CONFORMATIONAL VARIATIONS OF HUMAN C1q IN DIFFERENT BUFFERS

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Abstract

C1q is a primary activator of Complement system and has a physiological role in maintaining the homeostasis. C1q is now acknowledged as a protein of complex functionality, capable of forming multiple binding sites for ligands of various nature by its C-terminal globular domains (gC1q), composed of the globular fragments of the three types of chains, ghA, ghB and ghC. The conformation of the globular fragments is critical for the formation of different binding sites underlying the functional activity of C1q. We studied the conformational flexibility of C1q and its globular fragments ghA, ghB and ghC in different routinely used buffers by the highly sensitive fluorescent spectroscopy. We compared the conformational state of C1q in PBS, pH 7.2; TBS, pH 7.2; carbonate buffer, pH 9.6; NaHCO3, pH 7.2 and HEPES, pH 7.2. HEPES buffer appeared most favourable solute in terms of conformational stability of C1q. The ghB of all three globular fragments followed most closely the native protein.

Key words: C1q, conformation, HEPES

Introduction. The immune system relies on specialized components to orchestrate responses to threats and maintain homeostasis. At the front of this immune orchestra stands human C1q, a structurally complex protein renowned for its pivotal role in complement activation and immune modulation [1,2]. C1q is the recognition molecule of the C1 complex of Complement and the bridge between innate and acquired immunity. C1q is a hexameric molecule that appears as a bouquet-of-tulips-like structure. It is composed of 18 polypeptide chains
(6A, 6B, and 6C), and the N-terminal parts of these chains associate to yield the collagen-like region (CLR), whereas the C-terminal portion of the A (ghA, 28 kDa), B (ghB, 25 kDa), and C (ghC, 24 kDa) chains each contribute to the formation of six heterotrimeric globular domains (gC1q domain) [3]. The gC1q modules are arranged clockwise in the order ghA, ghB, ghC, when viewed from the top. In each gC1q the ghB module lies on the external part of the molecule, whereas the ghA and ghC modules are positioned inside [4]. The gC1q has a Ca$^{2+}$ ion exposed to the solvent, which makes an intricate network of ionic bonds that probably contributes to its high stability. The Ca$^{2+}$ ion in the gC1q structure is coordinated by six oxygen atoms, forming the Ca$^{2+}$-binding site which is asymmetrical relative to the trimer [5].

C1q is known to have a wide range of functional activity due to its ability to form binding sites for various specific ligands. This ability strongly depends on the conformational state of the protein. C1q continues to be intensively researched for new functional features by complex approaches including in vitro methods. Furthermore, C1q is used as a diagnostic molecule for autoimmune disorders marked by anti-C1q antibodies. The quantification of the autoantibodies is achieved by immunosorbent in vitro techniques. The objective of this study was to characterize the conformational state of human C1q in different buffers including PBS, TBS and HEPES. Given that C1q has an isoelectric point (pI) of 9.3, makes it one of the most positively charged proteins in human plasma [6].

Materials and methods. Proteins. Human C1q was purchased from Merck Millipore Calbiochem™, Darmstadt, Germany.

Expression and purification of proteins. The recombinant C1q globular head modules designated ghA, ghB and ghC were expressed as fusion proteins with MBP in E. coli BL21(DE3) transformed with pKBM-A, pKBM-B and pKBM-C coding for the globular fragments ghA, ghB and ghC, respectively. The expression of the recombinant proteins was induced by 0.5 mM IPTG for 5 h/25°C and by autoinduction in ZYP-5052 medium at 25°C and 225 rpm for 18–24 h [7]. The cells induced by both protocols were combined and lysed in 50 mM Tris, pH 8.0, containing 0.5 M NaCl, 1 mM EDTA, 1 mM benzamidine-HCl, 0.25% Tween 20, 0.25% Triton × 100, 0.25% NP-40 and 100 µg/ml lysozyme for 15 min on ice. The lysed cells were centrifuged and the supernatant, containing the recombinant globular fragments was subjected to affinity chromatography on Amylose resign in 20 mM Tris, pH 8.0, containing 0.5 M NaCl, 1 mM EDTA, 5% glycerol and 0.25% Tween 20. The bound proteins were eluted with 10 mM maltose. The purified proteins were extensively dialyzed against PBS (0.01 M Na$_2$HPO$_4$, 0.01 M NaH$_2$PO$_4$, 0.145 M NaCl, pH 7.4).

