Specificity of C-Reactive Protein for Choline Phosphate Residues of Pneumococcal C-Polysaccharide (35323)

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C-reactive protein (CRP) is an acute phase protein appearing in the serum of man during various pathological conditions, and frequently employed as a clinical index of acute inflammation. It is precipitated from the serum by pneumococcal C-polysaccharide in the presence of Ca ions. Although this precipitation reaction, which led to the discovery of CRP, has been known for many years, there is little information about the active site(s) in the molecule of C-polysaccharide participating in the reaction with CRP. It has been recently reported that choline phosphate is a unit of the macromolecular structure of C-polysaccharide (1, 2). In an attempt to determine the specificity of CRP for choline phosphate residues of C-polysaccharide, we conducted quantitative inhibition studies of CRP-C-polysaccharide precipitation. The substances tested for inhibitory effect included choline phosphate and a series of organic phosphate monoesters. The results of these experiments indicated that choline phosphate is the most active inhibitor of CRP–C-polysaccharide precipitation yet described, and suggested that this compound might provide the major reacting site of C-polysaccharide.

A single lot of CRP-positive serum (no. 9 E.M.), obtained 2 days following surgery from a patient with an intertrochanteric fracture of the femur, was used for all the inhibition experiments described below. Tested by capillary precipitation against a commercial antiserum (Hyland Labs, Los Angeles, California), this serum gave a 4+ reaction for CRP. The results with this serum were representative of data obtained also with other CRP-positive sera. C-polysaccharide was prepared by the method of Anderson and McCarty (3) from a III R strain of pneumococcus. The following substances obtained from commerical sources were tested for inhibitory effect without further purification: Choline phosphate chloride, *o*-phosphoryl-ethanolamine, DL-α-glycerophosphate, β-glycerophosphate, L-α-glycerophosphorylcholine, choline base, uridine-5′-monophosphate (UMP), adenosine-5′-monophosphate (AMP), and cytidine-5′-monophosphate (CMP). Saline containing 0.01% $CaCl_2$ and buffered with 0.01 *M* imidazole, pH 7.5, was used as diluent for all reagents. The pH of all solutions was adjusted to 7.5 if required.

In a typical experiment, 0.2-ml aliquots of serum were mixed with increasing amounts of inhibitor from 0.015 to 2.0 μmoles dissolved in 0.1 ml of buffer. After 30-min incubation at 37°, the quantity of C-polysaccharide required for maximal precipitation in the absence of inhibitor was added in 0.1-ml volume buffer. This was 10 μg in the case of serum no. 9 E.M. The tubes were incubated for an additional 30 min at 37° and for 24 hr at 4°. Controls included serum plus inhibitor alone and serum plus buffer alone. The precipitates were sedimented by centrifugation at 3600 rpm for 30 min in the cold, washed two times with 2.0 ml of ice-cold buffer, and the protein N content was determined by a modified Folin-Ciocalteu procedure read at 650 mμ (4). The controls gave negative or minimal OD readings. Percentage inhibition was calculated as:

$$1-\frac{(\text{OD of precipitate in presence of inhibitor})-(\text{OD of serum–buffer control})}{(\text{OD of precipitate in absence of inhibitor})-(\text{OD of serum–buffer control})}\times 100.$$

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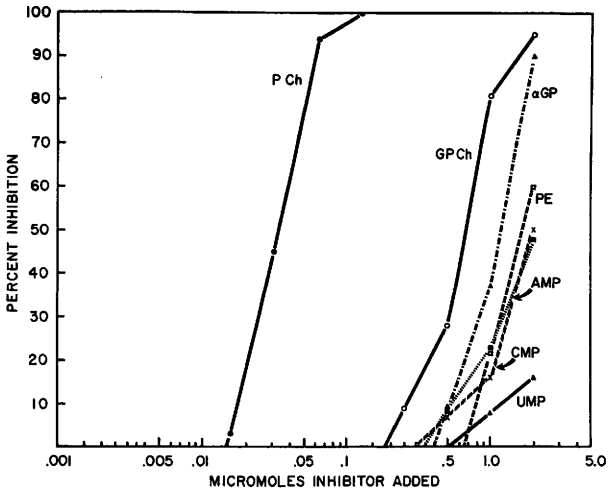

FIG. 1. Quantitative inhibition of CRP–C-polysaccharide precipitation by the following compounds: PCh, choline phosphate (phosphorylcholine); GPCh, L-α-glycerophosphorylcholine; α GP, DL-α-glycerophosphate; PE, phosphoryl-ethanolamine; AMP, adenosine-5′-monophosphate; CMP, cytidine-5′-monophosphate; UMP, uridine-5′-monophosphate.

