Chaperone-Like Effect of Polyzwitterions on the Interaction of C1q with IgG

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The amphiphilic polyzwitterion (PZ) poly(ethylene oxide-b-*N*,*N*-dimethyl(methacryloyloxyethyl)ammonium propanesulfonate), zwitterionic surfactant (ZS) *n*-dodecyl-*N*,*N*-dimethyl-3-ammonium-1-propanesulfonate, and zwitterionic monomer (ZM) *N*,*N*-dimethyl(methacryloyloxyethyl)ammonium propanesulfonate were analyzed for their suggested chaperone-like effect on the interaction of C1q and IgG. Our results proved that the PZ retarded the C1q interaction with IgG, demonstrating a specific protein-folding helper effect. The ZS enhanced this interaction, when the ZS concentration was lower than the critical micelle concentration (*CMC*), and retarded it, when the ZS concentration was above the *CMC*. The ZM, with no self-assembling ability, did not influence this interaction. These results support the hypothesis of a hydrophobic interaction between Pts and hydrophobic domains of partly denatured protein molecules. The amphiphilic self-assemblies, formed by polyzwitterionic macromolecules or zwitterionic surfactants, have the ability to transform the hydrophobic domains of the protein molecules into hydrophilic ones, covering them with their hydrophilic parts.

Key words: C1q Complement Component, Immunoglobulin G, Polyzwitterion, Zwitterionic Surfactant

Introduction

The complement system is the major effector of the innate humoral immunity. The activation of complement is known to be triggered by three different pathways and results in killing of pathogens either by opsonization or by direct lysis. C1q, the recognition molecule of the classical complement pathway, binds to the Fc fragment of immunoglobulins (IgG and IgM) in immune complexes, thus activating the complement system (Kishore and Reid, 2000). The C1q recognition and binding of IgG have been a focus of research for a long time. The IgG-binding motif of C1q was demonstrated to have a charged nature. Two basic residues within IgG are necessary for C1q binding (Burton *et al.,* 1980; Duncan and Winter, 1988), and the binding efficiency is dependent on the ionic strength (*I*), thus implying an electrostatic interaction between C1q and IgG (Marques *et al.,* 1993; Kaul and Loos, 1997; Nauta *et al.,* 2003; Kojouharova *et al.,* 2004). Alternatively, there is evidence for hydrophobic motifs involved in the interaction of C1q with IgG and other ligands (Malmsten *et al.,*1996). To clear this inconsistency we studied the interaction of C1q with IgG in the presence of the amphiphilic polyzwitterion (PZ) poly(ethylene oxide-b-*N*,*N*-dimethyl(methacryloyloxyethyl)ammonium propanesulfonate), zwitterionic surfactant (ZS) *n*-dodecyl-*N*,*N*-dimethyl-3-ammonium-1-propanesulfonate, and zwitterionic monomer (ZM) *N*,*N*-dimethyl(methacryloyloxyethyl)ammonium propanesulfonate (Fig. 1).

Previously we proved that in PZ aqueous solution with high *I* values, PZ macromolecules transform into PZ micelles (as a result of self-assembling), whose core is composed of a macromolecular backbone and segments between the zwitterionic charges in all of the side chains, while their shell consists of both charges together with their counterions (Georgiev *et al.,* 2005, 2006; Atanasov *et al.,* 2006). These PZ self-assemblies are of great importance for *in vitro* preservation of the biopolymer macromolecule native states

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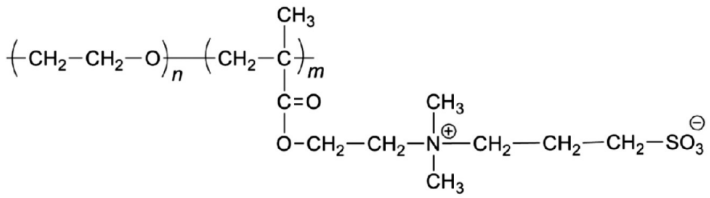
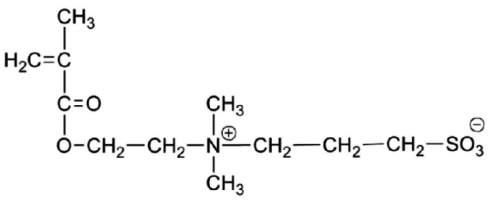
PZ



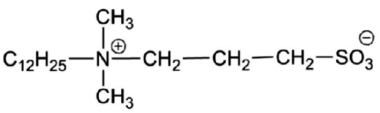
Poly(ethylene oxide-b-*N*,*N*-dimethyl(methacryloyloxyethyl)ammonium propanesulfonate)

