REGISTRATION OF THE INTERACTION BETWEEN C1q HUMAN COMPLEMENT DERIVATIVES AND IMMUNOGLOBULINS BY ELISA - ROLE OF THE SOLID PHASE

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ABSTRACT

The interaction of C1q, the first subcomponent of the human complement, with its immunoglobulin ligands from immune complexes is the crucial step in the activation of the classical complement pathway. Thus the mechanism of these interaction and the factors, which influence them, are from high interest. In the present study the effect of immobilization of the interacting proteins on a solid support in ELISA was investigated. The obtained results lead us to the conclusion, that the immobilization process may have a significant influence on the binding activity of tested proteins, especially when hydrophobic interactions are involved.

Introduction

Enzyme-linked immunosorbent assay is a widely used method for detection of protein-protein interactions. One of the two interacting macromolecules is immobilized on the solid support (PVC plates etc.) and the other is dissolved in the fluid phase. The method is based on the assumption that the immobilization process does not cause significant changes in the state of exposition of the amino-acid residues, which are directly involved in the recognition process, so the active groups of the immobilized protein retain their binding activity towards the protein from the fluid phase.

The human C1q complement subcomponent is a complex protein, composed of 18 polypeptide chains (6A, 6B, and 6C) (1, 2). This protein plays a key role in the recognition of immune complexes, containing immunoglobulins IgG or IgM and thus in the initiation of the classical complement pathway (CCP) (3).

In order to reveal the discreet structure and nature of its immunoglobulin-binding sites we have recently expressed the C-terminal globular head regions of human C1q A, B and C chains in *E. coli* as soluble fusion proteins with maltose-binding protein (MBP) and have analysed their IgG/IgM-binding properties (4, 5).

The purpose of the present study is to compare IgG/IgM-binding capacity of the three C1q-derivatives (rghA/B/C), assayed by different ELISA systems in order to detect the influence of immobilization of each of the interacting partners (C1q, rgh-MBP and Ig) on their binding activity.

Abbreviation: CCP - classical complement pathway; gC1q - the globular head region of C1q; CLR - the collagen–like region of C1q; MBP - maltose-binding protein; MBP-ghA/B/C – recombinant fusion proteins, containing maltose-binding protein linked to the globular head region of A, B and C chains, respectively.

Materials and Methods

1. Intracellular expression and purification of the rgh fragments of C1q as fusion proteins with MBP

The globular head regions of A-chain (rghA, residues 88-223), B-chain (rghB, 90-226) and C-chain (rghC, 87-217) were expressed in *E. coli* HB101 as fusion proteins with MBP. The purification of the expressed recombinant proteins was performed as described previously (5), using affinity chromatography on amylose resin. Additional purification was obtained by ion-exchange chromatography on Q-Sepharose. The fusion protein was eluted with 0.1-1.0 M NaCl gradient. The purity of the expressed proteins was proved by SDS-PAGE (6).

2. Solid–phase binding assays

2.1. ElISA for the detection of the IgG/IgM-binding activity of the rgh-fragments of C1q

The interaction of rghC1q derivatives and immunoglobulins were analysed by two ELISA systems:

In the first one (type I), different amounts of the recombinant proteins rghA/B/C were coated on microtitre plates (0.0625, 0.125, 0.25, 0.5, 1, 2 and 4 μg/well for IgG and 0.0313, 0.0625, 0.125, 0.25, 0.5, 1, and 2 μg/well for IgM) in SC buffer, pH 9.6, which were then blocked with 1% w/v BSA in PBS for 1 h at 37°C. After washing, the plates were incubated with HAIgG (10 μg/well) or IgM (15 μg/well) in PBST (PBS, containing 0.05% Tween 20), pH 7.4, overnight at 4°C. Following washing goat anti-human IgG-HRP/AP or anti-human IgM-HRP/AP respectively was added and the reaction was developed using OPD or pNPP. A_{490}/A_{405} was measured, respectively. MBP was used as a negative control.

