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Mode of interaction of different polyanions with the first (C1, $C\bar{1}$) the second (C2) and the fourth (C4) component of complement*

IV. ACTIVATION OF C1 IN SERUM BY POLYANIONS

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Summary. Treatment of serum with dextransulphate, polyvinylsulphate or polyanetholsulphonate resulted in a dose-dependent activation of C1 and of C3; this was found for normal serum as well as for C4-deficient guinea-pig serum. Activation of C1 and C3 occurred at the same concentration of polyanions. The consumption of C3 in C4 deficient serum and the requirement of factor D of the alternative pathway indicate that C3 is activated via the alternative pathway.

INTRODUCTION

Polyanions (PA) behave as potent-inducing substances of the alternative pathway of complement activation (Hadding, Dierich, König, Limbert, Schorlemmer & Bitter-Suermann, 1973; Burger, Hadding, Schorlemmer, Brade & Bitter-Suermann, 1975) although the mechanism of PA action is not yet clear. Recently we demonstrated that PA interfere with the components of the classical pathway in at least three different ways: PA bind to C1q, (Raepple, Hill & Loos, 1976); PA prevent the consumption of C4 and C2 by $C1\bar{s}$, by interfering with its C4 and C2 binding site(s) (Loos, Volanakis & Stroud, 1976b); PA inhibit the binding of C2 to EAC4 by sequestering the Mg^{2+} ions (Loos, Volanakis & Stroud, 1976a). Based on the finding that PA bind to C1q it was of interest to find out whether addition of PA to serum leads to an activation of C1. Therefore the effect of polyvinyl sulphate (PVS), dextran sulphate (DS) and polyanethol sulphate (liquoid) on serum C1 was tested and compared with the activation of C3 by these substances via the alternative pathway.

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The complement nomenclature follows the WHO recommendations (*Bull. Wld Hlth Org.* 1968, **39**, 939).

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MATERIALS AND METHODS

The methods for the preparation of sheep erythrocyte (E), cell intermediates, antisera and buffer are given in Rapp & Borsos (1970). Pools of fresh-frozen normal guinea-pig and C4-deficient guinea-pig serum were used. The titration of C1, C4 and C2 with guinea-pig C–EDTA as the source of late-acting components was described in Rapp & Borsos (1970); with the haemolytic C1 test only the activated form of C1, $C\bar{1}$, can be measured

whereas precursor C1 or serum C1 is not detectable under these conditions. The C3 assay was performed in the immune haemolysis test system as described by Bitter-Suermann, Dierich, König, Hadding (1972). Guinea-pig serum depleted of factor D (RD) was prepared as described by Dierich, Hadding, König, Limbert, Schorlemmer & Bitter-Suermann (1974). DS, sodium salt. mol. wt 500·000, catalogue no. 18707 and PVS, potassium salt, molecular weight: n × 162·6, catalogue no. 33426 were purchased from Serva, Heidelberg, Germany; polyanethol sulfonate (liquoid), batch No. G 607277 was a gift from Hoffmann-La-Roche, Grenzach, Germany.

All polyanions were dissolved in veronal-buffered saline (VBS), $\mu = 0{\cdot}15$; pH = 7·3 without metal ions and gelatine to a concentration of 10 mg/ml; these stock solutions were stored at −20°C. The dilutions of the PA were made in veronal-buffered saline with sucrose (VBS-S) containing gelatine (0·1%), Ca^{2+} (0·15 mM) and Mg^{2+} (1 mM), μ = 0·065, pH 7·3. The concentrations of PA are given in μg/ml dilution buffer added to serum; therefore, the final concentrations of PA in the reaction mixtures is the half of the indicated amounts.

RESULTS

To test whether PVS, DS or polyanethol sulphonate are able to activate C1 in serum, equal volumes of different PA concentrations were mixed with undiluted guinea-pig serum and incubated for 15 min at 37°. Afterwards the different sera samples were diluted in VBS-S and tested for C1 and C3 activity. Fig. 1 shows the effect of different PVS concentrations on C1 in serum in comparison to buffer-treated controls. It can be seen that PVS in serum lead to a concentration dependent consumption of C3 similar to the described effect of DS (Burger *et al.*, 1975). At the same concentration where C3 consumption occurred an increase of C1 activity was found in comparison to the buffer-treated control.

