Conformational changes in C1q upon binding to IgG oligomers

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The interaction between C1q and immune complexes is inhibited by 1-anilino-8-naphthalenesulfonate (ANS) in the concentration range of 2–4 mM. ANS binds to C1q with a 20-fold higher affinity than to IgG [(1986) Mol. Immunol. 23, 39–44] and therefore it is possible to label only C1q with ANS in the presence of IgG. Under such conditions no inhibition is observed. Addition of monomer IgG to a solution of C1q-bound ANS did not significantly alter the fluorescence of the ANS. However when oligomeric IgG was added there was a 2-fold increase in fluorescence over the same IgG concentration range. When C1q was pretreated with diethylpyrocarbonate there was little change in the fluorescence when IgG oligomers were added to C1q:ANS solutions. These results suggest that C1q undergoes conformational changes upon binding to IgG oligomers.

C1q *Immunoglobulin* *1-Anilino-8-naphthalene sulfonate* *Conformational change* *Diethylprocarbonate* *Binding inhibition*

1. INTRODUCTION

The first component of the classical complement pathway, C1, is a complex of three glycoproteins; an immunoglobulin recognition unit, C1q, and a Ca^{2+}-dependent tetramer of two zymogens, $C1r_2C1s_2$ [1]. The activation of the classical complement pathway is initiated by binding of C1, via C1q, to aggregated IgM or IgG [2]. This process is followed by auto-catalytic activation of C1r to give C1r [3]. The proteolytic activity of C1r is then directed against C1s, which in turn becomes a serine protease, responsible for activation of complement proteins C4 and C2 [2].

The trigger for auto-catalytic activation of C1r remains unclear, but it has been suggested that this process occurs as a consequence of altered interactions between C1q and C1r, caused by structural changes within C1q attendant upon its binding to aggregated immunoglobulin [4].

In an attempt to detect structural changes within C1q upon binding to aggregated IgG, we have exploited the fact that the fluorescent probe, 1-anilino-8-naphthalenesulfonate (ANS) binds to C1q with 20-fold higher affinity than to IgG [5]. The data presented below show that there is a change in the fluorescence of C1q-bound ANS when the protein binds to oligomers of IgG. Thus, it would seem that there is a structural change in C1q when it binds to IgG, and this change may be related to the process of C1 activation.

2. MATERIALS AND METHODS

2.1. *Materials*

ANS (Mg salt) was obtained from Pierce (Rockford, IL) and diethylpyrocarbonate was from Sigma. Human C1q was purified from serum and labelled with ^{125}I, using lactoperoxidase, by the procedures of Tenner and co-workers [6]. Anti-ovalbumin rabbit IgG and immune complexes (prepared at the equivalence IgG : ovalbumin ratio)

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were obtained as in [7]. Human IgG was prepared as in [8]; 250 mg of IgG was chemically crosslinked by dithiobis(succinimidylpropionate), with a crosslinker:IgG ratio of 1.8:1 (mol/mol), as in method B of [9]. The IgG oligomers were fractionated by gel filtration over Sephacryl S-300, equilibrated with 0.01 M phosphate, 0.15 M NaCl, pH 7.2. Samples containing predominantly IgG trimers and tetramers were pooled and stored at 4°C prior to use. Such oligomers have been shown to be stable for up to 2 months at −70°C [9]. Samples containing monomeric IgG were treated similarly.

2.2. *Methods*

The immunoglobulin binding function of C1q was inactivated by treating the protein with diethylpyrocarbonate as in [10]. The binding of ^{125}I-labelled C1q to immune complexes was measured using the methods of [7]. The fluorescence of protein-bound ANS was measured at 490 ± 5 nm, following excitation at 390 ± 5 nm, using an Aminco SPF-500 spectrofluorimeter, with the cell-holders thermostatted at 37°C.