The second ELISA system (type II) involved coating of microtitre plates with different amounts immunoglobulins (0.0313, 0.0625, 0.125, 0.25, 0.5, 1 and 2 μg/well). After blocking (with 1% w/v BSA in PBS for 1h at 37°C) the plates were incubated with the recombinant proteins rghA/B/C (2 μg/well) in PBST, pH 7.6, overnight at 4°C. The plates were washed and incubated with mouse anti-MBP antibodies and anti-mouse IgG-HRP conjugate. OPD substrate system was used and A_{490} was measured. MBP was used as a negative control.

3. pH-dependence of the interaction of IgG with rghA/B/C (wild types and mutants)

ELISA plates were coated with 1 μg/well rghA/B/C in SC buffer pH 9.6 and any residual binding sites were blocked with 1% w/v BSA in PBS for 1 h at 37°C. After washing, plates were incubated with 10 μg/well HAIgG (in citric-phosphate buffer - 0.05M sodium citrate, 0.05M Na_2HPO_4, 0.14M NaCl, 0.05% Tween20 for the pH-range 3 - 8.5 or in carbonate buffer - 0.05M $NaHCO_3$, 0.05M Na_2CO_3, 0.14M NaCl, 0.05% Tween20 for the pH-range 8.5 – 12). The bound proteins were detected by anti-IgG-HRP conjugate (1:1000) using OPD. Alternatively ELISA plates were coated with HAIgG (10 μg/well). After blocking the plates were incubated with biotinylated MBP-ghA in PBS with pHs in the range 3.0 – 7.8. Bound MBP-ghA was detected using Extravidine, conjugated with AP (1:30 000) and pNPP as a substrate (0.5 mg/ml). The analysis was made by fitting the data plots with the closest sigmoid curve using the data analysis software MSExcel and Microcal Origin ver. 6.0. The sharpness of the fit was calculated on the basis of the following equation $\Delta n_{H^+}=\partial \log(Y/1-Y)/\partial$ pH, where Y is arbitrary value of IgG binding.

Results and Discussion

pH-dependence of the interaction between IgG and rghA/B/C

The obtained results are shown on **Fig. 1a**, **Fig. 1b.** The curves are bell-shaped and have inflexed-points at 4.0-5.0 and 6.0-7.0 in the acidic and alkaline range respectively. The three recombinant proteins show different pH-dependent behaviour in the interaction with IgG. rghB and rghC have comparable pH-profiles and reach a maximum HAIgG-binding at pH 4.8. Un-

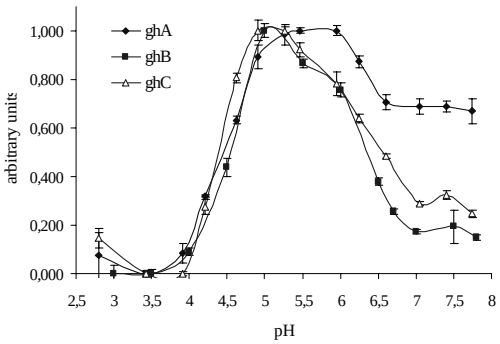

Fig. 1a. pH-dependence of the rghA/B/C–IgG interaction.

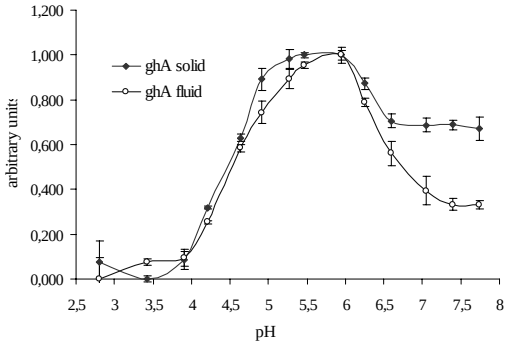

Fig. 1b. pH-dependence of the rghA, being immobilized and in the fluid phase.

