THE RECONSTITUTION OF HUMAN C1, THE FIRST COMPLEMENT COMPONENT

Binding of C1r and C1s to C1q influences the C1q conformation

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1. Introduction

The first component of the classical complement cascade, C1, consists of 3 subunits, C1q, C1r and C1s, which are held together by Ca^{2+} [1]. The collagen-like protein C1q serves as the recognition site of C1 for the Fc part of immunoglobulins in antigen–antibody complexes. Upon binding of C1q to the Fc portion the activation of native C1 to $C\overline{1}$ is initiated through the conversion of the zymogens C1r and C1s to active serine esterases [2,3]. Since C1q is known to be enzymically inactive the mechanism of the activation of C1r is not understood [4,5]. Two possible activation mechanisms have been suggested:

(1) 'Internal activation', postulating a conformational change within the C1q molecule upon binding to immune complexes leading directly to the activation of C1r. Active $C1\overline{r}$ cleaves the zymogen C1s forming the active $C\overline{1}$-molecule [3];
(2) The suggested formation of an uncleaved C1r intermediate designed as C1r* able to interact with the second C1r within the C1 molecule leading to the formation of $C1\overline{r}$ [6].

The two theories were developed from different experimental situations. The former deals with the activation of native C1 as present in normal serum, whereas the other is based on in vitro studies of native C1 generated from its isolated subcomponents C1q, C1r and C1s in the presence of Ca^{2+}. However, native serum C1 behaves differently from C1 generated from the isolated subcomponents [7] perhaps due to the different behaviour of native and isolated C1q [8,9].

* The nomenclature follows the recommendation of the WHO Committee on Complement Nomenclature: Immunochemistry (1970) 7 137–142

A conformational change occurs within the C1q molecule upon binding to antigen–antibody complexes [10]. Here, we characterize the interaction of isolated C1r and C1s with fluid phase C1q and cell-bound C1q.

2. Materials and methods

2.1. *Complement components and assays*

Sheep erythrocytes (E) sensitized with anti-E rabbit antibodies (A) were prepared as in [11]. Cell intermediates EA and EAC4 as well as the appropriate buffers, e.g., veronal-buffered saline, VBS (ionic strength 0.15 (pH 7.3)) and veronal-buffered saline with sucrose, VBS-S (ionic strength 0.065 (pH 7.3)) were prepared as in [12]. In addition veronal-buffers of different pH and ionic strength (adjusted by addition of NaCl) were used.

Human C1q was purified as in [13] and stored at −70°C in 0.5 mM Tris–HCl buffer (pH 7.5) containing 300 mM NaCl and 20% glycerol; in this buffer system C1q remained stable even for 4 h at 30°C (in preparation). Partially purified C1r and C1s (C1r–C1s) were prepared as in [10]. Using this preparation method C1r–C1s were still in the precursor form. Therefore reconstituted C1 was activatable by immune complexes (e.g., EA) of by trypsin. $C2^{gp}$ and $C4^{gp}$ were isolated by a slightly modified method [14]. Guinea pig serum pretreated with EDTA (C-EDTA) was used as source for the late acting components C3–C9 [12]. The rate of hemolysis, calculations and cell concentrations used were as in [15].

Transfer tests were performed as follows: EAC1, prepared by incubation of EAC1q with C1r and C1s as described below were washed in VBS-S, and resuspended in VBS, and incubated for 10 min at 30°C.

The cells were removed by centrifugation and C1 activity was determined in the supernatant. Activation of native C1 by trypsin was done as in [16].

2.2. *Reassociation of C1*

Three different methods were used for the reassociation of C1:

(1) C1q was preincubated with C1r and C1s for 10 min at 30°C. Afterwards EA or EAC4 were added in the same buffer as the C1 subcomponents and further incubated for 10 min at 30°C. The cells were washed and the cell-bound C1 was determined by successive addition of C4, C2, C-EDTA or C2, and C-EDTA, respectively.

(2) C1q, C1r and C1s were incubated together with the target cells for 10 min at 30°C. Cell-bound C1 activity was determined.

(3) EAC1q or EAC1q4 were prepared by incubation of EA or EAC4 with purified C1q for 10 min at 30°C in VBS-S. The cells were washed, resuspended in VBS-S, and incubated with C1r and C1s (diluted in VBS-S). EAC14 or EAC1 were then washed, resuspended in VBS-S, and C1 cell-bound was determined.

3. Results

C1q, prepared as in [13], was found to be rather unstable even when stored at −70°C in VBS or VBS-S. As mentioned in section 2 a buffer system containing glycerol was found to stabilize C1q. This enabled us to study the physicochemical parameters for the reassociation of purified C1q with partially purified native C1r and C1s (table 1).

