# INHIBITION EXPERIMENTS WITH PNEUMOCOCCAL C AND DEPYRUVYLATED TYPE-IV POLYSACCHARIDES*

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ABSTRACT

Phosphorylcholine, a component of the group-specific "somatic" C-polysaccharide of pneumococcus, has previously been shown to be a potent inhibitor of the precipitation of C-reactive protein (CRP) by C-polysaccharide as well as of the reaction between C-reactive myeloma proteins and the polysaccharide. We now find that phosphorylcholine is a fairly strong inhibitor of the homologous C-polysaccharide anti-C reaction as well as of the precipitation of anti-C by depyruvylated, pneumococcal type-IV, capsular polysaccharide. D-Glucose and 2-acetamido-2-deoxy-D-galactose are poor inhibitors. The surmise that phosphorylcholine might be an important antigenic determinant of C-polysaccharide is therefore confirmed by direct evidence.

## INTRODUCTION

The writers recently showed that the type-specific, capsular polysaccharide (S-IV) of pneumococcus (Pn) type-IV was converted by loss of pyruvic acid into a derivative (dp-IV) which not only reacted massively with antibodies to pneumococcal group-specific C-polysaccharide, regardless of type, but also precipitated the C-reactive protein which appears in human infections and inflammations[1]. S-IV is composed of D-galactose, pyruvic acid, and the *N*-acetyl derivatives of 2-amino-2-deoxy-D-galactose, 2-amino-2-deoxy-D-mannose, and 2-amino-2,6-dideoxy-L-galactose. C-substance obtained by autolysis or detergent-induced lysis of pneumococci contains 2-acetamido-2-deoxy-D-galactose phosphate, 2-acetamido-2-deoxy-D-glucose, *N*-acetylmuramic acid, amino acids, glucose, and choline[2,3]. In addition, ribitol phosphate and a mono-*N*-acetyldiaminotrideoxyhexose have been detected in C-polysaccharide prepared by extraction with trichloroacetic acid[4]. 2-Acetamido-2-deoxy-D-galactose, the only sugar common to both C-substance and S-IV, was shown to inhibit the C anti-C reaction less well than the corresponding phosphate[2].

In the meantime, phosphorylcholine (PC) has been found to be an excellent inhibitor of the reaction between C and C-reactive protein[5], as well as of the precipi-

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tation of C by certain myeloma proteins[6]. As far as is known, these proteins are not actually elicited in animals by C-polysaccharide. Inhibition of these reactions, while providing evidence for receptors accommodating PC on the surfaces of C-reactive protein and reactive myeloma proteins, does not necessarily indicate the presence of similar receptors on anti-C globulin, nor does it testify as to the importance of PC as an actual antigenic determinant of C-polysaccharide; that is, a portion of the molecule reactive with antibodies stimulated by the pneumococcal, group-specific antigen.

Direct evidence is now given that PC is, indeed, an antigenic determinant of C-polysaccharide.

## MATERIALS AND METHODS

The quantitative estimations of antibody nitrogen given in Tables I and II were carried out as in earlier papers[7,8] with antisera diluted so as to yield approximately the desired amount of antibody at equivalence, or, in the case of the dp-IV anti-C reactions, at maximal precipitation. The tubes containing serum–saline controls and mixtures with inhibitor were allowed to stand in ice–water for 40–60 min before addition of polysaccharide.

Inhibition of the reaction of C-substance with CRP by phosphorylcholine and phosphoethanolamine was assayed by a micromethod employing $^{125}I$ trace-labelled crystalline CRP; labelled by the Chloramine T method[9], it gave a specific activity of

TABLE I

INHIBITION OF PRECIPITATION[a] IN PNEUMOCOCCAL *C* ANTI-*C* SYSTEMS BY 2-ACETAMIDO-2-DEOXY-D-GALACTOSE AND BY PHOSPHORYLCHOLINE (PC)

| *Inhibitor added* (μM) | *Antiserum* | *Total vol.* (*ml*) | *At 0°* (*days*) | *Antibody N pptd.* (μg) | *Inhibition* (%) |
|---|---|---|---|---|---|
| None | R1238[b] | 0.5 | 6 | 28[c] | |
| PC, 22.5 | R1238 | 0.5 | 6 | 19.5 | 30 |
| PC, 11 | R1238 | 0.5 | 6 | 18.5 | 34 |
| None | R1238 | 0.5 | 7 | 29.5 | |
| GalNAc, 30 | R1238 | 0.5 | 7 | 21.5 | 27 |
| PC, 11 | R1238 | 0.5 | 7 | 19.5 | 34 |
| PC, 6 | R1238 | 0.5 | 7 | 21 | 29 |
| None | R1238 | 0.5 | 7 | 28.5[d] | |
| PC, 45 | R1238 | 0.5 | 7 | 17.5 | 39 |
| PC, 22.5 | R1238 | 0.5 | 7 | 20.5 | 28 |

[a]In separate experiments, 33μM D-glucose failed to inhibit precipitation of anti-C in anti-PnI 1057 or anti-PnVII 1074 by C-polysaccharide. [b]Serum of rabbit immunized with non-encapsulated pneumococci. [c]The C-polysaccharide used was prepared from non-encapsulated pneumococci originally of type II. [d]C-polysaccharide used was isolated as a by-product in the preparation of the capsular polysaccharide of Pn type-IV.

