MODULATION OF THE FORMATION OF THE AMPLIFICATION CONVERTASE OF COMPLEMENT, C3b,Bb, BY NATIVE AND COMMERCIAL HEPARIN*

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Commercial heparin has long been known to possess anti-complementary as well as anti-coagulant activity. Early studies identified several possible sites of inhibition by heparin ranging from classical early (1) to late component functions (2, 3), and recent studies continue to reveal more than one possible site of inhibitory action. Heparin directly inhibits C1q binding to immune complexes (4), interaction of $C\bar{1}s$ with C4 and C2 (5), and binding of C2 to C4b (6). Based on the demonstration of the anti-thrombin III cofactor activity of heparin (7), the potentiation by heparin of the effect of the $C\bar{1}$ inhibitor on $C\bar{1}$ has been noted (8). Heparin also inhibits formation or binding of the trimolecular complex, C5b67, as measured by the reactive lysis phenomenon (9). Finally, heparin has been shown to inhibit cobra venom factor (CoVF)[1]-dependent C3 inactivation in whole serum (10), thereby indicating an effect on the generation or action of the C3 convertase formed from cobra venom factor, B and $\bar{D}$.

Amplified C3 cleavage by the C3b-dependent C3 convertase (C3b,Bb) may well determine whether the initial activation of the complement sequence by the classical or by the alternative pathway eventuates in effective utilization of the terminal components. The action of heparin was therefore examined on the generation and natural regulation of the amplification convertase. These

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[1] *Abbreviations used in this paper:* C3b,Bb, C3b-dependent C3 convertase; C3bINA, C3b inactivator; C3NeF, C3 nephritic factor; C-EDTA, rat serum diluted 1:20 in 0.04 M EDTA-GVB; CoVF, Cobra venom factor; CoVFBb, CoVF-dependent C3 convertase; DGVB, half-isotonic Veronal-buffered saline, pH 7.5 containing 0.1% gelatin and 2.5% dextrose; $DGVB^{++}$, DGVB with 0.5 mM magnesium and 0.15 mM calcium; EAC4b,3b, sheep erythrocyte sensitized with specific antibody and bearing the major cleavage fragments of C4 and C3; EDTA, ethylenediamine tetraacetate; EDTA-DGVB, DGVB containing 0.01 M EDTA; EDTA-GVB, isotonic Veronal-buffered saline containing 0.1% gelatin and 0.04 M EDTA; P, properdin; RPMC, rat peritoneal mast cell; Z, average number of hemolytic sites/cell.

studies employed not only commercial heparin, a degraded hog glycosaminoglycan mixture of average 12,000 mol wt, but also native rat peritoneal mast cell (RPMC) heparin, a 750,000 mol wt proteoglycan (11). Both native and commercial heparin profoundly modulate C3b-dependent amplification of the complement system by inhibition of C3b,Bb formation and prevention of β1H-mediated decay-dissociation of C3b,Bb.

Materials and Methods

Cobra venom (Reptile Institute, Inc., Silver Spring, Fla.), Azure A (Fisher Scientific Co., Somerville, N. J.), Dowex 1-Cl 100–200 mesh (Bio-Rad Laboratories, Richmond, Calif.), Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, N. J.), carboxymethyl cellulose (CM-52) (Reeve Angel, Clifton, N. J.), Metrizamide (Accurate Chemical & Scientific Corp., Hicksville, N. Y.), and cetyltrimethyl ammonium bromide (Sigma Chemical Co., St. Louis, Mo.) were obtained as indicated. Aqueous preservative-free commercial sodium heparin, 1,000 U (6.077 mg) per ml, was obtained from Fellows Medical Division, Chromalloy Pharmaceuticals, Oak Park, Mich. as manufactured by Elkins-Sinn, Inc., Cherry Hill, N. J.

Native Heparin and Heparin Assays. Commercial and RPMC heparin were quantitated by metachromasia with Azure A by a modification (12) of the method of Jaques et al. (13) and by uronic acid content using the carbazole reaction as modified by Bitter and Muir (14). The commercial and RPMC heparin showed similar dose responses and were quantitated from a reference curve linear between 10 and 100 μg/ml established with a second commercial heparin preparation containing 130 U/mg (Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. J.). The anti-coagulant activities of commercial and RPMC heparin were determined in a modified partial thromboplastin time assay (15) which gave a linear dose response from 1 to 10 μg with the second commercial heparin preparation used as the standard.