The percentage inhibition plotted against log concentration of each inhibitor tested is presented in Fig. 1. Choline phosphate was found the most effective inhibitor, producing 50% inhibition at 0.03 μmole. Choline base was not inhibitory up to 5.0 μmoles. The amount of each compound required for 50% inhibition and its relative inhibitory power compared with choline phosphate are shown in Table I.

The naturally occurring L form of α-glycerophosphorylcholine was a considerably less effective inhibitor than choline phosphate with a relative inhibitory power of 0.045. This finding can perhaps be explained by the steric hindrance offered by the glycerol linkage or by its secondary phosphate structure. However, this compound was more inhibitory than the phosphate monoesters, DL-α-glycerophosphate, CMP, and AMP, which, as a group, demonstrated only small differences in inhibitory power—0.025, 0.015, and 0.014, respectively. The inhibitory capacity of these latter compounds for CRP was attributable to their primary phosphate ester group. However, β-glycerophosphate gave only minimal inhibition at 5.0 μmoles, presumably due to steric hindrance by esterification to the β-OH group. Interestingly, o-phosphoryl-ethanolamine, a structural analogue of choline phosphate had a relative inhibitory power of 0.018, very similar to that of other organic monoesters. The importance of the three methyl groups of choline for determining the specificity of the inhibition is thus underlined.

The chemical composition and the structure attributed to the C-polysaccharide macromolecule have differed considerably from one report to another, due in part to differences in procedures used for its isolation. It is generally agreed that C-polysaccharide is a component of the pneumococcal cell wall.

TABLE I. Amount Inhibitor Required for 50% Inhibition.

	Amount (μmoles)	Relative inhibitory power[a]
Choline phosphate	0.03	1.0
L-α-Glycerophosphorylcholine	0.66	0.045
DL-α-Glycerophosphate	1.2	0.025
β-Glycerophosphate	>5.0	—
o-Phosphoryl-ethanolamine	1.65	0.018
Adenosine-5′-monophosphate	2.1	0.014
Cytidine-5′-monophosphate	2.0	0.015
Uridine-5′-monophosphate	>2.0	—
Choline base	>5.0	—

[a] Molar ratio of choline phosphate to inhibitor compound at 50% inhibition.

Gotschlich and Liu (5) presented data indicating that C-polysaccharide, prepared from autolyzed cell walls by a procedure derived from that of Anderson and McCarty (3), contained at least two cross-linked polymers—a mucopeptide polymer containing muramic acid phosphate and a polymer of *N*-acetyl-galactosamine phosphate. They indicated the latter to be the major antigenic determinant of the reaction of pneumococcal polysaccharide with specific antibody and also presented evidence that this polymer reacted selectively with CRP. Precipitation of CRP–C-polysaccharide was shown to be inhibited by various phosphate monoesters, including UMP (6), AMP, CMP, and α-glycerophosphate (7). This inhibition was found related to the direct binding of CRP to phosphate monoesters in the presence of Ca ions. It was postulated by Gotschlich and Edelman (7) that the specificity of the reaction of CRP with C-polysaccharide might extend to the secondary phosphate groups of the proposed structure of C-polysaccharide.

Tomasz (2) first reported that choline was a covalently linked component of pneumococcal cell walls and of C-polysaccharide. Presence of choline was shown to play an important role in defining the biological properties of the pneumococcal cell surface with respect to cellular adhesion, genetic transformation, and autolytic susceptibility (8). More recently, Brundish and Baddiley (1) published data supporting the view that C-substance isolated by trichoroacetic acid extraction is a ribitol–teichoic acid complex containing choline phosphate. They reported that the composition of their preparation included phosphate, *N*-acetyl-D-galactosamine, D-glucose, N-acetyl-diaminotrideoxyhexose (9), ribitol, and choline in the molecular proportion 2:1:1:1:1:1. They proposed a structure in which choline phosphate was a unit of the polymer attached through a phosphodiester linkage to a hydroxyl group on either sugar or ribitol.

Our findings provide evidence that the choline phosphate present in C-polysaccharide provides in the least a major determinant group for the precipitation reaction with CRP. The inhibitory capacity of other phosphate monoesters was of significantly lower magnitude and could be attributed to the phosphate ester group held in common with choline phosphate. The specificity of CRP for other of the phosphorylated residues present in pneumococcal C-polysaccharide remains to be assessed quantitatively. It is further relevant that while specific antibody to C-polysaccharide exhibits specificity directed mainly to *N*-acetyl-galactosamine-6-phosphate residues (5), several mouse myeloma sera have been recently described by Leon (personal communication), which exhibited reaction with C-polysaccharide with specificity for choline phosphate residues as indicated by inhibition studies.

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