ZM



N,*N*-Dimethyl(methacryloyloxyethyl)ammonium propanesulfonate

ZS



n-Dodecyl-*N*,*N*-dimethyl-3-ammonium-1-propanesulfonate

Fig. 1. Chemical structures of the PZ, ZM and ZS.

and cell membranes. This effect is a result of the interaction between the PZ micelle cores with the hydrophobic domains, exposed after perturbation of the native state of protein globules or cell membranes. When the hydrophobic cores interact with hydrophobic domains of the protein globule or cell membrane, the hydrophilic shell of the PZ micelle covers the hydrophobic "pocket" and the protein globule or membrane surfaces become hydrophilic again. This is just the transformation that hampers or prevents further undesired conformational transitions in the protein macromolecules, responsible for protein aggregation, as well as hydrophobic interactions, responsible for cell adhesion. Thus, due to this effect of PZ self-assemblies the PZs are considered to act as protein-folding helpers or chaperone-like macromolecules (Goldberg *et al.,* 1996; Vuillard *et al.,* 1998; Bezancon *et al.,* 2003; Georgiev *et al.,* 2006).

The binding of both hydrophilic and hydrophobic parts of the PZ macromolecules is of great importance for the PZ unique chaperone-like effect. Free ZM molecules do not form such amphiphilic self-assemblies and therefore cannot act as chaperones. However, it is possible that a ZS could acquire such ability after micellization, when its concentration exceeds the critical micelle concentration (*CMC*). The verification of these PZ, ZM and ZS abilities to control the hydrophobic interaction between C1q and IgG is the main objective of this work.

Experimental

Materials

N,*N*-Dimethyl(methacryloyloxyethyl)ammonium propanesulfonate (DMAPS) and monomethoxy ethyleneglycol with the molecular weight 2000 Da (Merck, Germany) were used without previous purification. All other reagents (CuBr, Na_2HPO_4, NaH_2PO_4, NaCl, Tween 20) of analytical grade were purchased from Merck (Germany).

The following buffers were used: PBS, phosphate-buffered saline (0.01 M Na_2HPO_4, 0.01 M

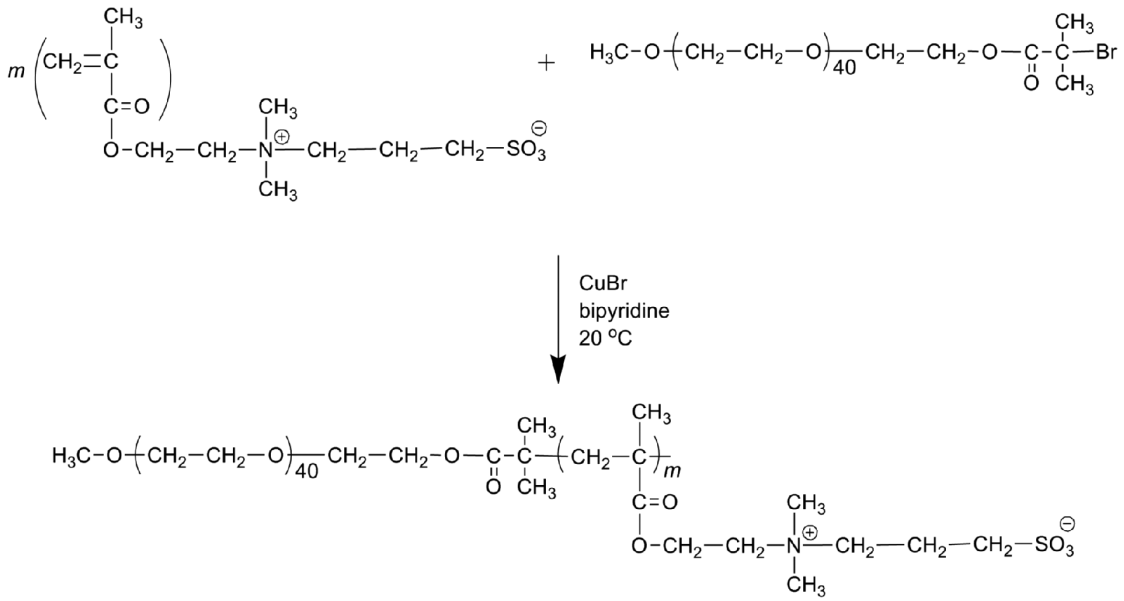


Fig. 2. Schematic presentation of the synthesis of the polyzwitterionic block-copolymer.