Rat mast cells, obtained by lavage of 40–50 rat peritoneal cavities, were pooled, and 1-ml samples were each centrifuged through 22.5% metrizamide cushions of 2 ml (16), yielding 90–95% final purity. The mast cells were frozen and thawed six times and extracted with 1 M NaCl. The extract was applied to a 1 × 20 cm column of Dowex 1 equilibrated in 1 M NaCl and the column was eluted sequentially with 1, 3, and 4 M NaCl (11). The 3 M NaCl eluate, which contained the RPMC heparin as determined by metachromasia, was dialyzed against 10 liters of distilled water, lyophilized, redissolved in 2 M NaCl and chromatographed on a 1 × 50 cm column of Sepharose 4B equilibrated in 2 M NaCl (11). The RPMC heparin filtered with an average mol wt of approximately 750,000 and was pooled, dialyzed, lyophilized, and redissolved as required in 0.15 M NaCl for complement studies or further purification. Additional purification utilized a 1 × 10 cm column containing CM-52 equilibrated in 1% cetyltrimethyl ammonium bromide. The heparin precipitated when applied to the column (17) and was eluted at 2.4 M NaCl by application of a concave logarithmic gradient from 0.15 to 5 M NaCl. The heparin was separated from the residual cetyltrimethyl ammonium bromide by the sequential addition of potassium iodide to a final molarity of 0.01 and an equal volume of chloroform (17). The heparin, which remained in the aqueous phase, was dialyzed against 10 liters of distilled water, lyophilized, and resuspended in 0.15 M NaCl. The RPMC heparin anti-coagulant activity per microgram, after the Sepharose 4B (11) or CM-52 step, was 15–18% of the activity of commercial heparin based on uronic acid content.

Mast cell granules were isolated from 90 to 95% purified rat mast cells. The mast cells were suspended to a concentration of 4×10^6 cells per ml in 0.0075 M Tris, pH 7.4, containing 0.01 M NaCl, 8×10^{-4} M magnesium chloride and 10 μg/ml deoxyribonuclease. The cells were frozen and thawed six times, and the debris was sedimented at 400 *g* for 10 min at room temperature. The supernate containing granules was decanted and sedimented at 3,000 *g* for 20 min at 4°C (12). The resulting granule pellet was stored at −70°C until it was resuspended in 0.0075 M Tris, pH 7.4, assayed for uronic acid content and used for complement studies.

Complement Components and Assays. Half-isotonic Veronal-buffered saline, pH 7.5, containing 0.1% gelatin and 2.5% dextrose (DGVB), DGVB with 0.5 mM magnesium and 0.15 mM calcium ($DGVB^{++}$), DGVB containing 0.01 M ethylenediamine tetraacetate (EDTA) (EDTA-DGVB), and isotonic Veronal-buffered saline containing 0.1% gelatin and 0.04 M EDTA (0.04 M EDTA-GVB) were used as diluents in hemolytic assays.

C3 (18-20), B (21), D̄ (22), properdin (P) (23), β1H (24), CoVF (25), and C3b inactivator (C3bINA) (26) were purified to homogeneity and quantitated as described. C3b was generated by incubating purified C3 with CoVF-dependent C3 convertase (CoVFBb) bound to Sepharose 4B (27); the C3b thus generated was devoid of hemolytic C3, B, and D̄ activities. Guinea pig C1, guinea pig C2 (28), and C3 nephritic factor (C3NeF) (29) were functionally purified and quantitated as described. Rat serum was obtained from Microbiological Associates, Bethesda, Md.

EAC4b,3b cellular intermediates (30) were prepared at a ratio of 100 μg C3 per 10^9 EAC1,4b,2a cells unless otherwise noted. B hemolytic activity in reaction mixtures of purified proteins (26) was assayed as described (31).

