Effect of Ionic Carbohydrate-Containing Biopolymers on Complement Activation

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We studied *in vitro* effects of charged polysaccharides on the classic and alternative pathways of complement activation. The complement system was affected by substances having different charges. Our findings suggest that the conformation of polysaccharide molecules, but not their charge, plays a primary role in the interaction with C1 and C3 complement components followed by initiation of cascade enzymatic reactions.

Key Words: *complement; classic pathway; alternative pathway; polysaccharides*

New natural immunostimulators attract much recent attention. Marine hydrobionts are the promising source of these substances. Here we studied the effects of biopolymers carrying different charges from marine hydrobionts on the classic (CP) and alternative pathways (AP) of complement activation.

MATERIALS AND METHODS

We used agar, agarose Ia, ethylene glycol-bis(β-aminoethyl ether)-N,N,N,N'-tetraacetic acid (EGTA), ethylenediaminetetraacetate (EDTA, Sigma), highly and low esterified apple pectin (Herbstreith und Fox), zymosan A (Reakhim), Zosterine from marine grass *Zostera marina*, high-molecular-weight λ- and κ-carrageenases from *Chondrus armatus*, low-molecular-weight λ-carrageenan obtained from high-molecular-weight λ-carrageenan by enzymatic hydrolysis, *o*-carboxylated chitin derivatives, chitosans with various N-acetylation degrees (N-ac) and molecular weights, N-succinyl chitosan synthesized from chitosan of *Camchaticus peralithodes* king crab shells, translam obtained from *Laminaria cichorioides* seaweed laminarin by enzymatic synthesis, mitilan isolated from *Crenomytilus grayanus* mussels (Pacific Institute of Bioorganic Chemistry), chitosan hydrochloride from *C. paralithodes*, and sodium alginate from *L. japonica* (Vostokfarm).

Complement activation by CP was performed as described previously [1]. Complement activation by AP was performed by the method of Y. Adachi *et al.* [5] with modifications [3].

RESULTS

We studied *in vitro* effects of charged polysaccharides (PS) on complement activation by CP and AP. Polyatomic cation exchangers included chitosans with various N-ac degrees and molecular weights. Polyatomic anion exchangers included carboxylated (sodium alginate, Zosterine, pectins, *o*-carboxymethyl and *o*-carboxyethyl chitin derivatives, and N-succinyl chitosan) and low sulfonated (agar and agarose) and highly sulfonated PS (high- and low-molecular-weight λ-carrageenans and high-molecular-weight κ-carrageenan). Neutral compounds included zymosan, high-molecular-weight mitilan α-glucan, and translam β-1,3;1,6-glucan.

High-molecular-weight λ- and κ-carrageenans in concentrations of 5-20 mg/ml displayed high activity and inhibited hemolysis by more than 50% (Table 1). High-molecular-weight λ-carrageenan was more potent in complement activation by AP that zymosan [6]. It should be emphasized that low-molecular-weight

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λ-carrageenan had no effect on complement activation by CP, but in a dose of 20 mg/ml this agent was active in complement activation by AP. Anionic *o*-carboxymethyl and *o*-carboxyethyl chitin derivatives inhibited complement activation by CP and AP. All anionic PS dose-dependently inhibited hemolysis. Positively charged high-molecular-weight chitosan displayed pronounced activity in complement activation by CP; the effect of chitosan decreased with increasing the degree of N-ac (Table 1). Commercial preparation of chitosan hydrochloride was inactive in complement activation by both pathways, which was probably related to low degree of N-deacetylation. Other PS had practically no effects of complement activation.

Polycationic and neutral polymers had no effect on complement activation by AP. Not all negatively charged PS display their activity in complement activation by AP and CP. Most anionic PS were inactive, which indicated that the activity of PS depends not only on their charge.

Chitin derivatives and carrageenans displaying pronounced activity possess gel-forming properties, which probably contributes to their effects on complement activation. However, negatively charged Zosterine, pectins, and agar having gel- and sol-forming properties did not modulate complement activation by both pathways.

Previous experiments with glucans showed that complement activation by AP is triggered by high-molecular-weight PS (above 250 kDa) [5]. The same interrelation was found between complement activation by CP and the molecular weight of λ-carrageenan. However, low-molecular-weight λ-carrageenan in a dose of 20 mg/ml inhibited complement-dependent hemolysis in complement activation by AP. This activity was probably related to the formation of high-molecular-weight associates in the presence of bivalent cations [2,4], which trigger cascade enzymatic reactions in the complement system.

Among sulfonated PS, only carrageenans displayed activity in complement activation by both pathways. λ-Carrageenan was more potent than κ-carrageenan probably due to additional sulfate groups. Agar and agarose were inactive in complement activation by both pathways, probably due to low content of sulfates or presence of L-galactose residues in their molecules.

Carboxylated chitin derivatives were active in complement activation by both pathways. Chitin carboxymethyl was more potent than chitin carboxyethyl in inhibiting hemolysis dependent on complement activation by AP. However, N-succinyl chitosan had no effect on complement activation by AP and CP. Probably, the conformation of chitin derivatives differs from that of N-succinyl chitosan. Our findings suggest that the conformation of glycan molecules, but not the charge of PS, is responsible for the formation of specific complexes with C1 and C3 complement components followed by their dissociation into active complement components.

TABLE 1. Residual Hemolysis during Complement Activation by PS (%, $X \pm m$)

Substances	Molecular weight, kDa	Complement activation			
		CP		AP	
		20 mg/ml	10 mg/ml	20 mg/ml	10 mg/ml
Chitosan					
high-molecular-weight	200	9.1±4.8	47.0±4.4	89.3±2.3	90.1±3.2
4% N-ac	25	61.1±5.7	98.7±9.3	100.0±6.8	98.4±3.9
19% N-ac	25	70.6±2.8	89.5±9.9	91.4±6.6	95.5±8.4
55% N-ac	25	99.8±4.1	99.3±4.5	100.0±3.9	95.3±5.8
low-molecular-weight	10	93.1±7.2	94.3±9.0	100.0±2.8	98.6±5.3
N-succinyl chitosan	200	93.0±3.8	94.0±3.1	98.2±6.5	98.4±3.5
Chitin-O-CH_2COOH	200	57.3±5.5	60.0±6.4	37.0±2.1	48.0±2.1
Chitin-O-CH_2CH_2COOH	200	67.9±8.8	92.2±8.9	50.0±4.1	82.1±3.6
κ-Carrageenan					
high-molecular-weight	>500	5.0±2.1	5.3±2.0	42.0±0.4	65.0±1.1
λ-Carrageenan					
high-molecular-weight	>500	4.9±1.1	5.3±0.9	14.0±1.5	69±7
low-molecular-weight	25-35	89.5±5.7	99.0±9.2	31.0±2.2	81.3±9.1
Zymosan	Insoluble	99.2±6.0	99.5±4.8	33.0±2.9	65.1±3.5

Neutral PS mitilan α-glucan and branched half-synthetic translam β-glucan had no effect on complement activation by AP and CP. Probably, these PS do not interact with complement components (as differentiated from standard AP activator water-insoluble zymosan β-glucan).

The unique conformation of various PS probably determines their interaction with the corresponding complement components followed by their activation and initiation of cascade reactions. This property contributes to the directional regulation of immune system reactivity.

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