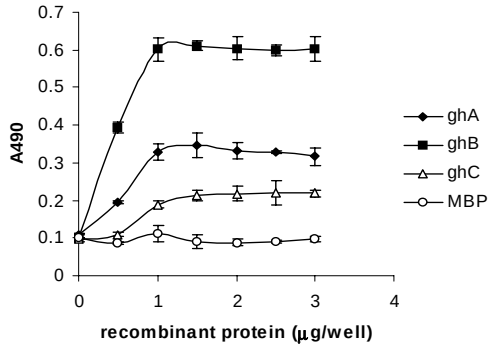

Fig. 2a. Interaction of the recombinant ghA/B/C with HAIgG (ELISA type I, with immobilized immunoglobulin).

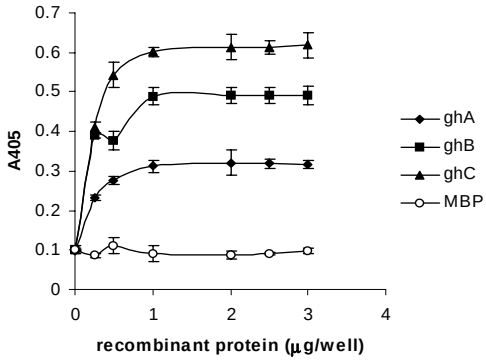

Fig. 2b. Interaction of the recombinant ghA/B/C with IgM (ELISA type I, with immobilized immunoglobulin).

like rghB and rghC, the maximum HAIgG-binding for rghA is at pH 6.0. When immobilized, rghA interacts with HAIgG in a pH independent manner at pH values above 6.5. On the other hand, when it is in the fluid phase, its binding to immobilized HAIgG is pH dependent and resembles the one of rhB and rghC.

The lack of any pH-dependence for rghA in the pH-range 6.5-7.5 is of high interest. This brings us to the suggestion that the A-chain contributes to the interaction of C1q with IgG mainly through hydrophobic forces.

The recombinant globular head B shows the strongest pH-dependence, followed by rghC so they are very likely to promote chiefly the electrostatic nature of the interaction between C1q and IgG. This is in good correspondence with the fact, that the B-chain possesses the highest number of charged residues, which are supposed to be crucial for the IgG-recognition, as we reported in a recently published study (5).

IgG/IgM recognition and binding

The IgG/IgM-binding of the recombinant globular heads (rghA/B/C) were analyzed by two ELISA systems. In the first one, different amounts of all tested recombinant proteins were coated on microtiter plates and incubated with HAIgG or IgM (**Fig. 2a.** and **Fig. 2b.**).

All the recombinant globular head fragments bind IgG and IgM in a dose depen-

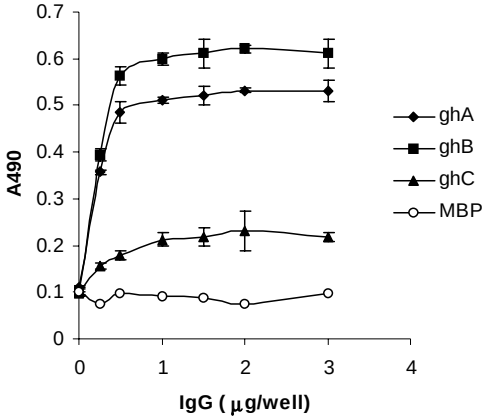

Fig. 3a. Interaction of the recombinant ghA/B/C with HAIgG (ELISA type II, with the immunoglobulin being in the fluid phase).