Results

Inhibition of Formation of Amplification Pathway Convertases. Dose-response effects of both commercial and RPMC heparin on the formation of C3b,Bb, stabilized by P or C3NeF or unstabilized, and on the convertase formed without D̄ so as to contain uncleaved B were studied. 1 ml reaction mixtures yielding approximately one convertase site per cell consisted of: 0.45 ng B, 0.10 μg D̄, and 0.65 μg P per 10^8 EAC4b,3b for formation of EAC4b,3b,Bb,P; 1.2 ng B, 0.10 μg D̄, and 0.079 U C3NeF per 10^8 EAC4b,3b for EAC4b,3b,Bb,NeF; 4.5 ng B and 0.10 μg D̄ per 10^8 EAC4b,3b for EAC4b,3b,Bb; and 224 ng B per 10^8 EAC4b,3b for EAC4b,3b,B. A 0.1-ml portion of each mixture was added to 0.1 ml $DGVB^{++}$ alone or containing commercial or RPMC heparin and incubated 30 min at 30°C with shaking. Three-tenths ml of a 1:20 dilution of rat serum in 0.04 M EDTA-GVB (C-EDTA) was added to each reaction mixture, and incubation was continued for 60 min at 37°C. After addition of 1.5 ml saline, percent lysis was measured and the average number of hemolytic sites per cell (Z) were calculated (Fig. 1). Formation of all four types of convertase sites was inhibited by heparin in a dose-related manner, with the EAC4b,3b,B being the least sensitive. The commercial and RPMC heparin had similar activities on a weight basis. Dialysis of commercial heparin against cation-containing ($DGVB^{++}$) or noncation-containing (DGVB) buffer overnight at 4°C did not diminish its inhibitory action, indicating that chelation of cations was not the mechanism of its inhibitory effect.

It was necessary to introduce heparin with the C-EDTA to exclude a significant inhibitory effect of heparin upon the developing reagent. 1 ml $DGVB^{++}$ containing 1×10^8 EAC4b,3b, 1.87 ng B, 0.10 μg D̄, and 0.65 μg P was incubated with shaking for 30 min at 30°C to form EAC4b,3b,Bb,P. One tenth-ml portions were then added to 0.1 ml 0.04 M EDTA-GVB alone or containing varying amounts of commercial heparin, followed immediately by addition of 0.3 ml C-EDTA. Incubation was continued for 60 min at 37°C, and percent lysis was determined. A 0.1 ml portion of the starting mixture was also incubated with 8.66 μg commercial heparin during convertase formation and developed with 0.1 ml 0.04 M EDTA-GVB and 0.3 ml C-EDTA. Commercial heparin, 86.6 μg per 10^7 cells, inhibited hemolysis by C-EDTA of the erythrocytes bearing the preformed convertase by only 8%, whereas 1/10 this concentration per 10^7 cells gave 99% inhibition of convertase formation.

To analyze further the site of heparin action on convertase formation, the quantity of cell-bound C3b was varied and the amount of B input adjusted to produce approximately one hemolytic site per cell. EAC4b,3b were prepared at

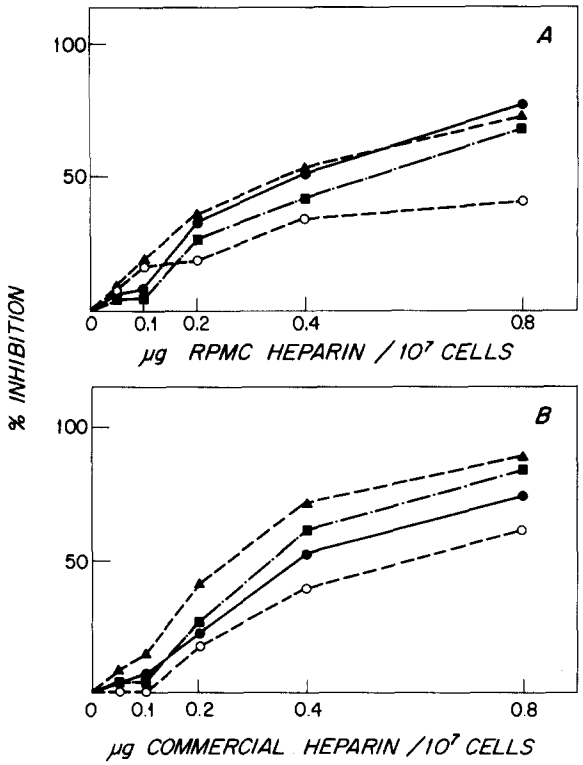

FIG. 1. Dose-response effects of native (A) and commercial (B) heparin on the formation of EAC4b,3b,Bb,P (●——●), EAC4b,3b,Bb,NeF (▲——▲), EAC4b,3b,Bb (■——■), and EAC4b,3b,B (○——○).