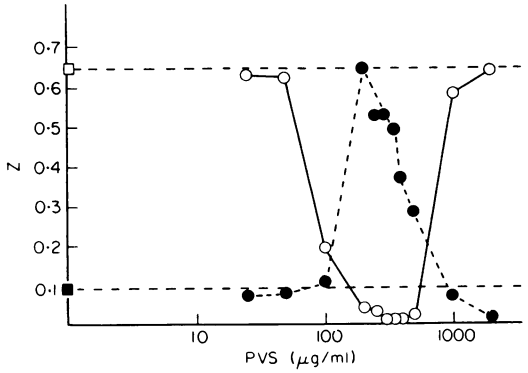

Figure 1. Effect of increasing amounts of PVS upon C1 and C3 haemolytic activities in normal guinea-pig serum. Equal volumes of undiluted serum were incubated with PVS for 10 min at 37°; after this time each sample was tested for haemolytic C1 (●– – –●) and C3 (○——○) activities and compared to buffer-treated controls (C1: ■; C3: □).

To find out whether C3 consumption is the result of the already described activation effect of PA on the alternative pathway or due to the classical pathway via C1, C4 and C2, similar experiments were performed in C4-deficient guinea-pig serum. The results are shown in Fig. 2 for liquoid and DS.

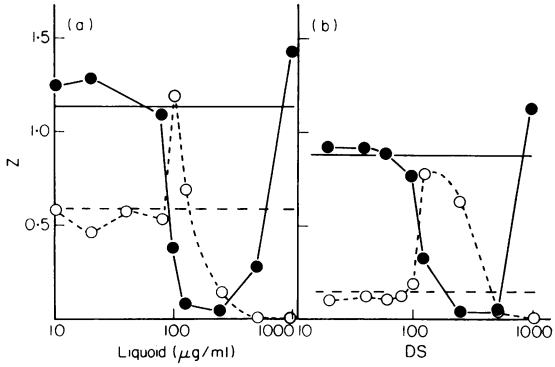

Figure 2. Effect of increasing amounts of DS liquoid, (a) or DS (b) upon C1 and C3 haemolytic activities in C4-deficient guinea-pig serum. Equal volumes of undiluted C4-deficient guinea-pig serum were incubated with liquoid or DS for 10 min at 37°; after this time each sample was tested for haemolytic C1 (○– – –○) and C3 (●——●) activities and compared to buffer-treated controls: C3 (——) and C1 (– – –).

In both cases an increase of C1 activity and C3 consumption was found indicating that C3 consumption was not triggered via the fourth component of the classical pathway. Furthermore, besides the increase of C1 activity a reduction of C1 was found at higher PA concentrations.

If PA activate C1 and C3 in serum independently, then in a factor-D-depleted serum they should cause an increase of C1 activity but no consumption of C3. Furthermore it was of interest to test whether the activation of C1 also leads to an increased reduction of C2, one of the natural substrates of $C\bar{1}$. After treatment of a factor-D-depleted serum

with the three different substances as already mentioned at 37° the samples were checked for C1, C2 and C3 activity. As shown in Fig. 3 for PVS

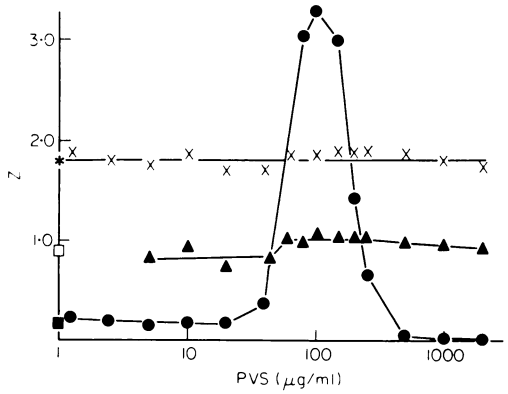

Figure 3. Effect of increasing amounts of PVS upon C1, C2 and C3 haemolytic activities in a normal guinea-pig serum which was depleted of factor D of the alternative pathway (RD serum). Equal volumes of RD serum were incubated with PVS for 10 min at 37°; after this time each sample was tested for haemolytic C1 (●——●), C2 (×——×) and C3 (▲——▲) activities and compared to buffer-treated controls: C1 (■); C2 (*); C3 (□).

an increase of C1 activity was observed, whereas no reduction of C2 or C3 activity was detectable.