Fluorescence measurements. Steady state fluorescence spectra were recorded with a Shimadzu RF-5000 spectrofluorometer (Japan), equipped with 10 mm quartz cuvette holder. The measurements were done in a Helma quartz cuvette. Total fluorescence was calculated after correction for dilution. In order to
minimize inner filter and self-absorption effects, in all experiments the absorbance of the samples at the excitation wavelength was kept less than 0.05 OD. All fluorescence measurements were carried out at 25 °C, using 295 nm wavelength of excitation for the protein in order to avoid the influence of Tyr residues on the Trp emission and the observed emission spectra between 300 nm and 500 nm is the result of the emitted photons from the Trp residues.

The samples were prepared with concentrations of C1q 0.3 µM and their globular fragments in concentrations of 0.3 µM. We first measured the absorption spectra of the proteins from 240 nm to 340 nm in the analysed solvents. We determined the concentrations of the initial protein solutions spectrophotometrically using the following molar extinction coefficients: 
\[
\varepsilon_{280}(\text{C1q}) = 0.05 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}, \\
\varepsilon_{280}(\text{ghA}) = 0.08 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}, \\
\varepsilon_{280}(\text{ghB}) = 0.0688 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}, \\
\varepsilon_{280}(\text{ghC}) = 0.0768 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}.
\]
In order to accurately measure the fluorescence intensity, each measurement was repeated 3–5 times.

The emission spectra for the fluorescence studies were measured at an excitation wavelength of 295 nm. We recorded the fluorescence intensity and emission maximum of the proteins in the different buffer solutions. We monitored the fluorescence emission spectrum in the range from 300 nm to 500 nm (λ_{exc} = 295 nm).

**Results and discussion.** We used fluorescence spectroscopy to characterise the alterations in the dynamics of C1q structure when dissolved in different buffers. The three amino acids, tryptophan (Trp), tyrosine (Tyr) and phenylalanine (Phe), which are the main contributors to the protein emission are located mostly in the globular part of the sequences of A, B and C chains [8]. For this reason, the globular fragments ghA, ghB and ghC were also included in the analysis as individual proteins. The relevance of their functional activity is well documented [3,9,10].

We studied the conformational changes in the proteins using Trp fluorescence by measuring their fluorescence intensity in the following buffers: PBS, pH 7.2; TBS, pH 7.2; carbonate buffer, pH 9.6; NaHCO\(_3\), pH 7.2 and HEPES, pH 7.2. PBS and TBS are routinely used in immunosorbert assays as they best represent the physiological conditions in human serum, but unlike HEPES, they tend to precipitate Ca\(^{2+}\)-containing aqueous solutions. The NaHCO\(_3\), pH 7.2 was included, regarding the fact that human blood contains a buffer of carbonic acid (H\(_2\)CO\(_3\)) and bicarbonate anion (HCO\(_3\)\(^-\)) in order to maintain the blood pH between 7.2 and 7.4. The carbonate buffer, pH 9.6 was also studied as it is the medium in which any protein is dissolved when being prepared for immobilization on polystyrene plates.

The fluorescence spectrum of C1q was with the highest fluorescence intensity and emission maximum position was registered at 344 nm in HEPES (Fig. 1). Interestingly, when C1q was in PBS, the registered emission maximum position was at 339 nm, which showed nonpolar microenvironment of the fluorophores and presence of class II tryptophans, partially “buried” within the protein structure. This finding was accompanied by a decrease in the fluorescence intensity.