a ratio of 1 μg C3 (low), 10 μg C3 (medium), or 100 μg C3 (high) per 10^9 EAC1,4b,2a (30). Low, medium, and high EAC4b,3b, 2×10^8, were mixed with 0.2 μg $\bar{D}$, 1.3 μg P, and 90, 9 and 0.75 ng B, respectively, in 2 ml $DGVB^{++}$. One tenth-ml portions of each mixture were added to 0.1 ml $DGVB^{++}$ alone, to $DGVB^{++}$ containing RPMC heparin, and to $DGVB^{++}$ containing commercial heparin and incubated for 30 min at 30°C. C-EDTA was then added, and the convertase sites were developed. The cells prepared with the least amount of C3 and requiring the highest amount of B to be lysed were profoundly inhibited by both RPMC and commercial heparin, while cells prepared with the highest amount of C3 and requiring the least amount of B to be lysed were relatively resistant to inhibition by heparin (Fig. 2).

The kinetics of inhibition by heparin of formation of unstabilized convertase were then examined. Two-tenths μg of B alone, with 7.8 μg commercial heparin, and with 3.9 μg commercial heparin were pre-incubated in 1.8 ml $DGVB^{++}$ for 10 min at 30°C. Three portions containing 1.8×10^8 EAC4b,3b and 0.18 μg $\bar{D}$ in 1.8 ml $DGVB^{++}$ were also preincubated. These reactants were then brought to 37°C and mixed together at time zero. At timed intervals thereafter, 0.2-ml samples were removed and added to C-EDTA to develop convertase sites. The T_{max} was 5 min and the Z_{max} was 0.84 in buffer (Fig. 3). The T_{max} was not appreciably changed by the two concentrations of commercial heparin, whereas the Z_{max} was reduced to 0.33 and 0.52, respectively.

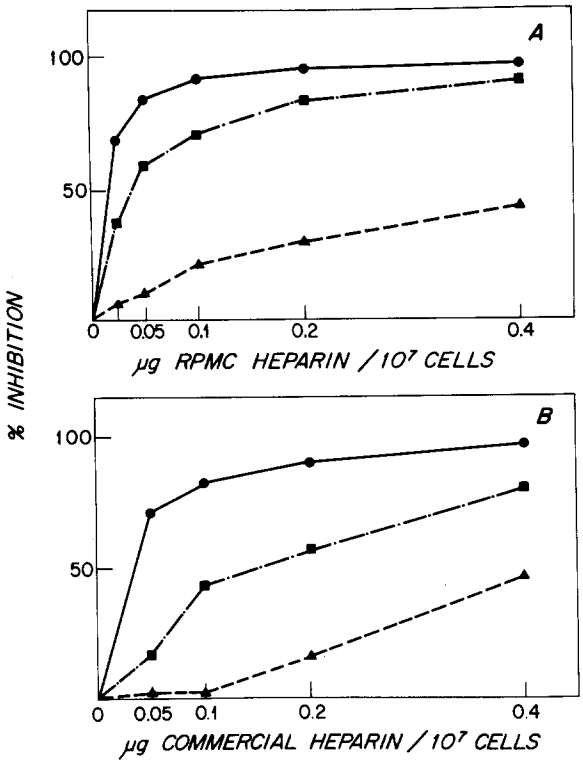

FIG. 2. Dose-response effects of native (A) and commercial (B) heparin on the formation of EAC4b,3b,Bb,P using EAC4b,3b prepared with low (●——●), medium (■——■), and high (▲——▲) C3 inputs. The number of hemolytic sites per cell were 1.4, 1.7, and 1.6, respectively.

The fluid phase interaction of C3b and B was examined to determine if heparin inhibition was due to impaired B utilization. Reaction mixtures containing 0.5 μg C3b and 20 ng D̄ alone and with incremental concentrations of commercial or RPMC heparin in 150 μl DGVB^{++} were prewarmed at 30°C, and at time zero 1.1 μg B in 50 μl DGVB^{++} at 30°C was introduced. Incubation was continued at 30°C, and at timed intervals 10 μl was removed from each reaction mixture, added to 0.5 ml ice cold DGVB^{++}, and assayed for residual B (Fig. 4). RPMC heparin at 4 μg per reaction mixture and commercial heparin at 0.9 μg and 4.3 μg per reaction mixture inhibited fluid phase consumption of B, thereby indicating that heparin decreased convertase formation by decreasing B utilization.