NaH_2PO_4, 0.145 M NaCl, pH 7.4); TPBS, PBS containing 0.05% Tween 20; AP, alkaline phosphatase buffer (100 mM Tris(hydroxymethyl)aminomethane, 150 mM NaCl, 5 mM $MgCl_2$, pH 9.6).

C1q was isolated from human plasma by affinity chromatography on IgG-Sepharose, followed by affinity Protein G chromatography (Reid and Porter, 1976). The protein concentration was estimated by measuring the absorption at 280 nm and using the A_{280} (1 t%, 1 cm) coefficient of 6.82. The purity of C1q was assessed by SDS-PAGE (15 l%) under reducing conditions where it appeared as three bands, corresponding to the A, B, and C chains of 34, 32, and 27 kDa, respectively.

Heat-aggregated IgG (HAIgG) was used as a model for immune complexes.

Synthesis of double hydrophilic copolymer

First the oligoethylene oxide macroinitiator was synthesized as already described (Matyjaszewski and Xia, 2001). The macroinitiator was used for radical polymerization with atom transfer to produce the second polyzwitterionic block of copolymer according to Ma *et al.* (2002) (Fig. 2). The synthesized block-copolymer was dialyzed against water for 42 h and lyophilized.

ELISA assays

The principal scheme for the ELISA assays was as follows: The microtitre plates were coated with IgG (20 μg/well) in PBS and the remaining binding sites were blocked with 1 t% BSA. Then the plates were incubated with C1q (8 μg/well) in PBS containing different concentrations of the PZ or ZM or ZS. The bound human C1q was detected by subsequent incubations with rabbit polyclonal anti-C1q (DAKO) and anti-rabbit IgG-alkaline phosphatase conjugate (Sigma). After the incubations the enzyme reaction was carried out with *p*-nitrophenyl phosphate dissolved in AP. The absorbance was read at 405 nm. All the incubations were carried out for 1 h at 37 °C. After each incubation the wells were washed three times with TPBS.

Results and Discussion

The effect of PZs, ZMs and ZSs on the interaction of C1q with IgG was analyzed by ELISA. The results for the effect of PZs, assessed at different concentrations of zwitterionic monomer units (C_{PZ},mu = 0.05, 1.50, 25.00 and 50.00 mM) are shown in Fig. 3. The comparison between A_{405nm} as a measure of the IgG-C1q interaction in the presence of IgG (A_S) and in the absence of IgG (A_C)

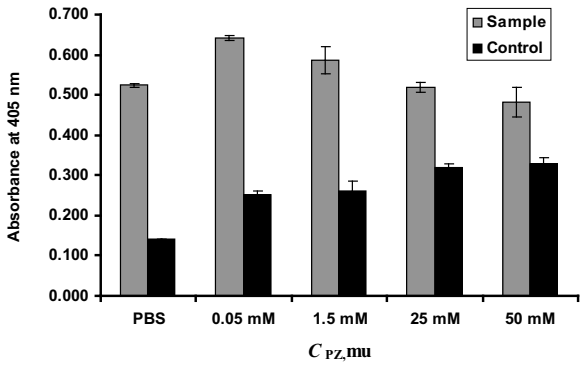


Fig. 3. ELISA assay for IgG binding of human C1q in the presence of different PZ monomer unit concentrations (C_{PZ},mu). C1q (8 μg/well) in PBS containing different concentrations of the PZ was added to IgG-coated plates (20 μg/well). The plates were incubated for 1 h at 37 °C and rabbit anti-human C1q antibody (1 μg/well) was added and incubated again for 1 h. The amount of bound C1q was visualized by alkaline phosphatase conjugated to goat anti-rabbit IgG antibodies.

is presented as the ratio between A_S/A_C at a given concentration of PZ monomer units (C_{PZ},mu) and A_S/A_C in the absence of them: $g_N = [A_S/A_C]_{PZ}/[A_S/A_C]_0$ (Table I). The results show that g_N decreases monotonically as the C_{PZ},mu increases, which is in full agreement with our expectation for a chaperone-like effect due to the PZ self-assemblies in the buffer aqueous solutions.

The results for the interaction of C1q with IgG in the presence of ZMs indicate that the ZM concentration did not influence the g_N ratio, confirming that free ZM molecules do not have the ability to form amphiphilic self-assemblies and to take part in the retardation of the hydrophobic C1q interaction with IgG (Table I).