530 A. Kapogianni, V. Bogoeva, G. Cholakova et al.
The emission maximum as well as the fluorescence intensity are characteristic of conformational transitions in the protein molecule. When C1q was dissolved in HEPES, we found partial exposure of tryptophan residues on the surface of the protein molecule, thus monitoring structural changes in C1q. Tryptophans located in the interior of the protein molecule changed their location, which was accompanied by an enhancement of the fluorescence intensity. Confirmation of all these recorded changes was also the presence of a small, the so-called “red” offset/shift of the fluorescence spectrum. The fluorescence curves of C1q in TBS, NaHCO₃ and carbonate buffer with pH 9.6, overlapped, indicating that the influence of ions in these solutions did not induce significant conformational changes. Due to this fact, we continued the experiments with HEPES, PBS and TBS, disregarding NaHCO₃ and carbonate buffer with pH 9.6.

Similar to C1q, ghB exhibited the highest fluorescence intensity and emission maximum was registered at 346 nm in HEPES (Fig. 2B), similar to the behaviour of the native molecule. The ghA resembled C1q only in relation to the registered highest fluorescence intensity and emission maximum at 344 nm in HEPES was measured (Fig. 2A). The ghC (Fig. 2C) exhibited a unique pattern – the highest fluorescence intensity in PBS and the lowest in TBS. The fact that ghA, ghB and ghC showed a specific pattern of behaviour clearly demonstrated the fact that the three globular fragments composing gC1q could have individual functional activity thus contributing to the remarkable variability of C1q ligands. That is
the reason why the three globular fragments are being considered functionally independent modules of C1q.

Human serum contains 1.25 mM Ca\(^{2+}\), which is expected to affect the serum proteins’ conformation. Accordingly, many functional assays include Ca\(^{2+}\) in the buffer medium. Next, we analysed the influence of Ca\(^{2+}\) on the conformational state of C1q and its globular modules while dissolved in HEPES (Fig. 3). The presence of CaCl\(_2\) in HEPES did not change the emission maximum but decreased the fluorescence intensity suggesting that the presence of Ca\(^{2+}\) stabilized the conformation of the proteins. The native protein showed the slightest degree of change, probably because its intact globular domains already have a Ca\(^{2+}\) bound per gC1q, while ghB showed the most pronounced change, suggesting that its structure is sensitive and affectable by the lack of Ca\(^{2+}\). The ghC showed low sensitivity to the presence of Ca\(^{2+}\). Interestingly, ghA revealed the opposite behaviour by the increased fluorescent intensity in the presence of Ca\(^{2+}\).

The comparison between the four proteins (Fig. 4) outlined differences in the degree of sensitivity of their conformation to the presence of Ca\(^{2+}\). We registered the highest fluorescence intensity for ghB and ghA, while the intensity of the intact protein was lowest.

There were no significant changes in the fluorescence emission maximum recorded in the 344–347 nm range in HEPES. This indicated low conformational
Fig. 3. Fluorescence of C1q (A), ghA (B), ghB (C) and ghC (D) in: HEPES (blue) and HEPES with 1.25 mM CaCl₂ (orange)

Fig. 4. Fluorescence of C1q (orange), ghA (red), ghB (blue) and ghC (green) in HEPES with 1.25 mM CaCl₂
changes due to surface location of the tryptophan amino acids. These initial studies focused our attention on the further use of HEPES buffer.

**Conclusion.** The physiological functionality of proteins is maintained by their conformational flexibility and C1q is a classic example in this regard. We monitored the conformational fluctuations of the protein structure in different buffer solutions in order to proceed with further experiments, choosing the most suitable one, where C1q is conformationally compact and stable. HEPES buffer appeared the most favourable solute in terms of conformational stability of C1q.

The globular domains of C1q are the mediators of most of its physiological functions. The three fragments, composing one gC1q, behaved in a different way when dissolved in the tested buffers thus revealing their individual requirements for conformational stability. The ghB of all three proteins followed most closely the intact protein. Both ghB and ghC showed similar way of sensitivity to the presence of Ca$^{2+}$ with C1q, while ghA revealed a greater extent of dependence on the presence of Ca$^{2+}$. This set the foundation for future investigation of the interaction of C1q with different ligands.

**REFERENCES**


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