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Trimeric reassembly of the globular domain of human C1q

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Abstract

C1q is a versatile recognition protein which binds to a variety of targets and consequently triggers the classical pathway of complement. C1q is a hetero-trimer composed of three chains (A, B and C) arranged in three domains, a short N-terminal region, followed by a collagenous repeat domain that gives rise to the formation of (ABC) triple helices, each ending in a C-terminal hetero-trimeric globular domain, called gC1q, which is responsible for the recognition properties of C1q. The mechanism of the trimeric assembly of C1q and in particular the role of each domain in the process is unknown. Here, we have investigated if the gC1q domain was able to assemble into functional trimers, in vitro, in the absence of the collagenous domain, a motif known to promote obligatory trimers in other proteins. Acid-mediated gC1q protomers reassembled into functional trimers, once neutralized, indicating that it is the gC1q domain which possesses the information for trimerization. However, reassembly occurred after neutralization, only if the gC1q protomers had preserved a residual tertiary structure at the end of the acidic treatment. Thus, the collagenous domain of C1q might initialize the folding of the gC1q domain so that subsequent assembly of the entire molecule can occur.

Keywords: C1q; Assembly mechanism; Trimer; Folding; Function

1. Introduction

The classical pathway of complement is one of the routes of innate immunity, and is triggered by C1, a multi-molecular complex which combines the ability to bind pathogens and to convert a recognition signal into specific proteolytic activities [1]. As the recognition subunit of C1, C1q is the first player in the complement cascade and is able to recognize a plethora of different targets, including viruses, bacteria and even misfolded proteins such as amyloid and prions [2–7]. Though, the mechanism of such versatile recognition is unknown, it is clearly related to the structure of C1q which is a heterotrimer comprising three chains, A, B and C of similar lengths (~220 residues) and of homologous amino acid sequences. Each chain comprises a short N-terminal region, involved in the formation of A–B and C–C inter chain disulfide bonds, followed by a repeating collagen-like sequence which assembles into six ABC triple helices. These six triple helices first associate to form a "stalk" and then, due to interruptions in the repeating Gly-Xaa-Yaa collagen-like sequence, diverge to form six "arms" [8,9]. Each arm merges at its C-terminal end into a globular "head" region consisting of ABC heterotrimers, known as gC1q and responsible for the recognition properties of C1q [10–12]. The C-terminal domains assembled from gC1q modules are also found at the C-terminal end of various other collagen-containing proteins such as types VIII and X collagens and adipocyte complement-related protein-30 [12,13]. These proteins share similar macromolecular organization with C1q, i.e. a short N-terminal domain followed by collagenous repeats forming triple helices and ending with a trimeric globular C-terminal domain. The folding and the assembly processes leading to the formation of such a complex structure has not been addressed

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on the full-length protein, but for some members of the family, the C-terminal domain expressed in bacterial system or synthetic peptides from the C-terminal domain have been shown to trimerize in vitro [14,15]. In those cases, the sequence of the C-terminal domain contains the information required for trimerization and the domain constitutes an independent folding unit, or a so-called foldon. Interestingly, these proteins also contain the collagen-like triple helix, which is one of the few 3D-assembly motifs known to trigger obligatory trimerization [16,17]. Therefore, for the C1q molecule, it remains to be established if it is the C-terminal domain or the collagenous repeats domain which triggers the assembly of the entire C1 molecule. We addressed this question by using the heterotrimeric C-terminal domain of C1q (gC1q) which had been separated from the collagenous repeats by a collagenase treatment. The X-ray structure of human gC1q shows that it is stable as a trimer [18], indicating that whatever roles the collagenous repeats might have in the assembly process, maintaining the gC1q trimeric association is not one of them. In the present work, a protocol has been established to disassemble in vitro the gC1q trimer so that its subsequent ability to reassemble, upon neutralization, could be investigated. After various acidic treatments and subsequent neutralization, the structural states of gC1q have been analyzed using different spectroscopic methods and its stoichiometry has been determined by chemical cross-linking experiments coupled with SDS-PAGE (Sodium dodecyl-sulfate polyacrylamide gel electrophoresis) analysis. The data show that the gC1q modules have the ability to self-assemble into functional trimers, only if some residual tertiary structure is maintained at the end of the dissociation phase.

2. Materials and methods

2.1. Reagents and buffers

Type III collagenase from *Clostridium histolyticum* and human IgG purified from serum were obtained from SIGMA as well as all other chemicals. The buffers used were McIlVaine buffer (0.2 M di-sodium hydrogen phosphate, 0.1 M citric acid) at pH 3–7 and 0.1 M HCl/KCl at pH 1.0 or 1.7. All buffers were filtered through a sterile 0.22 μM filter before use.

2.2. Purification of the C1q globular domain

The globular domain of human C1q was generated essentially as described by Tacnet et al. [6]. Briefly, C1q was treated with collagenase (C1q:collagenase ratio=15:1, w/w) for 16 h at 37 °C and purification was achieved by high-pressure gel permeation on a TSK-G2000 SW column (LKB).