In a similar experiment performed at 0° in a factor-D-depleted serum, only a reduction of C1 activity was found in comparison to a buffer-treated control (Fig. 4). This finding indicates that the activation of C1 in serum is a temperature-dependent reaction.

DISCUSSION

These experiments show that incubation of normal guinea-pig serum with the polyanions (PA), DS, PVS and liquoid result in an increase of haemolytic C1 activity and in a consumption of haemolytic C3 activity. Both effects are concentration dependent; the optima are between 100–500 μg/ml. At higher PA concentration there is no C3 consumption, as described by Burger *et al.* (1975) and Hadding *et al.* (1973); also, at higher PA concentrations a reduction instead of an increase of haemolytic C1 activity was found.

In previous experiments (Raepple *et al.*, 1976) it was demonstrated that the PA tested interact directly with purified C1q, a sub-unit of the first component of complement. Based on these experiments an interpretation of the PA induced increase of haemolytic activity is possible. Addition of PA to serum leads to binding of C1 to PA via C1q. Binding of serum C1 to PA results in a temperature-dependent activation of C1 to C1̄, i.e. the conversion of the haemolytic non-active precursor C1 into the haemolytic active form C1̄. This interpretation is supported by the experiments with PVS and RD serum at 0° and at 37° (Figs 3 and 4). At 0° only binding and therefore reduction of C1 activity was found; at the same PA concentrations which show a binding at C1 at 0° an increase of C1̄ activity was found, when the same experiment was performed at 37°. At higher PA concentrations the inhibitory

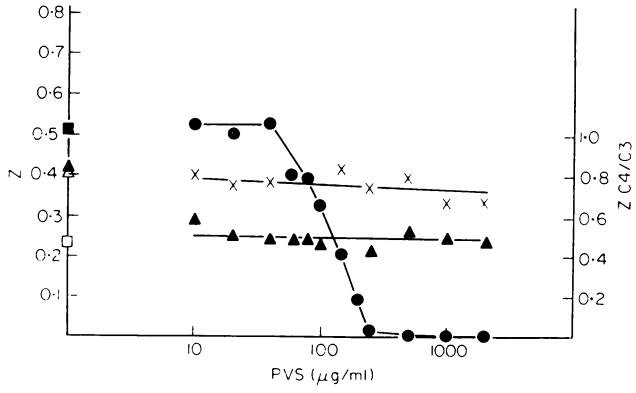

Figure 4. Effect of increasing amounts of polyvinyl sulfate (PVS) upon C1, C4 and C3 hemolytic activities in a factor D depleted normal guinea pig serum (RD serum) during preincubation for 10 min at 0°. C1 (●——●); C4 (×——×) C3 (▲——▲). Controls: C1 (■); C3 (□); C4 (△).

effect of PA overruled the activation effect of PA as shown in Fig. 2.

These findings and interpretations are also in good agreement with the experiments by Rent, Ertel, Eisenstein & Gewurz (1975) and Fiedel, Rent, Myhorman & Gewurz (1976). These authors demonstrated complement and C1 activation by interactions of PA and polycations using heparin–protamine complexes mixed in the source of complement. However preformed heparin–protamine complexes showed greatly reduced or no consumption of complement as well as of C1. This discrepancy may be explained by the experiments presented in this paper that PA alone are able to activate and/or consume C1. In the preformed heparin–protamine complex the polyanionic capacity of heparin is neutralized by protamine and therefore the effect of heparin on C1 is markedly reduced.

Although an increase of C1 activity was found in the RD serum no measurable C2 consumption was found. This may be because C4 and C2 consumption by $C1\bar{s}$ was shown to be markedly reduced in the presence of PA (Loos, Volanakis & Stroud, 1976). Despite the finding that C1 activation occurred at the same PA concentration as C3 consumption these experiments suggest that PA activates the complement system in its classical part (C1) as well as via the alternative pathway. However it cannot be excluded that the enzymes (i.e. $C1\bar{s}$) generated in one pathway influence or amplify the activation of the other pathway as described by Volanakis, Schulz & Stroud (1976).

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