The reconstitution of C1 was tested in two ways:

(i) Individual subcomponents were incubated in the fluid phase before the exposure to EAC4;

(ii) C1q was bound to EAC4 (EAC1q4) then incubated with C1r and C1s, so that the reassociation took place on the cell membrane.

Binding of C1q or binding of reconstituted native C1 to EAC4 are very fast reactions which are complete within <60 s. In contrast, the reassociation reactions of C1q with C1r and C1s to form C1 were observed to be much slower, taking up to 5 min including a lag-period. However, the time for generating 1 C1 site/cell ($Z = 63\%$ lysis) was independent of the concentrations of the C1 subcomponents.

The different reactions were also found to be sen-

Table 1
Physicochemical parameters of the reconstitution of native C1 from its subcomponents C1q, C1r and C1s

Parameter investigated	Reaction	Binding of C1q to EAC4: EAC4 + C1q → EAC1q4	Binding of preformed reconstituted C1 to EAC4: C1q + C1r–C1s → C1; C1 + EAC4 → EAC14	Reconstitution of C1 on EAC1q4: C1r–C1s + EAC1q4 → EAC14	Reconstitution of C1 in: the presence of EAC4: C1q + C1r–C1s + EAC4 → EAC14
$t^a_{63\%}$ 200 effective C1q molecules/cell		30 s	30 s	2.5 min	4 min
$t^a_{63\%}$ 20 effective C1q molecules/cell		60 s	60 s	3 min including a lag period	5 min including a lag period
Effective C1q molecules measured at:	0°C	2.9×10^{10}	1.0×10^{12}	1.0×10^{10}	6.5×10^{9}
	30°C	2.6×10^{10}	5.2×10^{12}	6.5×10^{11}	5.2×10^{12}
Effective C1q molecules measured at the ionic strength of:	0.065	1.5×10^{11}	1.5×10^{11}	6.9×10^{11}	3.4×10^{13}
	0.15	1.3×10^{9}	2.3×10^{10}	3.1×10^{10}	2.7×10^{12}
C1q-titer (effective molecules/cell)		n.d.[b]	45 000	5000	40 000

[a] $t_{63\%}$ is defined as the time leading to 63% lysis

[b] not determined; since 2 parameters are to be taken into consideration the pH-sensitivity of C1q binding and that of the C1r–C1s binding to EAC1q4

sitive to the ionic strength of the buffers, e.g., the binding of C1q to EAC4 was shown to be markedly reduced at physiological ionic strength (0.15). A similar dependency upon the ionic strength was observed when C1q and C1r–C1s were incubated together with EAC4. At low ionic strength (0.06) binding of C1q to EAC4 was temperature-independent between 0–30°C. In contrast, binding of C1r and C1s to bound C1q (EAC1q4) was temperature-sensitive and did not occur at 0°C.

No difference in the pH-optimum for the different reactions could be observed; the optimal pH value was found to be pH 7.6 as shown in fig.1. Incubation of C1q with C1r and C1s in the fluid phase in the presence of EAC4 resulted in a marked increase of $C\bar{1}$ activity compared to that generated by incubation of C1r and C1s with already bound C1q (EAC1q4). Since the C1r–C1s preparation contained the C1 subcomponents in their native form the question arose whether this difference in the $C\bar{1}$ activity was due to the inability of bound C1q to react with C1r and C1s or its inability to activate C1r and C1s within the reassociated C1 molecule. Therefore EAC14 prepared by incubation of EAC1q4 with C1r and C1s (EAC1qrs4) were treated with different amounts of trypsin which is known to convert C1 to $C\bar{1}$ [16].

As shown in fig.2, treatment of EAC1qrs4 with trypsin (0.05 μg/ml) resulted in a linear dose–response curve. The total number of hemolytic active C1 molecules could be increased 2.5-fold from 5000–13 600 effective $C\bar{1}$ molecules/EAC4. These results indicate the existence of at least 2 C1q populations generated upon binding of C1q to the target cells, one able to bind and activate C1r–C1s, another able to bind but not to activate C1r and C1s. However, the number of effective C1q/C1 molecules recovered was still 70% lower compared to the number of effective C1 mole-

