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A redox sensitivity-based method to quantify both pentameric and monomeric C-reactive protein in a single assay

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ABSTRACT

C-reactive protein (CRP) can exist in both pentameric (pCRP) and monomeric conformation (mCRP). Though serum pCRP is an established marker of inflammation, the diagnostic significance of mCRP remains unknown largely due to the lack of a reliable assay. The power and specificity of antibody-based assays are limited by the antibody reagents used and by the degree of cross-reactivity that may exist in detecting each antigen, as mCRP is known to be formed from the pentameric and both conformations usually coexist in clinical samples. Here, we describe an assay that measures both CRP conformations in simple samples in a single assay. This assay depends on the rationale that the intra-molecular disulfide bonds in pCRP resist reduction, while those in mCRP can be readily reduced. The distinct sensitivity of pCRP and mCRP to reduction can be easily detected and separated by electrophoresis. This assay may provide a means to study clinical correlation between pCRP and mCRP in clinical samples in the future and to evaluate their respective significance as disease markers.

1. Introduction

Serum pentameric C-reactive protein (pCRP), a non-specific diagnostic marker of inflammation, is composed of five identical globular subunits that non-covalently associated into a discoid structure with a central void (Pepys and Hirschfield, 2003). While clinical significance is defined for pCRP levels above 10 μg/ml, numerous studies have suggested pCRP levels between 3 and 10 μg/ml may be prediction of future risk for cardiovascular disease (Ridker et al., 2002; Rost et al., 2001; Ridker et al., 1997; Albert et al., 2002; Sakkinen et al., 2002). However, the causality link of pCRP to the underlying pathogenesis is not supported by large-scale genetic studies (Allin et al., 2010; Zacho et al., 2008) and by CRP transgenic or knock-out animal studies (Paul et al., 2004; Hirschfield et al., 2003; Kovacs et al., 2007; Mold et al., 2002).

Current evidence has shown that pCRP can be dissociated to its subunit, termed monomeric CRP (mCRP), at inflammatory foci when it encounters damaged cells (Ji et al., 2007; Mihlan et al., 2011), activated platelets (Molins et al., 2011), microparticles (Habersberger et al., 2012) and amyloid aggregates (Strang et al., 2012). The dissociation to mCRP accompanied with the loss of secondary structure elements and significant alterations in tertiary structure (Wang et al., 2011), which contributes to mCRP new epitope expression and enhanced pro-inflammatory activities (Ji et al., 2007; Wang et al., 2011; Ji et al., 2006, 2009; Braig et al., 2017). Immunohistochemical analyses have identified mCRP as the predominant CRP isoform present in inflamed tissues (Thiele et al., 2014; Eisenhardt et al., 2009; Schwedler, 2003; Thiele et al., 2015). Additionally, autoantibodies against mCRP were found in active lupus nephritis (Robey et al., 1985; Li et al., 2017),

Abbreviations: CRP, C-reactive protein; pCRP, pentameric C-reactive protein; mCRP, monomeric C-reactive protein; DTT, dithiothreitol; NEM, *N*-Ethylmaleimide; IAA, iodoacetamide; TCEP, Tris (2-carboxyethyl) phosphine hydrochloride solution; 4-VP, 4-Vinylpyridine; SDS, sodium dodecyl sulfate; CTAB, hexadecyl trimethyl ammonium Bromide; ELISA, enzyme linked immunosorbent assay

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which indicates mCRP antigens are naturally expressed in vivo. Related studies further showed that levels of mCRP autoantibodies positively correlate with the severity of lupus nephritis (Tan et al., 2008). All these studies underscore that mCRP is a naturally occurring isoform of CRP and that it has a significant role in host defense, inflammatory processes. Indeed, evolving studies strongly suggest that mCRP may be a more specific marker to underlying inflammatory and possible pathological processes.

