Activation of the binding of C1q to immune complexes by zinc

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$ZnSO_4$ promotes the binding of C1q to immune complexes over the same concentration range (10^{-5}–10^{-4} M) that it inhibits binding of C1 to cell-bound immunoglobulin [Biochem. Biophys. Res. Commun. (1981) 103, 856–862]. At higher concentrations (10^{-3}–2×10^{-2} M) $ZnSO_4$ inhibited the binding of C1q to immune complexes, [$K_i = (6 \pm 2) \times 10^{-3}$ M]. This inhibition could be correlated with a $ZnSO_4$-induced change in the tryptophan fluorescence of C1q [ΔF 25%, $K_d = (9.9 \pm 1.0) \times 10^{-3}$ M].

C1q *Immune complex* *Complement* *Zinc* *Fluorescence*

1. INTRODUCTION

The first step in activation of the classical complement pathway is binding of complement component C1 to aggregated immunoglobulin [1]. C1 is a macro-molecular complex containing 3 proteins: C1q, C1r and C1s. C1q is responsible for binding to immunoglobulins, while C1r and C1s are zymogens which are converted into serine proteases during activation of C1 [2]. The integrity of C1 is dependent on the presence of Ca^{2+} [3], but the effects of other divalent cations on the classical complement pathway have hitherto received little attention. C1 binding to cell-bound immunoglobulin is inhibited by Zn^{2+} over 10^{-5}–10^{-4} M [4]. The results presented below indicate that Zn^{2+} in this concentration range promotes the binding of C1q to immune complexes, while the binding is inhibited by Zn^{2+} over 10^{-3}–10^{-2} M.

2. MATERIALS AND METHODS

2.1. *Proteins*

C1q was prepared from human serum, and labelled with ^{125}I (using lactoperoxidase) using the procedure in [5]. Rabbit anti-ovalbumin IgG and equivalence ovalbumin:IgG immune complexes were prepared as in [6].

2.2. *C1q binding assay*

The binding of ^{125}I-labelled C1q to immune complexes was measured as in [7] with the following modifications: the reaction volume was 0.3 ml, the buffer was 0.03 M Tris–HCl (pH 7.2), containing 0.15% (w/v) gelatin, and the reaction time was 40 min. The effect of added $ZnSO_4$ on the ionic strength of the assay medium was corrected for by addition of NaCl to maintain the ionic strength at 0.15.

2.3. *Fluorescence measurements*

The effects of Zn^{2+} on the tryptophan fluorescence of IgG and C1q were determined using an Aminco SPF500 spectrofluorimeter, with the cell holder thermostatted at 37°C. The fluorescence of the protein samples (0.2–1.0 μM, in 0.05 M Tris–HCl, 0.1 M NaCl, pH 7.2) was measured at 340 nm, following excitation at 295 nm.

2.4. *Data analysis*

Kinetic constants for C1q binding to immune complexes, and Zn^{2+} binding to C1q were obtained by non-linear regression analysis [8] assuming that both binding processes are of the non-cooperative (Michaelis–Menten) type.

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3. RESULTS AND DISCUSSION

The dependence of C1q binding on $ZnSO_4$ concentration at various fixed immune complex concentrations, is shown in fig.1. Two different effects of $ZnSO_4$ are apparent; enhancement of C1q binding to immune complexes by $ZnSO_4$ over 10^{-5}–10^{-3} M, and inhibition of binding by $ZnSO_4$ over 10^{-3}–2×10^{-2} M. The effects of $ZnSO_4$ on the kinetic parameters of the binding process were investigated in both concentration ranges. The data shown in fig.2 show the dependence of bound C1q on the free C1q concentration, at various fixed $ZnSO_4$ levels in the low concentration range. The secondary plots (fig.2, insert) showed that only the slopes of the double reciprocal plots of fig.2 changed with $ZnSO_4$ concentration. Linear regression analysis of the slope replot gave an apparent activation constant for $ZnSO_4$ of $(1.1 \pm 0.1) \times 10^{-4}$ M.