Notably representative are the results for the hydrophobic interaction between C1q and IgG in the presence of ZSs (Table I). There are two essential characteristic features of this "g_N-C_{ZS}"

Table I. Comparison between the effects of the PZ, ZM and ZS on the interaction of C1q with IgG in terms of g_N values.

Concentration	$g_{N,PZ}$	$g_{N,ZM}$	$g_{N,ZS}$
PBS (control)	1.000	1.000	1.000
0.05 mM	0.683	1.224	1.269
1.5 mM	0.602	0.933	1.716
25 mM	0.436	0.908	1.256
50 mM	0.393	1.026	0.758

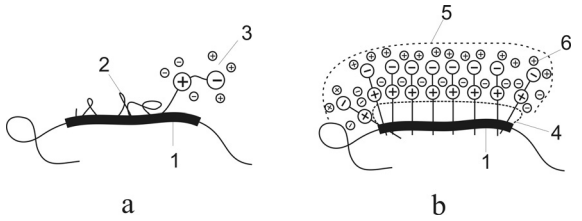


Fig. 4. Schematic presentation of the coating of the hydrophobic domain (C1q or IgG) (a) with an isolated ZS molecule ($C_{ZS} < CMC$), when the hydrophobic tail effect dominates over the ZS molecule head, and (b) with the ZS micelle ($C_{ZS} > CMC$), when the hydrophilic micelle shell covers totally the hydrophobic domain. 1, Hydrophobic domain of the protein molecule; 2, ZS molecule hydrophobic tail; 3, ZS molecule hydrophilic head; 4, hydrophobic ZS micelle core; 5, hydrophobic ZS micelle shell; 6, counterion atmosphere.

relationship: (i) The considerable augmentation of the C1q-IgG interaction at low ZS concentration and its retardation at high ones; (ii) its positive maximum. This more complex dependence could be attributed to the influence of the individual ZS micelles at low and high ZS concentrations, when $C_{ZS} > CMC_{ZS}$. In fact, if $C_{ZS} < CMC_{ZS}$, the hydrophobic domains of the free ZS molecules absorbed on the C1q or IgG enhance the hydrophobic interaction due to the dominance of the ZS hydrophobic tail effect over the ZS head (Fig. 4a). The increase of the hydrophobic surface due to the long hydrophobic tail of the ZS molecule is far greater than the decrease of this surface due to the hydrophilic molecule head. The higher ZS concentration should induce a stronger hydrophobic interactions between C1q and IgG. However, the situation changes drastically after reaching CMC_{ZS}. The ZS micelle formation covers the protein hydrophobic domain, totally transforming its hydrophobic surface into a hydrophilic one due to the micelle hydrophilic shell (Fig. 4b). As a result of this transition, the hydrophobic C1q interaction with IgG decreases gradually, and at higher ZS concentrations (high ZS micelle concentration) the retardation of this interaction becomes similar to that in the presence of PZs in solution. The g_N value becomes negative again. This similarity is a result of the fact that in both cases large amphiphilic self-assemblies are produced, and their effect on the protein hydrophobic surface is identical: transformation of the hydrophobic surface into a hy-

drophilic one. The very fine difference between both effects lies in the PZ self-assemblies formed at any PZ concentration (due to the binding of the zwitterionic monomer units), while the ZS micelle formation is a concentration-dependent self-assembling process and ZS micelles are only produced at ZS concentrations higher than *CM-*C_{ZS}. This is the reason for the difference between the g_N vs. C_{PZ} and g_N vs. C_{ZS} values.

Conclusion

Our results proved that the PZ retarded the interaction of C1q with IgG, demonstrating a specific protein-folding helper effect. On the other hand, the ZS enhanced this interaction if the ZS concentration was lower than the *CMC*, and retarded it when the ZS concentration was higher than the *CMC*. The ZM, with no self-assembling ability, did not influence this interaction.

After the first confirmation of the PZ protein-folding helper ability (Vuillard *et al.,* 1998; Bezancon *et al.,* 2003; Georgiev *et al.,* 2006), the results presented above support the hypothesis of hydrophobic interaction between the PZ and hydrophobic domains of partially denatured protein molecules (C1q, IgG, enzymes). The amphiphilic self-assemblies, formed by PZ macromolecules or the ZS, have the ability to transform the hydrophobic domains of the protein molecules into hydrophilic ones, covering them with their hydrophilic parts. The self-assembling of the PZ macromolecules is not a concentration-dependent process because of the binding of the zwitterionic monomer units within the polymer chain, while the ZS self-association is a concentration-dependent process and takes place only when the surfactant concentration is higher than the *CMC*. This different behaviour is the reason for the various effects of a PZ and ZS on the interaction of C1q with IgG, shown here for the first time. At the same time, our results confirm the contribution of hydrophobic motifs of C1q involved in its interaction with IgG.