The effect of a single high dose of commercial heparin on the decay rates of unstabilized and P or C3NeF-stabilized convertase was examined. Reaction mixtures containing 1.2×10^8 EAC4b,3b cells, 0.67 ng B, 0.12 μg D̄ and 0.77 μg P to form EAC4b,3b,Bb,P; 1.2×10^8 EAC4b,3b cells, 1.68 ng B, 0.12 μg D̄, and 0.095 U C3NeF to form EAC4b,3b,Bb,NeF; and 1.2×10^8 EAC4b,3b cells, 6.72 ng B and 0.12 μg D̄ to form EAC4b,3b,Bb, each in 1.2 ml DGVB^{++}, were prepared in duplicate and incubated for 30 min at 30°C. The cellular intermediates were washed twice in EDTA-DGVB, resuspended in 1.2 ml of EDTA-DGVB alone or in EDTA-DGVB containing 43.3 μg commercial heparin per 10^7

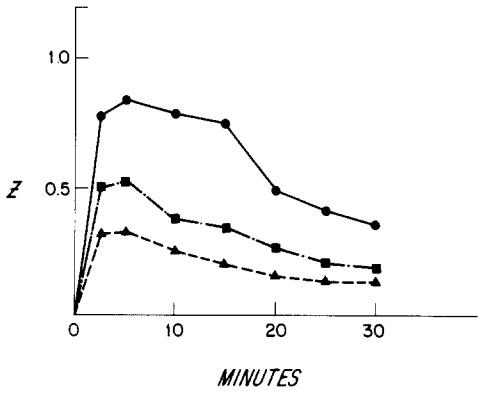

FIG. 3. Kinetics of generation of EAC4b,3b,Bb sites (Z) in DGVB^{++} (●——●) and in the presence of 0.22 μg (■——■) or 0.43 μg (▲——▲) heparin per 10^7 cellular intermediates.

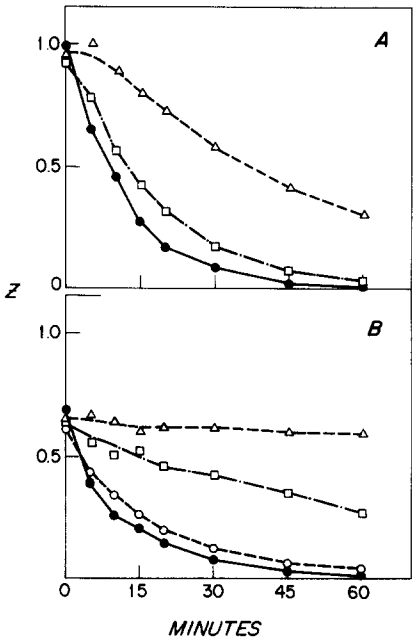

FIG. 4. Kinetics of B consumption by fluid phase C3b and D̄ alone (●——●) and in the presence of increasing concentrations of RPMC (A) or commercial (B) heparin. The amounts of RPMC heparin were 4 μg (△——△) and 0.8 μg (□——□) and of commercial heparin were 4.3 μg (△——△), 0.9 μg (□——□), and 0.2 μg (○——○) in the 0.2 ml reaction mixtures. Z expresses the residual fluid phase concentration of B in terms of the capacity to form hemolytic sites per cell.

cells, and decayed at 30°C. Two-tenths-ml portions were removed at timed intervals and added to 0.3 ml C-EDTA to develop residual convertase sites (Fig. 5). The half-lives of 5-1/2 min and 40 min of the unstabilized convertase and the C3NeF-stabilized convertase, respectively, were not changed by the presence of heparin. In contrast, the half-life of 48 min of the P-stabilized convertase decreased to 26 min in the presence of 43.3 μg heparin per 10^7