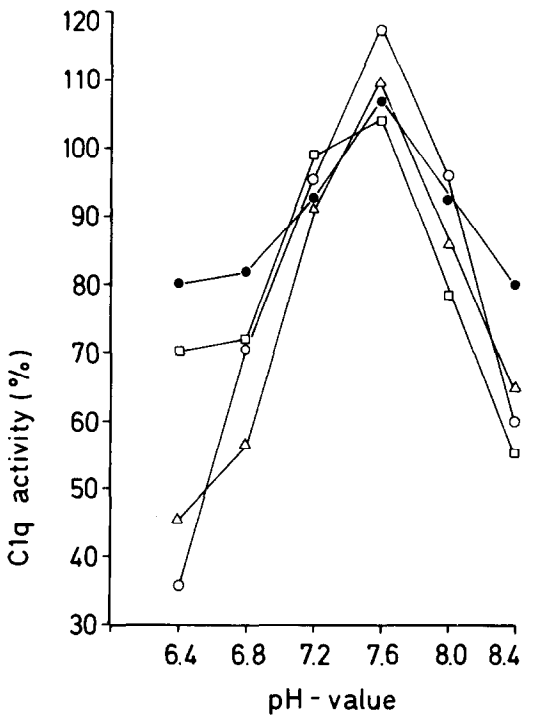

Fig.1. Effect of pH-variation on the reconstitution of C1 from its isolated subcomponents. The 100% values were measured at pH 7.3. (1) C1q, C1r and C1s were incubated together with EAC4 (100% = 45 000 effective C1q molecules/cell (○). (2) C1q was bound to EAC4 at different pH values. After washing EAC1q4 the cells were resuspended in VBS-S (pH 7.3) and EAC14 were formed at pH 7.3 (100% = 2400 effective C1q molecules/cell) (□). (3) C1q, C1r and C1s were preincubated for 10 min at 30°C. Reassociated C1 was determined by addition of EAC4 (100% = 730 effective C1q molecules/cell) (●). (4) EAC1q4 were incubated with C1r and C1s at different pH values (100% = 5300 effective C1q molecules/cell) (△).

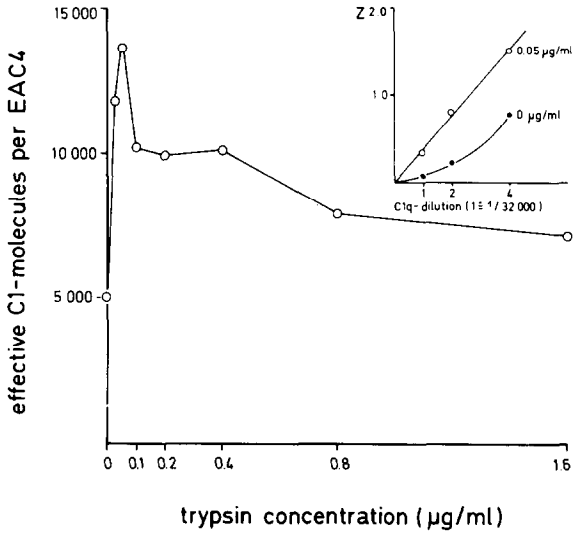

Fig.2. Activation of C1 bound by trypsin. EAC1q4 (1.3 × 10^8 cells/ml preincubated with different amounts of C1q) were incubated with C1r and C1s (20 molecules/cell) for 20 min at 30°C. The cells were washed, resuspended and incubated with different amounts of trypsin for 10 min at 30°C. After extensive washing bound $C\bar{1}$ was determined. Treatment of EAC14 with trypsin (0.05 μg/ml) changed the non-linear into a linear dose–response curve. Control experiments revealed that the C1q titer was 36 000 effective C1q molecules/cell using an assay in which C1q, C1r and C1s, and EAC4 were incubated together. Only 4100 effective C1q molecules/cell were measured when C1q was pre-bound to EAC4.