3. RESULTS AND DISCUSSION

3.1. *Inhibition of C1q binding to immune complexes by ANS*

The data shown in fig.1 indicate that ANS inhibits the binding of ^{125}I-labelled C1q to immune complexes over the concentration range 2–4 mM. There have been several reports (e.g. [11]) that commercial ANS preparations may be contaminated with bis-ANS, which may have a far higher affinity for proteins than ANS. This complication does not seem relevant in this case, because the ANS used in these experiments was pure as judged by high pressure liquid chromatography. A single absorbance peak at 340 nm was seen when samples of ANS, in 0.05 M NH_4HCO_3, pH 7, were applied to an LKB C-18 reverse phase column, developed with a gradient to 100% methanol.

We have previously reported that ANS is a competitive inhibitor, with respect to C1q, of the C1q:IgG interaction [12]. However, we have since shown [13] that it is not possible to draw such conclusions from such inhibition data, without independent direct measurements of the binding of the inhibitor to both proteins.

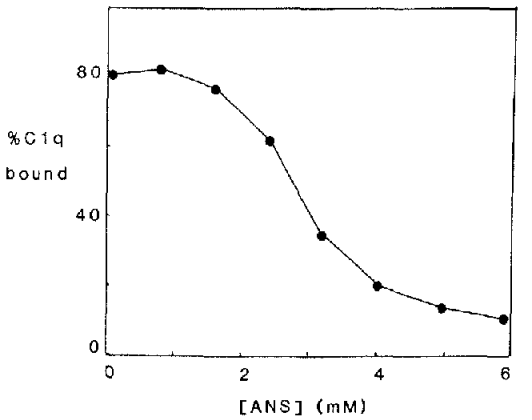

Fig.1. Inhibition of the C1q:IgG interaction by ANS. The percentage of input ^{125}I-labelled C1q (0.2 $\mu g \cdot ml^{-1}$, 3×10^5 cpm $\cdot \mu g^{-1}$), bound to immune complexes (100 $\mu g \cdot ml^{-1}$, in 0.05 M Tris-Cl, 0.1 M NaCl, 0.15% (w/v) gelatin, pH 7.2) at the indicated ANS concentrations was determined by the methods of [7].

In this context, Alcolea and co-workers [5] have shown that ANS binds to C1q with approx. 20-fold higher affinity than to IgG. They concluded from this that it was probable that the documented [12] inhibition of the C1q:IgG interaction by ANS arose from binding of ANS to C1q. However, the data of fig.1 show that ANS inhibits this interaction in the millimolar range; at these concentrations of ANS the high affinity binding sites on both IgG and C1q detected by Alcolea and co-workers would be completely saturated with ANS.

Thus, it would appear that the inhibition of the C1q:IgG interaction arises from relatively weak interactions of one or both of the proteins with ANS; it does not seem possible to deduce which is involved from the ANS binding data reported by Alcolea and co-workers.

3.2. *Conformational changes in C1q upon binding to IgG*

ANS binds to C1q with an affinity constant of 2×10^6 M^{-1}, and to IgG with an affinity constant of 9×10^4 M^{-1} [5]. It would be expected that addition of monomer IgG to a solution of C1q-bound ANS should have no effect on the fluorescence of the sample, because, firstly, monomer IgG binds to C1q with low affinity [14], and, secondly, a ma-

jority of the ANS molecules will remain bound to their high affinity C1q binding sites.

The data shown in fig.2 are consistent with this prediction, in that, although ANS was able to bind to monomer IgG in the absence of C1q (as judged by an increase in ANS fluorescence with increasing IgG concentration), there was little change in the fluorescence of the samples when monomer IgG was added to ANS in the presence of C1q. However, when oligomeric IgG was added to samples of C1q-bound ANS there was a 2-fold increase in their fluorescence, over the same IgG concentration range (fig.3). Evidence that this change was a consequence of an IgG:C1q interaction was obtained when the effects of adding oligomeric IgG to C1q which had been pretreated with diethylpyrocarbonate were examined. This reagent has been shown to inactivate specifically the immunoglobulin recognition sites of C1q, by modification of histidine residues [10]. The data in fig.3 show that, firstly, pretreatment of C1q with diethylpyrocarbonate did not lead to a difference in the fluorescence of protein-bound ANS, and secondly, there was little change in the fluorescence of ANS bound to diethylpyrocarbonate-treated C1q upon addition of oligomeric IgG. Therefore it would appear that the changes in ANS fluorescence with unmodified C1q arose from structural changes in C1q upon binding to aggregated IgG.