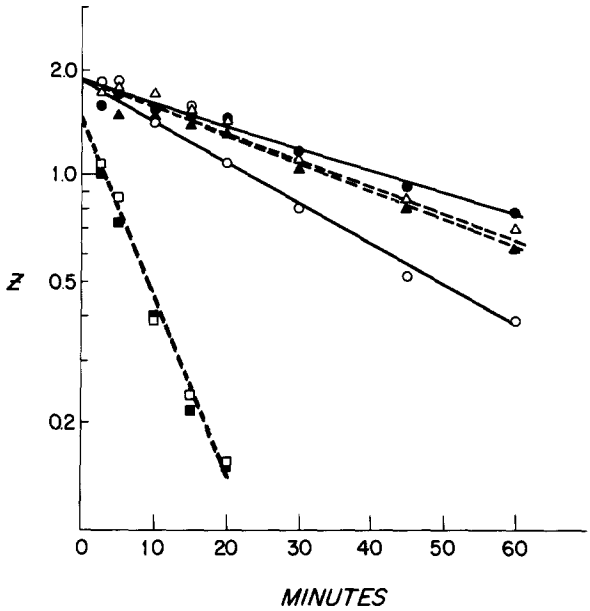

FIG. 5. Decay of hemolytic sites on EAC4b,3b,Bb (■——■), EAC4b,3b,Bb,P (●——●) and EAC4b,3b,Bb,NeF (▲——▲) in buffer alone and in the presence of 43.3 μg commercial heparin per 10^7 cells, respectively (□——□, ○——○, △——△).

cellular intermediates, suggesting that this high dose of heparin had no direct effect on the convertase but rather had an effect on P.

Effect of Heparin on the Regulatory Proteins, βlH, and C3bINA. The effect of heparin on βlH and C3bINA was also determined to assess further the capacity of heparin to modulate convertase expression. 2×10^8 EAC4b,3b, 1.5 ng B, 0.20 μg D̄, and 1.3 μg P in 2 ml $DGVB^{++}$, or 2×10^8 EAC4b,3b, 56 ng B, and 0.20 μg D̄ in 2 ml $DGVB^{++}$ were incubated for 30 min at 30°C to form EAC4b,3b,Bb,P and EAC4b,3b,Bb, respectively. One-tenth-ml portions of each intermediate were added to 0.2 ml EDTA-DGVB alone or EDTA-DGVB containing 16 ng βlH, or varying dilutions of commercial heparin, or both. The reaction mixtures were incubated for an additional 15 min at 30°C, and residual convertase sites were developed. βlH alone inhibited 58% of the P-stabilized sites and 56% of the unstabilized sites (Fig. 6). Increasing amounts of heparin inhibited the βlH effect on the unstabilized convertase, with 8.7 μg/10^7 cells fully inhibiting βlH acceleration of decay. With the P-stabilized convertase, low concentrations of heparin inhibited βlH-dependent decay, whereas higher amounts of heparin were directly inhibitory, presumably through removal of P-stabilization.

Seven-tenths μg C3bINA, 0.7 μg C3bINA and incremental amounts of commercial heparin, and incremental amounts of commercial heparin alone, in 0.2 ml $DGVB^{++}$, and the buffer alone were incubated for 15 min at 30°C. One-tenth ml $DGVB^{++}$ containing 1×10^7 EAC4b,3b was added to each mixture, and incubation was continued for 30 min at 37°C. 3 ml ice cold $DGVB^{++}$ was then added to each mixture, and the cells were washed twice in the same buffer. The cells were resuspended in 0.2 ml $DGVB^{++}$ containing 0.056 ng B,

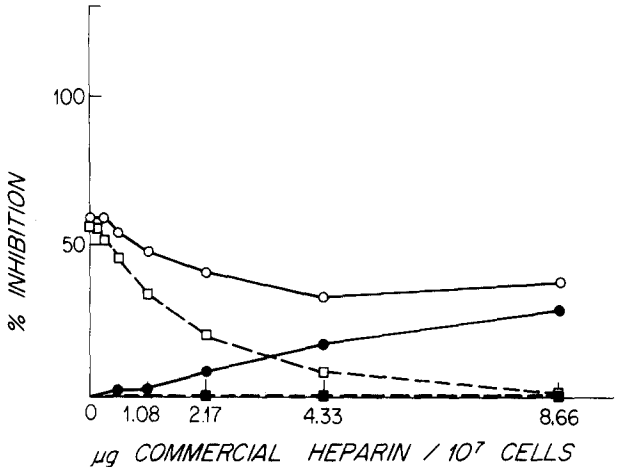

FIG. 6. Dose-response effects of commercial heparin on inhibition of EAC4b,3b,Bb,P (●——●) and EAC4b,3b,Bb (■——■) and on inhibition of these intermediates in the presence of a constant amount of β1H (○——○ and □——□, respectively). Percent inhibition refers to residual sites per cell (Z) as compared to decay in buffer alone.