Recent work successfully quantified human serum mCRP using ELISA techniques based on commercially available reagents (Zhang et al., 2018). This indicated that mCRP not only exists in inflammatory tissue but also in serum. Furthermore the study demonstrated that mCRP appears to be a more reliable marker than pCRP in several skin-related autoimmune disorders (Zhang et al., 2018). Despites the probable specific nature of mCRP, accurate quantification of it through immunoassay remains challenging. It has been suggested that the mCRP concentration detected by ELISA is usually a small percentage of pCRP (Zhang et al., 2018). However, the previous experiments showed that the concentration of total CRP detected by protein cleavage isotope dilution mass spectrometry is 10 times than that of pCRP assayed by the ELISA kit (Williams and Muddiman, 2009). Such a difference may be attributable to the interaction of mCRP with diverse proteins or lipids in serum (Habersberger et al., 2012; Li et al., 2016), which will significantly affect its recognition with antibodies and ELISA assays. Additionally, ELISA assay must carefully address the potential for cross-reactivity which exist between pCRP and mCRP (Zhang et al., 2018; Potempa et al., 2015).

The clinical significance of mCRP remains indefinite largely due to the lack of a reliable assay. Accordingly, developing a method that accurately and precisely quantifies mCRP and effectively discriminates mCRP quantification from pCRP quantification is warranted. Herein, we describe a method for the discrimination of mCRP from pCRP so that we can measure both CRP conformations in a single assay. This method utilizes the distinctive sensitivities of mCRP and pCRP to reducing agents. Structural packing of pCRP protects the sole intrachain disulfide bond, found at the base of a conformational cleft (Shrive et al., 1996) (Fig. 1) from reduction. Structural packing of mCRP is looser, such that the intrachain disulfide bond is more freely susceptible to reduction. Such sensitivity difference can be easily detected by electrophoresis and may even be used as a basic principle to develop more accurate quantitative methods in the future.

2. Materials and methods

2.1. Reagents

Pentameric CRP (purity > 97%) purified from human ascites was purchased from the BindingSite (Birmingham, UK; catalog number: BP300.X) and repurified with *p*-Aminophenyl Phosphoryl Choline Agarose (Thermo Fisher Scientific, IL, USA; catalog number: 20307). Purified pCRP was stored in TBS (10 mM Tris, 140 mM NaCl, pH 7.4) containing 2 mM $CaCl_2$ to prevent spontaneous dissociation (Ji et al., 2007). mCRP was prepared by treatment of purified pCRP with urea chelation (Potempa et al., 2015). Mouse anti-human mCRP monoclonal antibody 3H12 was generated as described previously (Ying et al., 1989). HRP-labeled goat anti-mouse IgG (H + L) was purchased from Abcam (Cambridge, UK; catalog number: ab6789). All other reagents were from Sigma-Aldrich unless otherwise stated.

2.2. SDS-PAGE and immunoblotting

pCRP or mCRP was incubated with reducing agents (DTT or TCEP) and blocked with alkylating reagent (NEM, IAA, 4-VP) to quench the reducing reaction and to prevent the reoxidation of free thiols. All these steps were conducted at 37 °C. Samples were then mixed with loading buffer and heated in boiling water for 10 min. Boiled samples were loaded on 12% denatured SDS-PAGE gels, and electrophoresis performed using BioRad electrophoresis apparatus under constant voltage of 120 V at 25 °C. If employing special SDS-PAGE gels with 1/20 content of SDS, samples cannot be boiled after mixed with loading buffer and electrophoresis buffer has 1/20 SDS as well (Taylor and van den Berg, 2007). On completion of electrophoresis, samples were transferred to PVDF membrane and blocked with 5% non-fat milk in TBST (20 mM Tris, 150 mM NaCl, pH 7.4, 0.05% Tween-20). The membrane was incubated with 3H12 mAb (1:1000) and followed by HRP-labeled goat anti-mouse IgG (H + L) (1:30000) in 1% BSA TBST. The ratio of reduced CRP subunit (having a larger apparent molecular weight and being indicative of the mCRP conformer) was calculated as a percent of total CRP subunit band intensity (i.e. band intensity of reduced CRP divided by the total CRP subunit band intensity (i.e. reduced CRP subunit band intensity plus non-reduced CRP subunit (having a smaller apparent molecular weight and being indicative of the pCRP subunit) X 100%). Band intensities were analyzed using ImageJ software.