The results shown in fig.3 show the dependence of bound C1q on free C1q concentration at $ZnSO_4$ levels between 1×10^{-3} M and 1.5×10^{-2} M. Again, only the slopes of the double reciprocal plots changed with $ZnSO_4$ concentration: linear regression analysis of the slope replot (fig.3, insert) gave an apparent inhibition constant of $(6 \pm 2) \times 10^{-3}$ M.

Addition of $ZnSO_4$ over $10^{-5} \times 10^{-4}$ M to either

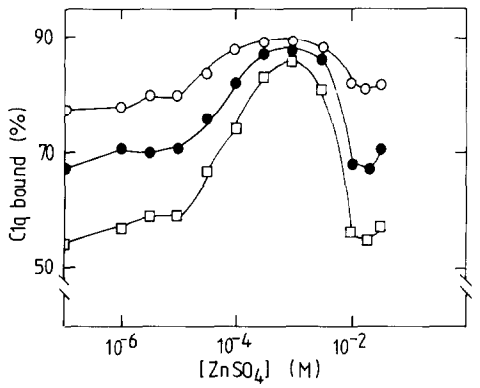

Fig.1. The effect of $ZnSO_4$ on C1q binding to immune complexes. The binding of ^{125}I-labelled C1q (0.1 μg/ml, 6×10^5 cpm/μg) to 10 μg/ml (□—□), 20 μg/ml (●—●), and 40 μg/ml (○—○) immune complexes was measured at the indicated $ZnSO_4$ concentrations. $ZnSO_4$ had no effect on the amount of C1q precipitated (5 ± 2%) in the absence of immune complexes.

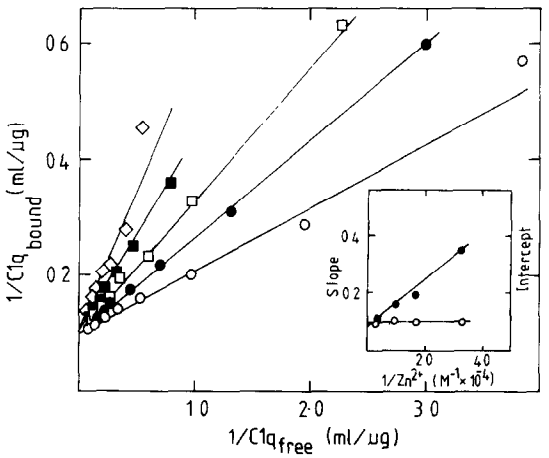

Fig.2. Enhancement of C1q binding to immune complexes by $ZnSO_4$. The binding of ^{125}I-labelled C1q (9×10^3 cpm/μg) to 60 μg/ml immune complexes was measured in the absence (◇—◇) and in the presence of 3×10^{-5} M (■—■), 6×10^{-5} M (□—□), 1×10^{-4} M (●—●) and 3×10^{-4} M (○—○) $ZnSO_4$. The lines shown were obtained by non-linear regression analysis [8] on the primary data. Insert: dependence of the slopes (●—●) and intercepts (○—○) of the double reciprocal plots on $ZnSO_4$ concentration.