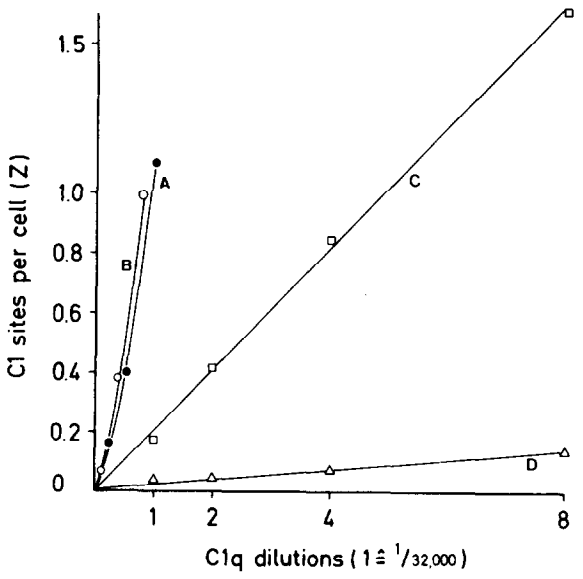

Fig.3. Transfer test performed with reconstituted C1 on EAC1. The incubation conditions were as follows: (1) C1q, C1r and C1s (20 effective molecules/cell) were incubated together with EA (1.3×10^8 cells/ml). C1 bound to EA (EAC1) was transfered to EAC4 (1.3×10^8 cells) (□). (2) EAC1q was incubated with C1r and C1s (20 effective molecules/cell). C1 formed on EA cells was transfered to EAC4 (1.3×10^8 cells/ml) (△). (3) EAC1q were incubated with C1r and C1s (20 effective molecules/cell). During the transfer of C1 to EAC4 further C1r and C1s (20 effective molecules/cell) was added (●). (4) C1q incubated with C1r and C1s in the presence of EAC4 (1.3×10^8 cells/ml) (○).

cules generated upon incubation of C1q with C1r and C1s in the fluid phase.

To find out the total number of C1q and C1 molecules bound to the sensitized erythrocytes, transfer tests were performed using EAC1q as in section 2. One transfer test series was slightly modified: the C1q/C1 released from EAC1 using VBS was further incubated with additional C1r and C1s in the presence of EAC4 at low ionic strength. Hereby, C1 was generated in the fluid phase and bound directly to EAC4 (fig.3). The C1q/C1 titer was found to be 4.7×10^{12} effective molecules (fig.3(A)). This titer is comparable to the amount of C1q/C1 measured by incubation of C1q with C1r and C1s in the presence of EAC4 ((B), 5.1×10^{12} effective molecules). (C) 8.9×10^{11} effective molecules were measured when C1q was incubated with C1r and C1s in the presence of EA. However, only 7.3×10^{10} effective C1q/C1 molecules were found when the tests were performed with preformed EAC1q (D).

4. Discussion

We have investigated the interaction of purified C1q with C1r and C1s in the fluid phase and on cell surfaces. Binding of C1q and C1 to EA or EAC4 is a temperature-independent and fast reaction taking place within seconds. The observed lag before onset of C1 esterase activity after binding is due to the fact that at least part of both C1r and C1s were still in the precursor forms and had to undergo internal activation which is temperature-sensitive [3]. Binding of C1 or C1q to EA or EAC4 was markedly reduced at physiological ionic strength (0.15) compared to the binding affinity at 0.065 ionic strength. Binding and reassociation were highly pH-sensitive (pH 7.6) being found to be optimal for the assay conditions tested.

The reconstitution step of C1 on the membrane of the target cells, i.e., binding of C1r and C1s to already bound C1q (EAC1q4) was completely prevented at low temperature, which agrees with [17]. The temperature-dependency might be explained by a conformational change within C1q and/or C1r and C1s. This explanation is also supported by observations showing that bound native serum C1 interacts in a temperature-dependent reaction with the pentosan polysulfoester, Sp 54. At low temperature Sp 54 did not react with C1, but raising the temperature results in an inactivation of bound serum C1 by Sp 54. Further analysis of this effect revealed that the subcomponent C1q was the part of the C1 molecule sensitive to Sp 54 [8]. A conformational change occurs within the C1q molecule upon binding of C1q to target cells at 30°C [10]. Therefore, the observed temperature effect on the reconstitution of C1 on the membrane is assumed also to be due to the C1q molecule, which is not altered on the EA and EAC4 at low temperature, and therefore cannot react with C1r and C1s. Raising the temperature causes a reversible energy-dependent conformational change within the C1q molecule which is now able to react with C1r and C1s. However, comparing C1 reconstituted in the fluid phase to that reconstituted on the target cells EAC1q4 demonstrates that binding of C1q to the cells also influences its reactivity towards C1r and C1s and its ability to activate reconstituted native C1. This might be explained by the results in fig.3, which shows that C1q exists in at least 3 different populations on the cells. This is probably due to the distribution and arrangement of IgG molecules on the cell surface: One C1q population which binds and activates C1r and C1s; another which

only binds but does not activate C1r and C1s and a third population which is unable to bind C1r and C1s. The latter is, however, only detectable when C1q is released into the fluid phase in the presence of C1r and C1s. This indicates that those C1q molecules assume a conformation which allows interaction of the C1q with C1r and C1s to form macromolecular C1. The number of C1q molecules detected under these conditions is identical with the number of effective C1q molecules found when reassociation of C1 took place in the fluid phase before exposure to the target cells.

Two conclusions can be made from these observations:

1. Binding of C1q to the Fc portion of the antibody influences its reactivity towards C1r and C1s and ability to activate these C1 subcomponents.
2. Binding of C1r and C1s to C1q in the fluid phase seems to change or fix the C1q conformation so that C1q is then able to bind only to such binding sites which can trigger the internal activation.

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