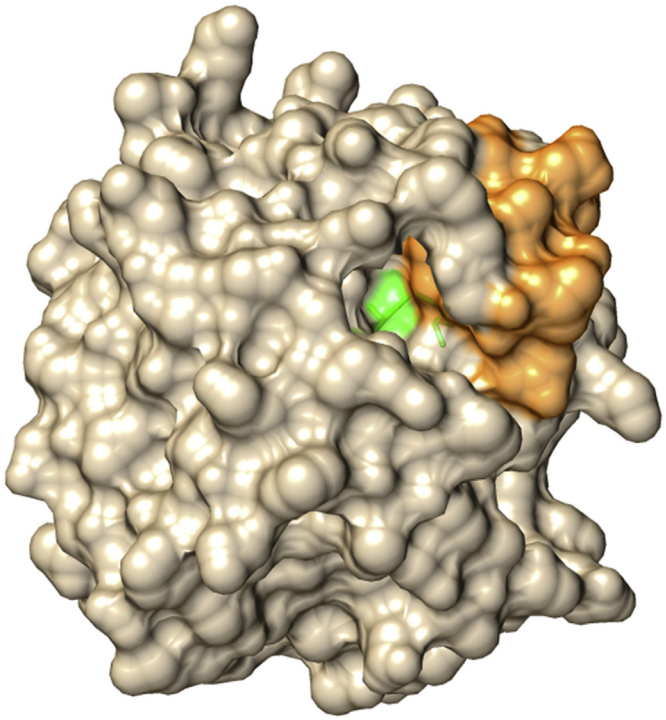

Fig. 1. Intrachain disulfide bond (green) covered by α-helix (aa 168–176, orange) is hidden in cleft of pCRP subunit (PDB: 1B09). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.3. Statistical analysis

All experiments were repeated three times. Data are presented as means ± SE. Statistical analysis was performed by one-way ANOVA. Differences of $P < .05$ were considered significant.

3. Results

3.1. Quality control of purified CRPs

Quality of purified mCRP and pCRP were analyzed by a special SDS-PAGE system, in which the content of SDS used is one-twentieth of the standard recommended levels for SDS-PAGE analyses (Taylor and van den Berg, 2007). The reduced SDS concentration electrophoresis system has been shown to clearly discriminate different conformation of CRPs. The result showed purified pCRP is too large to enter into the 12% gel,

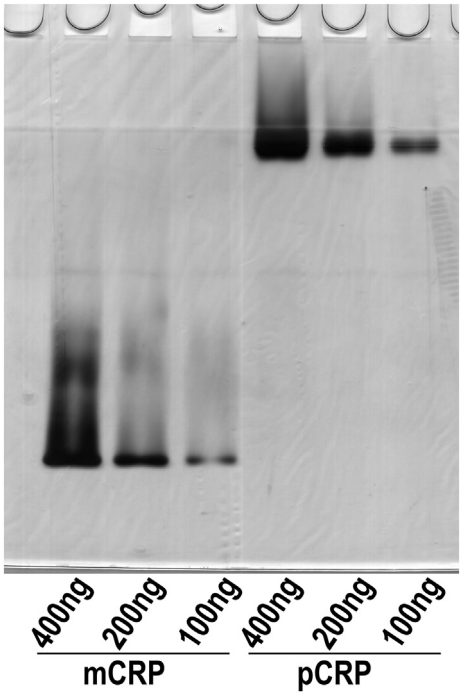

Fig. 2. Quality analysis of purified mCRP and pCRP by 12% SDS-PAGE with 1/20 content of SDS. Purified mCRP or pCRP was mixed with loading buffer with 1/20 content of SDS. Then samples without heated were separated under constant voltage of 120 V at 25 °C. After electrophoresis, gel was stained by silver. Pentameric CRP resided in the interface between stacking gel and separating gel, whereas mCRP can enter the separating gel. Result is a representative of three independent experiments.

residing at the interface of the stacking gel and separating gel. In contrast, purified mCRP readily migrated into the separating gel (Fig. 2). The result demonstrated that purified mCRP or pCRP was single configuration, and did not mingled with each other. This is a guarantee of reliable experiment results.