2.3. Production of recombinant gC1qR

The strategy for the construction of a plasmid containing the full-length gC1qR cDNA was described in detail previously [19,20]. Briefly, the cDNA was subcloned downstream of the glutathione-*S*-transferase (GST) gene in the expression plasmid pGex-2T (Pharmacia Biotech Inc.), transformed into *Escherichia coli* BL-21 (DE3) and protein expression was induced by isopropyl β-D-1 thiogalacto pyranoside. The protein was expressed as a GST fusion product with GST at the N-terminus of gC1qR. The fusion product was purified on a glutathione-Sepharose 4B column, then cleaved by thrombin and the GST-free gC1qR was purified by FPLC using a Mono-Q ion exchange column. The single peak containing gC1qR was pooled, concentrated to about 1 mg/ml, and stored at −80 °C.

2.4. N-terminal sequence analysis

N-terminal sequence analysis of the purified C1q globular domain was performed after SDS-PAGE and electrotransfer using an Applied Biosystems model 477 A protein sequencer as described previously [21].

2.5. SDS-PAGE analysis

SDS-PAGE was performed on 15% gels using a Bio-Rad mini-Protean 3 cell system according to Laemmli [22]. Samples containing about 1 μg protein were loaded in each well and gels were silver stained.

2.6. Fluorescence spectroscopy

Fluorescence measurements were performed using a Cary eclipse Varian spectrofluorimeter. Both Tyr and Trp residues were excited at wavelength 280 nm whereas selective excitation of Trp residue was performed at wavelength 295 nm. Slit widths were 5 nm for both excitation and emission. When emission spectra were recorded, the Raman contribution for water was removed by subtraction of a buffer blank. When time-dependent changes in the Trp-fluorescence of gC1q were monitored, the fluorescence intensity was recorded at an emission wavelength of 344 nm. The gC1q trimer contains three tryptophans, two in the A protomer and one in the C protomer. It also contains 20 tyrosine residues, five in the A protomer, seven in the B protomer and eight in the C protomer. Protein samples were directly added to the cuvette containing appropriate buffer at a final protein concentration of 75 μg/ml, otherwise stated. The measurement was started at once and taken for 30 min. The dead time of the instrument is a few seconds (<10 s). The experiments were carried out at 23 °C. Samples were collected at different times of the kinetics of denaturation and of renaturation for subsequent analysis of their trimeric state by chemical cross-linking and SDS-PAGE.

2.7. Circular dichroism (CD)

Circular dichroism spectra were recorded on a Jasco J810 spectropolarimeter. A cell with a path length of 0.1 cm, a spectral band width of 4 nm, and a time constant of 1 s were used, and each spectrum was recorded as an average of 4 scans. Circular dichroism measurements were made on samples diluted from a protein stock solution in McIlVaine buffer at pH 7.0 or at pH 5.0 and in 0.1 M HCl KCl buffer at pH 1.7 to a final protein concentration of 0.2 mg/ml. Samples were mixed directly in the cuvette and scans were taken immediately. The Far-UV spectra of the samples were scanned again, after further 15 min incubation at each pH. The dead time of the instrument is a few seconds (<5 s). The experiments were performed at 23 °C.

2.8. Denaturation of the C1q globular domain

Native gC1q trimer (0.9 mg/ml) was diluted to a final protein concentration of 75 μg/ml in 0.1 M HCl/KCl at pH 1.7 or at pH 1.0 or in McIlVaine buffers at pH ranging from 3.0 to 7.0 and incubated for 15 or 30 min at 23 °C. The pH of the samples was measured with a microelectrode.

2.9. Renaturation of the C1q globular domain

Renaturation was performed after acidification either at pH 1.7 or at pH 5.0, followed by neutralization to provide renaturation conditions. For the treatment at pH 1.7, the gC1q native trimer (0.9 mg/ml) was diluted two-fold in 0.1 M HCl/KCl at pH 1.0 for 10 s, 2 min and 15 min followed by a 6-fold dilution in the McIlVaine buffer at pH 7.0. For the treatment at pH 5.0, the gC1q native trimer (0.9 mg/ml) was diluted two-fold in McIlVaine buffer at pH 5.0 for 10 s, 30 s or 15 min, followed by a 6-fold dilution in the McIlVaine buffer at pH 7.0. To test the role of the buffer in the denaturation and renaturation processes, the gC1q native trimer was diluted two-fold in PBS containing 0.1 M HCl/KCl at pH 1.0 so that after the dilution, the final pH was 5.0. To do so a 10-times dilution of 0.1 M HCl/KCl at pH 1.0 was necessary. This guaranteed that the denaturation occurred at the same protein concentration for both the buffer systems. The sample was incubated for 30 s at pH 5.0 then neutralized by a 6-fold dilution in the McIlVaine buffer at pH 7.0. Otherwise stated, the final protein