0.01 μg D̄ and 0.065 μg P and incubated for 30 min more at 30°C. C-EDTA was then added to develop the convertase sites. C3bINA inactivated 35% of the C3b sites on the cellular intermediate; and the presence of 5.4, 10.8, 21.7, and 43.3 μg commercial heparin did not alter this result. When the EAC4b,3b intermediate was incubated with heparin alone and then washed, its ability to be lysed was not affected.

Inhibition of Formation of Amplification Pathway Convertase by Isolated RPMC Granules. RPMC granules were suspended in 0.0075 M Tris at pH 7.4 and studied for their effect on the formation of the P-stabilized amplification convertase, C3b,Bb,P. 1 ml $DGVB^{++}$ containing 0.56 ng B, 0.10 μg D̄, 0.65 μg P and 1×10^8 EAC4b,3b was added in 0.1-ml portions to 0.1 ml $DGVB^{++}$ alone or to $DGVB^{++}$ containing incremental amounts of RPMC heparin or RPMC granules. The reaction mixtures were incubated for 30 min at 30°C. The cellular intermediates were then washed three times with 3 ml $DGVB^{++}$ and resuspended in 0.2 ml $DGVB^{++}$, and C-EDTA was added to develop residual hemolytic sites. The RPMC granule heparin, quantitated by uronic acid content, inhibited the formation of the amplification pathway convertase, C3b,Bb,P, in a manner comparable to solubilized, isolated RPMC heparin (Fig. 7).

Discussion

Native macromolecular rat mast cell and commercial hog heparin inhibit generation of the amplification convertase C3b,Bb, thus modulating a critical step in the complement sequence. The inhibitory action of heparin was not due to chelation of magnesium, since equilibration of heparin with calcium and magnesium-containing buffer did not diminish its inhibitory activity. Both native and commercial heparin inhibit generation of the D̄-independent C3

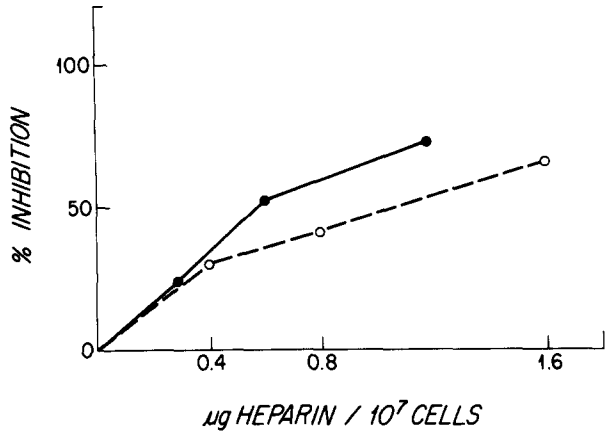

FIG. 7. Dose-response effects of RPMC heparin (O——O) and RPMC granules (●——●) on the formation of EAC4b,3b,Bb,P.

convertase, C3b,B, as well as the D̄-dependent convertase, C3b,Bb, even in the presence of P and C3NeF-stabilization, thereby suggesting an effect on the interaction of C3b and B rather than on D̄ or on the stabilizing principles (Fig. 1). Heparin was most active in impairing convertase formation on cellular intermediates formed with the lowest C3 input and developed with the highest B concentration, thereby suggesting an inhibitory action on the C3b binding site for B that is essential for convertase formation (Fig. 2). Heparin treatment of the EAC4b,3b intermediate followed by washing did not reduce site formation with B and D̄, thereby suggesting a reversible inhibitory action on C3b. The kinetics of unstabilized convertase (C3b,Bb) formation (Fig. 3) were not appreciably altered when heparin suppressed the total number of sites generated, and heparin did not accelerate decay of the unstabilized convertase (Fig. 5). Taken together, these studies indicate that native and commercial heparin, in a concentration of less than 1 μg per 10^7 cellular intermediates, act to prevent effective interaction of C3b and B, most likely by reversible action on C3b. This interpretation is strengthened by the demonstration that heparin does not inactivate B and, indeed, prevents B utilization in a fluid phase interaction of C3b, B, and D̄ (Fig. 4).