3.2. pCRP and mCRP have different sensitivity to reducing agent

Pentameric CRP and mCRP have different responses to DTT. Western blot was used to observe the distinction between mCRP and pCRP. Here we demonstrate that pCRP is resistant to DTT treatment at concentration between 0.1 and 30 mM (Fig. 3A). This characteristic resistance may be attributed to the pCRP's packing which wrap the disulfide bond of each subunit inside the structure and resist its contact with DTT. In contrast, the disulfide bond in the mCRP conformer is more easily accessible to the DTT reducing agent, leading to a reduced subunit that is more flexible and can move slower down the gel compared to a non-reduced subunit. The results also show that only a part of mCRP can be reduced at various concentration of DTT (Fig. 3A). This highlights the important aspect that suitable conditions for the maximum reduction of mCRP and minimum influence of pCRP need to be met for the measurement of mCRP and pCRP respectively.

3.3. Exploring the conditions for realizing the maximum reduction of mCRP

Based on the above results, we fixed the concentration of DTT at 30 mM, comparing the reduction ratio of mCRP at different incubation times. As no significant change ($P = .223$) was observed for reduction ratio of mCRP between 0.5 and 4 h (Fig. 3B). we chose to use a processing time of one hour at 37 °C for all subsequent evaluations. After incubation with 30 mM DTT for one hour at 37 °C, mCRP with different concentrations had a similar reduction ratio (Fig. 3C). Additionally, using IAA instead of NEM, or replacing DTT for TCEP and then blocking sulfhydryl with 4-VP, failed to improve the reduction ratio of mCRP (Fig. 4). Here, the results indicate that incomplete reduction of mCRP is

