 0.9 μg of D in 600 μl of veronal buffered saline containing 4 mM $MgCl_2$ at 37°C for 2 hr. The reaction mixture was then chromatographed on a Mono Q column equilibrated with 25 mM Tris-HCl pH 7.4. A linear gradient elution was applied using starting buffer and starting buffer containing 0.5 M NaCl. Ba and Bb were eluted at NaCl concentrations of 0.27 M and 0.21 M, respectively.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).
SDS-PAGE was performed using the Laemmli system (6).

Results and Discussion

After B was incubated at 37°C for 60 min in the presence of various concentrations of $CuCl_2$, reaction mixtures were subjected to SDS-PAGE. As shown in Fig. 1A, incubation of B with $CuCl_2$ led to the formation of a polymer in a dose dependent manner. The molecular weight of the polymer was estimated to be approximately 220 KDa. Therefore, poly B is likely a dimer. Under reducing conditions using 2-mercaptoethanol, the poly B band shifted, corresponding to that of B (data not shown). This indicates that B polymerization occuring in the presence of Cu^{2+} is via disulfide bond(s). Because B has been reported to possess one free sulfhydryl group on the Bb portion (7,8), we examined whether Bb was also polymerized in the presence of Cu^{2+}. As shown in Fig. 1B, a dimer of Bb (120 KDa) was formed as was seen for B. Ba, however, was not polymerized (data not shown). In addition, although the C3b molecule has one free sulfhydryl group as does B (9), polymerization of C3b in the presence of Cu^{2+} was not noted (data not shown).

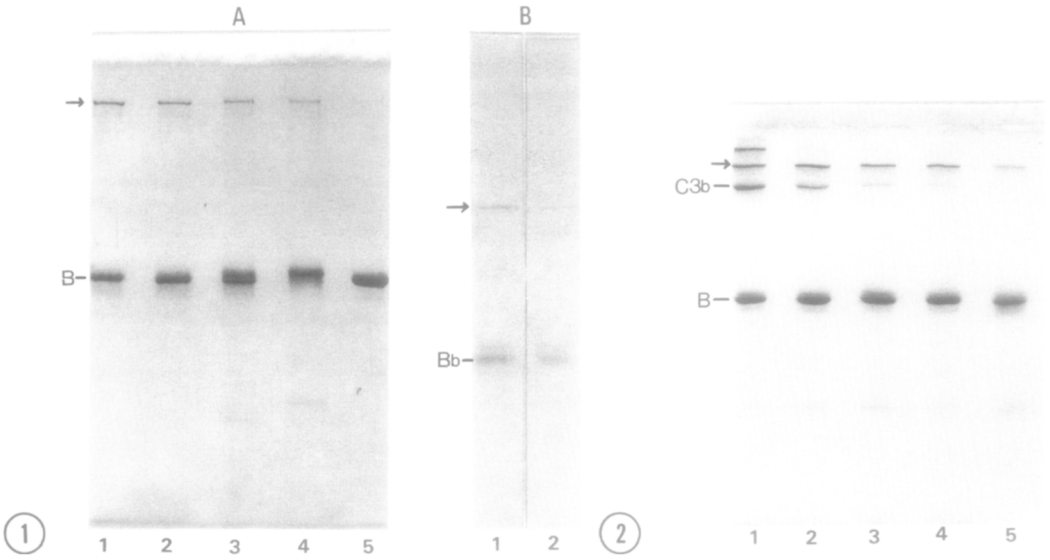

Fig. 1. Polymerization of B and Bb in the presence of Cu^{2+}.
(A) B (2 μg) was incubated with $CuCl_2$ at the concentrations of 5 mM (lane 1), 1 mM (lane 2), 0.5 mM (lane 3), 0.1 mM (lane 4) or no $CuCl_2$ (lane 5) in veronal buffered saline pH 7.4 at 37°C for 1 hr. The reaction mixtures were then subjected to SDS-PAGE followed by protein staining with Coomassie brilliant blue. The arrow indicates poly B.
(B) Bb (1 μg) was incubated with 0.1 mM $CuCl_2$ (lane 1) or no $CuCl_2$ (lane 2) in veronal buffered saline at 37°C for 1 hr. The reaction mixtures were then subjected to SDS-PAGE. The arrow indicates poly Bb.

Fig. 2. The stimulating effect of C3b on polymerization of B.
B (2 μg) was incubated with various amounts of C3b (4 μg, lane 1; 2 μg, lane 2; 1 μg, lane 3; 0.5 μg, lane 4; no C3b, lane 5) in the presence of 0.1 mM $CuCl_2$ in veronal buffered saline at 37°C for 1 hr. The reaction mixtures were then subjected to SDS-PAGE. The arrow indicates poly B.

Before the formation of C3 convertase of the alternative pathway, namely C3bBb, B first binds to C3b molecules. Magnesium ions have been reported to be unessential for the assembly (10,11). Therefore, we next examined whether C3b influences polymerization of B by Cu^{2+}. As shown in Fig. 2, C3b enhanced polymerization of B in a dose dependent manner, while C3b did not affect the polymerization of Bb (data not shown). In addition, a new band with higher molecular size than poly B appeared using 4 μg of C3b (Fig. 2, lane 1). This band has been found to represent a heterodimer consisting of B and C3b linked via disulfide bond(s) as revealed by immunoblotting analysis using anti-B and anti-C3 sera (data not shown). D is able to cleave B into Ba and Bb only when the complex forms between B and C3b. To explain this unusual proteolysis mech-

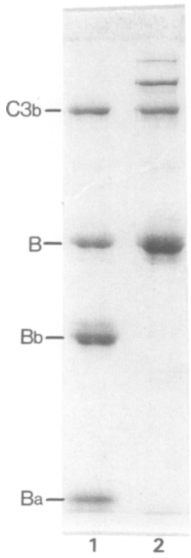

Fig. 3. Inhibition of cleavage of B by D in the presence of Cu^{2+}. Two μg of C3b, 4 μg of B, 60 ng of D and $MgCl_2$ (to a final concentration of 0.1 mM) in the absence (lane 1) or presence (lane 2) of $CuCl_2$ (to a final concentration of 0.1 mM) in veronal buffered saline were incubated at 37°C for 1 hr. The reaction mixtures were then subjected to SDS-PAGE.

anism, Lesavre and Müller-Eberhard (12) speculated that C3b induces B to fit into the substrate binding site of D. The stimulating effect of C3b on the polymerization of B in the presence of Cu^{2+} suggests a C3b-induced change in B.

Kaneko et. al. have already reported that Cu^{2+} inhibits C3 convertase activities of both the classical and alternative pathways. We confirmed their findings by examining C3 convertase formation. As shown in Fig. 3, Cu^{2+} at an equal concentration to Mg^{2+} completely prevented cleavage of B into Ba and Bb fragments by D in the presence of C3b. Although the exact inhibitory mechanisms by Cu^{2+} are unclear, a decrease in the amount of intact B due to the polymerization of B might be partly responsible for the impairment of C3 convertase activity, since polymerization of B by Cu^{2+} also occured even in the presence of Mg^{2+} (Fig. 3) and poly B possesses no B activity (data not shown).

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