Alcolea and co-workers [5] have shown that ANS binds to the globular head regions of C1q. Thus, our data imply that the binding of oligomeric IgG to C1q leads to changes in these sections of the molecule. The enhancement of the fluorescence of ANS attendant upon its binding to proteins has generally been interpreted as arising from decreased polarity of the environment of protein-bound ANS. Hence, the binding of oligomeric IgG to C1q may lead to alterations in the tertiary structure of hydrophobic sections within the globular heads of C1q. This conclusion should be viewed with some caution, however, in the light of the work of Weber and co-workers [15], who showed that changes in the fluorescence of protein-bound ANS do not always result from alterations in the polarity of its environment.

The above data, together with the demonstration of neoantigens in C1q after its binding to immune complexes [4], do not necessarily bear on the

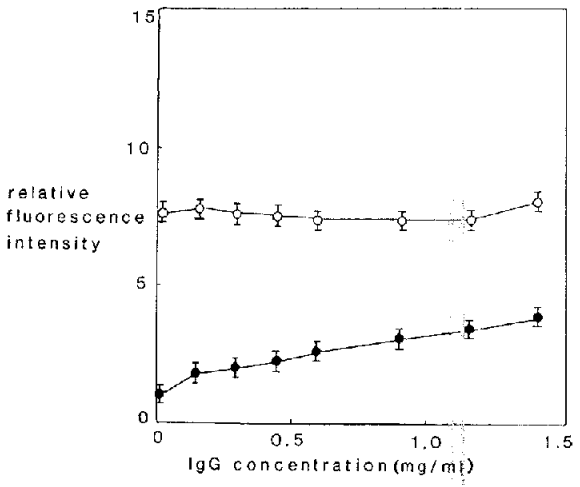

Fig.2. The fluorescence of ANS (0.2 μM) in the presence (○) and absence (●) of 0.1 mg·ml^{-1} C1q at the indicated monomeric IgG concentrations was determined as in section 2.2. The error bars indicate the extreme values of fluorescence noted over a 1-min observation period for each IgG concentration. Experiments were carried out twice.

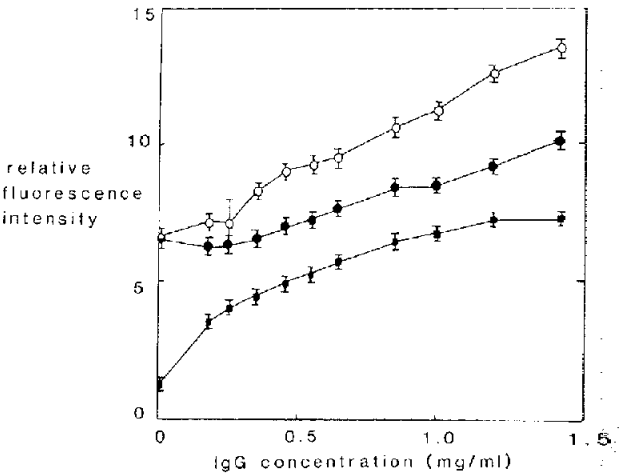

Fig.3. The fluorescence of ANS (0.2 μM): in the presence of 0.1 mg·ml^{-1} native C1q (○); 0.1 mg·ml^{-1} C1q which had been pretreated with 0.2 mM diethylpyrocarbonate for 15 min (●); and in the absence of C1q (■); at the indicated oligomeric IgG concentrations was determined as in section 2.2. The error bars indicate the extreme values of fluorescence noted over a 1-min observation period for each IgG concentration. Experiments were carried out twice.

question of the trigger for activation of C1r and C1s upon binding of the C1 complex to aggregated IgG. C1r and C1s bind to the collagenous regions of C1q, and it remains to be shown that the structural changes occurring in the C1q molecule upon its binding to IgG extend from the globular heads to the $C1r_2C1s_2$ binding site.

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