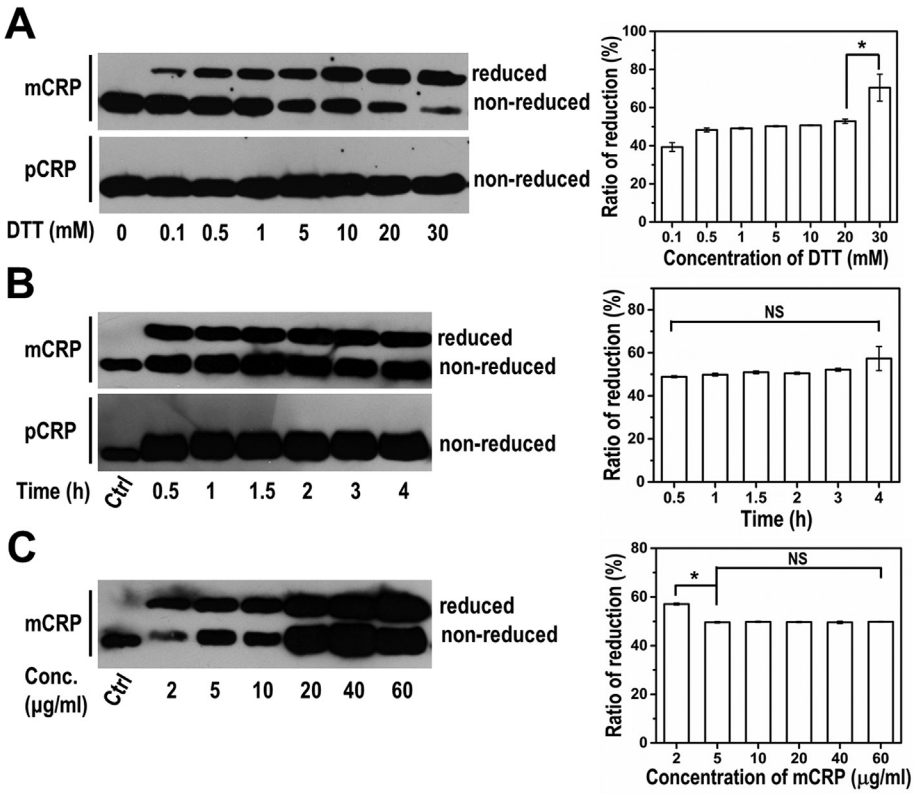

Fig. 3. Effect of mCRP and pCRP in response of DTT. **A)** mCRP or pCRP (4.2 μg/ml) was added to TBS-Ca (10 mM Tris, 140 mM NaCl, 2 mM $CaCl_2$, pH 7.4), and samples were reduced by DTT with indicated concentration for 1 h at 37 °C. Then samples were treated with NEM with six times DTT concentration for 30 min at 37 °C to block free thiols. **B)** mCRP or pCRP were reduced by 30 mM DTT for indicated time and then blocked with 180 mM NEM for 30 min at 37 °C. **C)** mCRP at the indicated concentration (Conc.) was added to TBS-Ca, and incubated with 30 mM DTT for 1 h at 37 °C and then blocked with NEM as above. Untreated mCRP or pCRP served as control (Ctrl). Representative western blot results are shown. The results are presented as means ± SE, $n = 3$. *$P < .05$. NS, no significant difference.

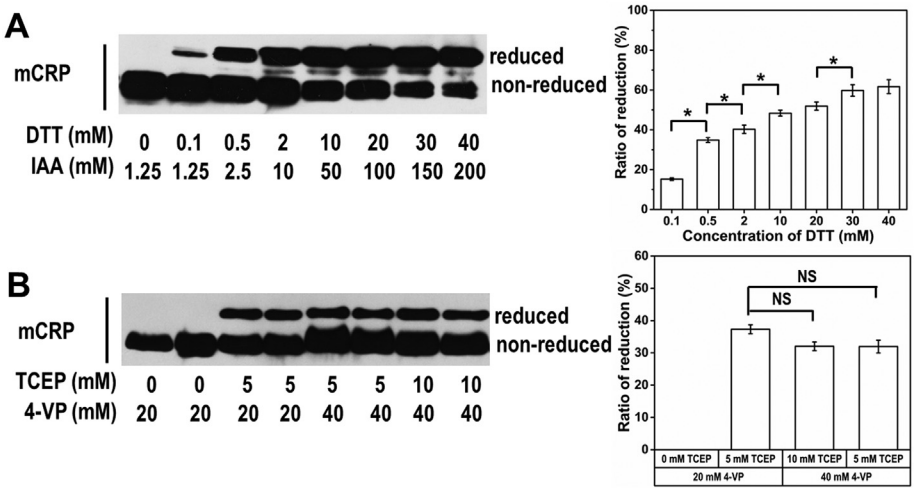

Fig. 4. Effect of different reducing agent and thiols blocking agent on reduction of mCRP. **A)** mCRP (4.2 μg/ml) was incubated with indicated DTT for 2 h at 37 °C and then blocked with indicated IAA for 30 min at 37 °C. **B)** mCRP (4.2 μg/ml) was incubated with indicated TCEP for 2 h at 37 °C and then blocked with indicated 4-VP for 30 min at 37 °C. Representative western blot results are shown. The results are presented as means ± SE, $n = 3$. *$P < .05$. NS, no significant difference.