The concentration of native or commercial heparin which inhibited C3b,Bb formation by about 50% on a cellular intermediate limited to about 1 site per cell was 0.3 to 0.6 μg/10^7 cells (Fig. 1). A concentration of 4.5 to 20 μg/ml inhibited the fluid phase utilization of B by C3b in the presence of D̄ by about 50% (Fig. 4). A much higher concentration of commercial heparin had previously been observed to inhibit the electrophoretic conversion of B by D̄ in the presence of C3b (32). These concentrations of 1-10 μg/ml are comparable to those in which the anti-coagulant and anti-thrombin III cofactor activities of native and commercial heparin are expressed (11). This concentration range is the same as that in which commercial heparin has been reported to inhibit the reactive lysis phenomenon (9) and the binding of hemolytic C1 (4), and to augment the action of the C1 esterase inhibitor (8). A concentration of heparin 50-fold greater than that required to inhibit formation of unstabilized and

stabilized convertases did not alter the decay rate of unstabilized or C3NeF-stabilized convertase (Fig. 5) but did accelerate the decay of the P-stabilized convertase, thereby implying a direct effect on P at this high dose.

Heparin in a concentration range of 0.54–8.66 μg per 10^7 cells inhibited the capacity of β1H to accelerate the decay of the unstabilized convertase (Fig. 6). This finding serves to distinguish further the action of heparin from that of β1H. Heparin interference with the β1H effect was also observed in the presence of the P-stabilized convertase, but interpretation of the result was more complex because of the capacity of heparin itself to accelerate decay of this form of stabilized convertase. Heparin had no effect on the inactivating action of C3bINA on cell-bound C3b. The finding that heparin could prevent convertase formation on one hand and theoretically increase convertase formation through supression of β1H regulatory action on the other may account for some of the apparently paradoxical effects of heparin seen in some patients with paroxysmal nocturnal hemoglobinuria (33).

Native rat mast cell heparin proteoglycan was as active on a weight basis in inhibiting convertase formation as commercial heparin, which represents glycosaminoglycan side chains cleaved from the original hog proteoglycan. Heparin in the mast cell granule is bound in large part to a highly cationic chymotrypsin-like protease, whose proteolytic activity is appreciably masked until the granule is solubilized (34) in high salt. In contrast, the mast cell granule did express anticomplementary activity on convertase formation (Fig. 7). A regulatory action of the mast cell via its granules on the alternative complement pathway could be relevant to the role of these two effector systems in helminthic infections. Helminths not only stimulate a marked IgE-dependent hypersensitivity response but are themselves direct activators of the alternative complement pathway (35, 36). Activation of the tissue mast cells would alter local vascular permeability to bring both plasma proteins as well as inflammatory cells to that site. Human skin contains between 5,000 and 10,000 mast cells per mm^3 (37) and there is at least 0.1 μg heparin per 10,000 rat mast cells (11, 34). Thus, if the human mast cell contains heparin in any amount comparable to that of the rat, the concentrations available in some tissues would appear to be sufficient to modulate the amplification phase of the alternative pathway.

Summary

Native rat mast cell macromolecular heparin proteoglycan and commercial hog heparin glycosaminoglycan chains inhibit generation of the amplification convertase, C3b,Bb. The inhibitory action of heparin is not due to chelation of magnesium. Heparin is most active in inhibiting convertase formation on cellular intermediates formed with the lowest C3b input and developed with the highest B concentration, thereby suggesting the receptor site for B on C3b as the point of heparin action. This interpretation is consistent with the demonstration that heparin prevents B utilization during the fluid phase interaction of C3b, B, and D.

Inhibition is observed also when C3b,Bb generation takes place on cellular intermediates in the presence of P or C3NeF, which yield stabilized forms of

the convertase. 50 times the concentration of heparin required to inhibit convertase generation does not accelerate the decay of the unstabilized or the C3NeF-stabilized convertases and has only a modest effect on the P-stabilized convertase. An additional effect of heparin is to impair β1H-mediated decay-dissociation of C3b,Bb. The concentration of native or commercial heparin which prevents convertase formation is in the same range as that required for the demonstration of its anti-coagulant and anti-thrombin III cofactor activities. The additional finding that this inhibitory action of heparin can be expressed by the isolated mast cell granule suggests that native heparin may contribute to the modulation of the amplification pathway of complement.

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