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Atanasov V., Vassileva E., Kamenova I., Ivanov I., and Georgiev G. (2006), Double hydrophilic block zwitterionic copolymer as an acid phosphatase folding helper. Ann. Univ. Sofia Chem. **98–99**, 317–325.

Bezancon N., Rabilloud T., Vuillard L., and Goldberg M. (2003), Physical-chemical features of non-detergent sulfobetaines active as protein-folding helpers. Biophys. Chem. **100**, 469–479.

Burton D. R., Boyd J., Brampton A. D., Easterbrook-Smith S. B., Emanuel E. J., Novotny J., Redmatcher T. W., van Schravendijk M. R., and Dwek R. A. (1980), The Clq receptor site on immunoglobulin G. Nature **288**, 338–344.

Duncan A. R. and Winter G. (1988), The binding site for C1q on IgG. Nature **332**, 738–740.

Georgiev G., Kamenska E., Vassileva E., Kamenova I., Georgieva V., Iliev S., and Ivanov I. (2005), Properties and biomedical application of polyzwitterions. Ann. Univ. Sofia Chem. **98–99**, 305–325.

Georgiev G., Kamenska E., Vassileva E., Kamenova I., Georgieva V., Iliev S., and Ivanov I. (2006), Self-assembly, antipolyelectrolyte effect, nonbiofouling properties of polyzwitterions. Biomacromolecules **7**, 1329–1334.

Goldberg M., Bezancon N., Vuillard L., and Rabilloud T. (1996), Non-detergent sulfobetaines: A new class of molecules that facilitate *in vitro* protein renaturation. Folding Des. **1**, 21–27.

Kaul M. and Loos M. (1997), Dissection of C1q capability of interaction with IgG: time-dependent formation of a tight and only partly reversible association. J. Biol. Chem. **272**, 33234–33244.

Kishore U. and Reid K. B. M. (2000), C1q: structure, function and receptors. Immunopharmacology **42**, 159–163.

Kojouharova M. S., Gadjeva M. G., Tsacheva I. G., Zlatarova A. S., Roumenina L. T., Tchorbadjieva M. I., Atanasov B. P., Waters P., Urban B. C., Sim R. B., Reid K. B. M., and Kishore U. (2004), Mutational analyses of the recombinant globular regions of human C1q A, B, and C chains suggest an essential role for arginine and histidine residues in the C1q-IgG interaction. J. Immunol. **172**, 4351–4358.

Ma I. Y., Lobb E. J., Billingham N. C., Armes S. P., Lewis A. L., Lloyd A. W., and Salvage J. (2002), Synthesis of biocompatible polymers. 1. Homopolymerization of 2-methacryloyloxyethyl phosphorylcholine via ATRP in protic solvents: An optimization study. Macromolecules **35**, 9306–9314.

Malmsten M., Lassen B., Van Alstine J. M., and Nilsson R. U. (1996), Adsorption of complement proteins C3 and C1q. J. Coll. Interf. Sci. **178**, 123–134.

Marques G., Anton L. C., Barrio E., Sanchez A., Ruiz S., Gavilanes F., and Vivanco F. (1993), Arginine residues of the globular regions of human C1q involved in the interaction with immunoglobulin G. J. Biol. Chem. **268**, 10393–10402.

Matyjaszewski K. and Xia J. H. (2001), Atom transfer radical polymerization. Chem. Rev. **101**, 2921–2990.

Nauta A. J., Bottazzi B., Mantovani A., Salvatori G., Kishore U., Schwaeble W. J., Gingras A. R., Tzima S., Vivanco F., Egido J., Tijsma O., Hack E.C., Daha M.R., and Roos A. (2003), Biochemical and functional characterization of the interaction between pentraxin 3 and C1q. Eur. J. Immunol. **33**, 465–473.

Reid K. B. M. and Porter R. R. (1976), Subunit composition and structure of subcomponent C1q of the first component of human complement. Biochem. J. **155**, 19–23.

Vuillard L., Rabilloud T., and Goldberg M. (1998), Interactions of non-detergent sulfobetaines with early folding intermediates facilitate *in vitro* protein renaturation. Eur. J. Biochem. **256**, 128–135.