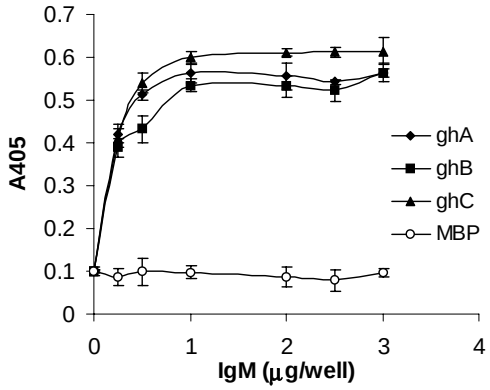

Fig. 3b. Interaction of the recombinant ghA/B/C with IgM (ELISA type II, with the immunoglobulin being in the fluid phase).

dent manner. No significant binding for the MBP-control protein was found. rghB shows the highest IgG-binding. The other two C1q-modules (rghA and rghC) have a lower IgG-binding capacity compared to rghB. In the case of IgM-binding the rghC is the most important chain, followed by rghB and rghA.

These two interactions were analyzed via another ELISA system, where the immunoglobulins were immobilized on the solid phase and ghC1q-modules were in the fluid phase. The obtained results show some deviances in compare with those, obtained by the first type of ELISA system.

In the case of IgG-binding rghA shows a higher IgG-binding activity, similar to that of rghB (**Fig. 3a**). Considering the results, obtained using this model system, we are justified in the suggestion, that rghA contributes to the IgG-binding properties of C1q in a comparable degree as rghB does.

The data from the IgM-binding test are shown on **Fig. 3b**). Here rghA exhibits also some slight divergences in compare with the previous used system. So we could draw the inference, that being in the fluid phase, rghA is capable of a more efficient interaction with IgG as well as with IgM in comparison with the case, when it is immobilized. This could be due to the influence of the immobilization. It is known that when a protein is adsorbed to a plastic surface its conformation could be altered due to conversion of native epitops to denaturated forms (7). This could lead to exposure of hydrophobic residues to the surface, changes in the position of charged residues and alteration of the interaction. rghA seems to be the most sensitive to surface effects. Since immobilized rghA is recognized by anti-MBP-, anti-rghA and anti-C1q antibodies (data not shown) and remains functionally active, we consider the observed alterations as not dramatic.

Considering the fact, that such a variation in the binding manner was observed solely for the A-chain, and that it is the only does one not demonstrating a pH-dependence for the IgG-recognition leads us to the suggestion, that rghA contributes to the immunoglobulin interaction of C1q mainly through hydrophobic forces.

In vivo immunoglobulins are immobilized on target cells and C1q, as a serum protein, participates in the interaction from the fluid phase. So the design of the second ELISA system seems to be more relevant to

the physiological conditions and thus providing more adequate results. This assumption is corroborated by our data, obtained previously by C1q-dependent hemolysis (3, 4). rghA and rghB were found to have similar inhibitory activity for the C1q-IgG interaction. In the C1q-IgM recognition rghA and rghC had also about the same inhibitory activity.

The presented data lead us to the conclusion, that the process of immobilization on the solid support may significantly influence the binding capacity of tested proteins, especially when hydrophobic interactions are involved. We suppose that the B-chain contributes to the ligand-recognition and binding mainly through its charged amino-acid residues, and that the other two chains are probably significant for their hydrophobic patches (8). Support for this hypothesis was recently provided by the revealed crystal structure of C1q. In the postulated C1q-model several hydrophobic amino acid residues are supposed to take part in ligand-recognition and binding (9), which is also reported from Tischenko et al. (10). Hydrophobic interactions are supposed to be important in the immune system. It is assumed, that receptors, activating the immune system, are evolved to recognize and react with suddenly exposed hydrophobic portions of molecules (hyppos) (11). It is important to mention the ability of C1q to interact with many hydrophobic target molecules, such as β-amyloid, lipid-A, gp41 from HIV-1, cardiolipin, etc. (8). These facts lead us to the hypothesis, that C1q could be not only a charge-recognition molecule (9), but also a hyppo-binding receptor.

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