TABLE II

INHIBITION OF PRECIPITATION IN DEPYRUVYLATED S-IV ANTI-C SYSTEMS[a] BY PHOSPHORYLCHOLINE

| *Inhibitor added (μM)* | *Antiserum* | *Total vol. (ml)* | *At 0° (days)* | *Antibody N pptd. (μg)* | *Inhibition (%)* |
|---|---|---|---|---|---|
| None | R1238 | 0.5 | 6 | 25.5[b] | |
| PC, 11 | R1238 | 0.5 | 6 | 28.5 | |
| PC, 6 | R1238 | 0.5 | 6 | 25.5 | |
| None | Anti-PnI 1057[c] | 0.5 | 10 | 27 | |
| PC, 45 | Anti-PnI 1057 | 0.5 | 10 | 22.5 | 17 |
| PC, 22.5 | Anti-PnI 1057 | 0.5 | 10 | 23.5 | 13 |
| None | Anti-PnVII 1074[d] | 0.5 | 9 | 34.5 | |
| PC, 45 | Anti-PnVII 1074 | 0.5 | 9 | 30 | 13 |
| PC, 22.5 | Anti-PnVII 1074 | 0.5 | 9 | 34 | |
| None | R1238 | 0.5 | 8 | 32[e] | |
| PC, 45 | R1238 | 0.5 | 8 | 22 | 31 |
| PC, 22.5 | R1238 | 0.5 | 8 | 29.5 | 8 |
| PC, 11 | R1238 | 0.5 | 8 | 30 | |

[a]Inhibition in these systems by 40μM 2-acetamido-2-deoxy-D-glucose was minimal. [b]Dp-IV prepared from S-IV in 0.01M HCl for 30 min at 100°. [c]Antiserum contains 288 μg of anti-C nitrogen per ml. [d]Antiserum contains 580 μg of anti-C nitrogen per ml. [e]Dp-IV prepared from S-IV in 0.01M HCl for 7 min at 100°.

60,000 c.p.m./μg. To 8 μg of CRP were added various concentrations of the inhibitors and either 1 μg of C-substance or 10 μg of dp-IV. The reactions were carried out in a total volume of 100 μl in Microfuge tubes (Beckman Instruments, Palo Alto, Calif.).

TABLE III

INHIBITION BY PHOSPHORYLCHOLINE AND PHOSPHOETHANOLAMINE OF PRECIPITATION OF CRP WITH C-SUBSTANCE AND WITH DEPYRUVYLATED S-IV (DP-IV)

| *Inhibitor* | *Concentration of inhibitor (μmoles/ml)* | *Inhibition[a] of reaction of CRP with* | |
|---|---|---|---|
| | | *C-polysaccharide (%)* | *dp-IV (%)* |
| Phosphorylcholine | 0.002 | 0 | 18 |
| | 0.005 | 8 | 49 |
| | 0.010 | 22 | 77 |
| | 0.020 | 43 | 92 |
| | 0.050 | 95 | 92 |
| Phosphoethanolamine | 0.010 | 0 | 0 |
| | 0.020 | 0 | 9 |
| | 0.050 | 2 | 20 |
| | 0.100 | 9 | 59 |
| | 0.200 | 17 | 80 |
| | 0.500 | 49 | 94 |
| | 1.000 | 84 | 95 |
| | 2.000 | 97 | 94 |

[a]To the nearest %.

After equilibration overnight at 4°, the precipitated CRP was sedimented by centrifugation for 5 min in a Microfuge (Beckman Instruments). The supernatant was discarded, and the radioactivity in the sediment was measured in a gamma scintillation counter.

## RESULTS AND DISCUSSION

From the studies cited[5,6] and from the data in Tables I–III, it is evident that phosphorylcholine (PC) is a far more-potent inhibitor of the precipitation of myeloma protein or C-reactive protein (CRP) by pneumococcal C-polysaccharide or depyruvylated, type-IV, capsular polysaccharide (dp-IV) than of the homologous C anti-C reaction or of the dp-IV anti-C cross-reaction.

The failure of PC to inhibit the C anti-C reaction more impressively is readily understandable, since PC is only a single component of this complex polysaccharide. Anti-C is therefore probably directed against several of the constituents of C-polysaccharide and any single component would not be expected to inhibit greatly, if at all. This has already been shown for a number of polysaccharide antibody systems, *e.g.* Refs. 10–12. Cross-reactions, which usually involve only a portion of the complete antigenic determinant, are more easily inhibited than homologous precipitations.

It has been shown that several very different phosphate monoesters inhibit the reaction of CRP with C-substance and that CRP has a binding site for phosphate monoester on each of its subunits[13]. The finding that phosphorylcholine is approximately 20 times more potent than phosphoethanolamine in inhibiting CRP is in agreement with the data of Kaplan and Volanakis[5]. Why this particular phosphate monoester, PC, is a more-potent inhibitor is as yet unexplained. It is also uncertain why depyruvylated S-VI should react with CRP, particularly since 2-acetamido-2-deoxy-D-galactose is the only component common to dp-IV and C.

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