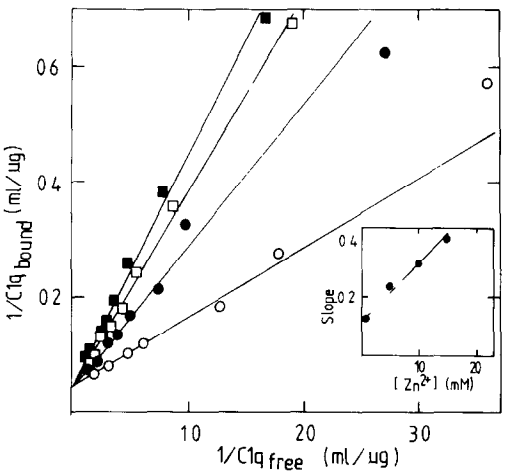

Fig.3. Inhibition of C1q binding to immune complexes by $ZnSO_4$. The binding of ^{125}I-labelled C1q (9×10^3 cpm/μg) to immune complexes was measured in the presence of $ZnSO_4$ at: 1×10^{-3} M (○—○); 5×10^{-3} M (●—●); 1×10^{-2} M (□—□); and 1.5×10^{-2} M (■—■). The lines shown were obtained by non-linear regression analysis [8] on the primary data. Insert: Dependence on the slope of the double reciprocal lines on $ZnSO_4$ concentration.

C1q or IgG had no significant effect on the tryptophan fluorescence of either protein. Furthermore, the tryptophan fluorescence of IgG was unaffected by $ZnSO_4$ over $10^{-3} - 2 \times 10^{-2}$ M.

However, addition of $ZnSO_4$ in this range to C1q was associated with changes in the tryptophan fluorescence of the protein (fig.4). Non-linear regression analysis on these data gave a maximum fluorescence change of 24.6 ± 1.5%, associated with an apparent dissociation constant for $ZnSO_4$ binding of $(9.9 \pm 1.0) \times 10^{-3}$ M.

The results shown in fig.2 indicate that Zn^{2+} over 10^{-5}–10^{-4} M promotes the binding of C1q to immune complexes. It would appear that this effect arises from a relatively subtle change in the structure of C1q, in that Zn^{2+} had no effect on the tryptophan fluorescence of the protein over 10^{-5}–10^{-4} M. On the other hand, the inhibition of C1q binding by Zn^{2+} which was characterised by an inhibition constant of $(6 \pm 2) \times 10^{-3}$ M could be correlated with a 25% decrease in the tryptophan fluorescence of the protein, with a dissociation constant of $(9.9 \pm 1.0) \times 10^{-3}$ M. Although it is not possible to deduce the extent of the structural change from the magnitude of the fluorescence change, this inhibition may be associated with a more extensive change in the structure of C1q than the enhancement of binding observed at lower Zn^{2+} concentrations. Although the Zn^{2+} mediated enhancement of C1q binding to immune complexes falls in the same concentration range (10^{-5}–10^{-4} M) as the Zn^{2+}-mediated inhibition of C1 binding to cell-bound immunoglobulin [4] it is not clear whether the two phenomena arise from similar mechanisms. The latter system is far more complex, as the binding of C1r and C1s to C1q influences the conformation of C1q [9,10], and the binding of C1q to aggregated immunoglobulin alters both its structure [11], and its affinity for the $C1r_2\ C1s_2$ tetramer [12]. Thus, although the Zn^{2+}-induced inhibition of C1 binding may arise from the same mechanism as the Zn^{2+}-mediated promotion of C1q binding to immune complexes, the possibility that the inhibition is mediated via C1r or C1s can not be excluded.

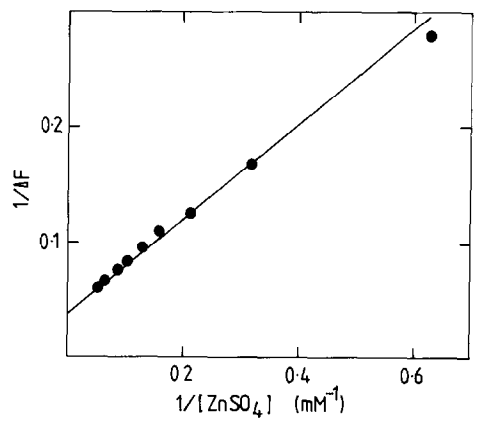

Fig.4. Effect of $ZnSO_4$ on the tryptophan fluorescence of C1q. The change in fluorescence of C1q (0.22 μM) following addition of $ZnSO_4$ was determined as in section 2. The line shown was obtained by non-linear regression analysis [8] on the primary data.

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