

Activation of the binding of C1q to immune complexes by zinc

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Received 11 June 1983; revised version received 4 August 1983

ZnSO₄ 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) ZnSO₄ inhibited the binding of C1q to immune complexes, [$K_i = (6 \pm 2) \times 10^{-3}$ M]. This inhibition could be correlated with a ZnSO₄-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 Ca²⁺ [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 Zn²⁺ over 10^{-5} – 10^{-4} M [4]. The results presented below indicate that Zn²⁺ in this concentration range promotes the binding of C1q to immune complexes, while the binding is inhibited by Zn²⁺ over 10^{-3} – 10^{-2} M.

2. MATERIALS AND METHODS

2.1. Proteins

C1q was prepared from human serum, and labelled with ¹²⁵I (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 ¹²⁵I-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 ZnSO₄ 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 Zn²⁺ 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 Zn²⁺ binding to C1q were obtained by non-linear regression analysis [8] assuming that both binding processes are of the non-cooperative (Michaelis-Menten) type.

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

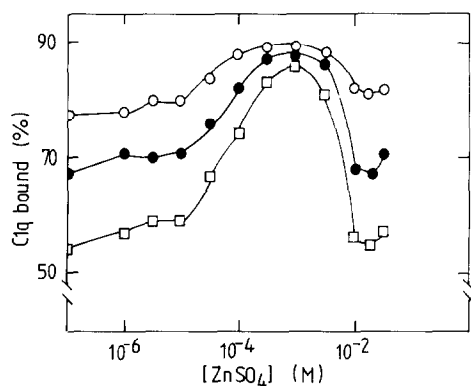


Fig.1. The effect of ZnSO_4 on C1q binding to immune complexes. The binding of ^{125}I -labelled C1q ($0.1 \mu\text{g/ml}$, $6 \times 10^5 \text{ cpm}/\mu\text{g}$) to $10 \mu\text{g/ml}$ ($\square$ — $\square$), $20 \mu\text{g/ml}$ ($\bullet$ — $\bullet$), and $40 \mu\text{g/ml}$ ($\circ$ — $\circ$) immune complexes was measured at the indicated ZnSO_4 concentrations. ZnSO_4 had no effect on the amount of C1q precipitated ($5 \pm 2\%$) in the absence of immune complexes.

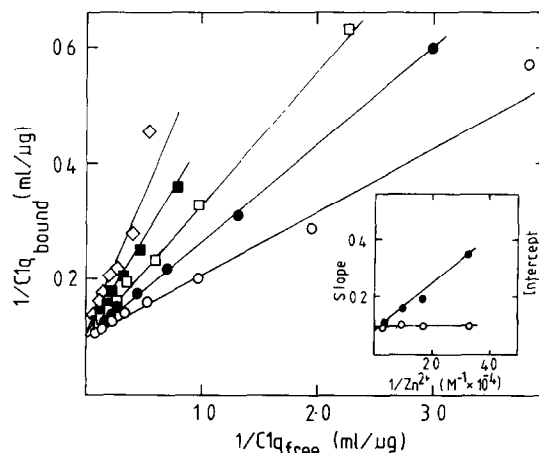


Fig.2. Enhancement of C1q binding to immune complexes by ZnSO_4 . The binding of ^{125}I -labelled C1q ($9 \times 10^3 \text{ cpm}/\mu\text{g}$) to $60 \mu\text{g/ml}$ immune complexes was measured in the absence ($\diamond$ — $\diamond$) and in the presence of 3×10^{-5} M ($\blacksquare$ — $\blacksquare$),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

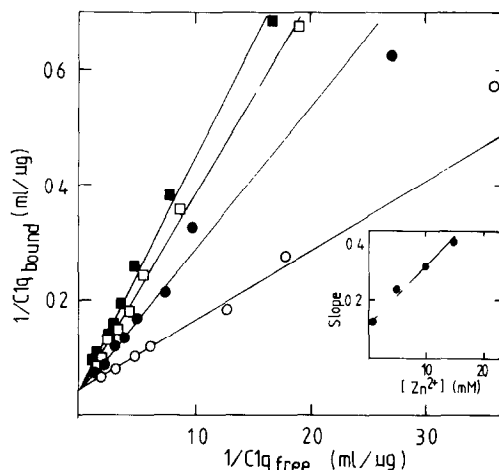


Fig.3. Inhibition of C1q binding to immune complexes by ZnSO_4 . The binding of ^{125}I -labelled C1q ($9 \times 10^3 \text{ cpm}/\mu\text{g}$) to immune complexes was measured in the presence of ZnSO_4 at: 1×10^{-3} M ($\circ$ — $\circ$); 5×10^{-3} M ($\bullet$ — $\bullet$); 1×10^{-2} M ($\square$ — $\square$); and 1.5×10^{-2} M ($\blacksquare$ — $\blacksquare$). 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 $24.6 \pm 1.5\%$, 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

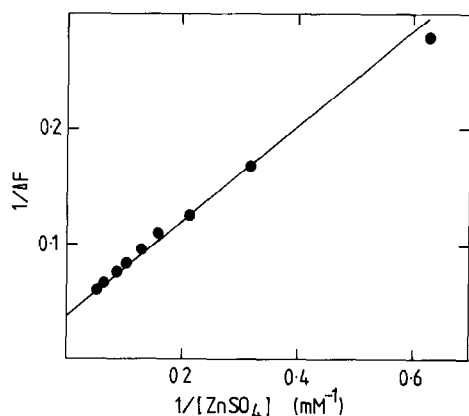


Fig.4. Effect of ZnSO_4 on the tryptophan fluorescence of C1q. The change in fluorescence of C1q ($0.22 \mu\text{M}$) following addition of ZnSO_4 was determined as in section 2. The line shown was obtained by non-linear regression analysis [8] on the primary data.

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 C1r₂ C1s₂ 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.

ACKNOWLEDGEMENTS

This work was supported by the Australian Research Grants Scheme. I am grateful to Dr A. Lovric (Red Cross Blood Bank, Sydney NSW) for arranging a supply of human serum for C1q preparations.

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