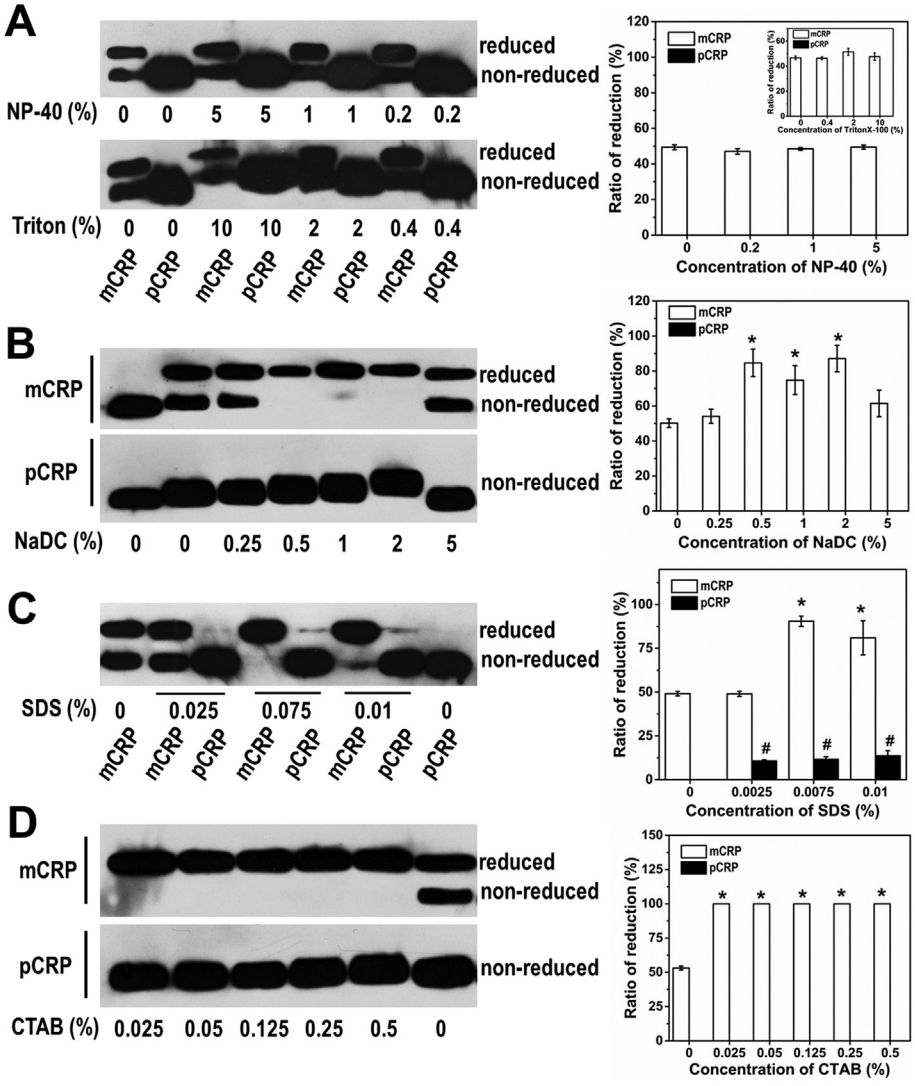

Fig. 5. Effect of different types of detergents on the reduction of mCRP. **A)** mCRP or pCRP (1 μg/ml) was incubated with 30 mM DTT and NP-40 or Triton X-100 (Triton) at indicated concentration for 2 h at 37 °C. Then samples blocked with 350 mM NEM for 30 min at 37 °C. mCRP or pCRP (2.5 μg/ml) was incubated with 30 mM DTT and sodium deoxycholate (NaDC) **B)**, SDS **C)**, CTAB **D)** at indicated concentration for 1 h at 37 °C, and then blocked with 200 mM NEM as above. Representative western blot results are shown. The results are presented as means ± SE, $n = 3$. *$P < .05$ vs. mCRP without detergent treated. # $P < .05$ vs. pCRP without detergent treated.

mainly attributable to the conformation of mCRP which hinders its reduction rather than the low efficiency of reducing and blocking agent.

3.4. Reduction of mCRP markedly enhanced by using of detergent

We additionally compared the effects of several detergent classes (non-ionic, anionic and cationic) to mCRP reduction. Our results demonstrate that, NP-40 ($P = .516$) and TritonX-100 ($P = .402$) have no facilitating effect on mCRP reduction (Fig. 5A). Sodium deoxycholate has a weak promotive effect on mCRP reduction, although this led to loss of mCRP due to precipitation of protein in the system (Fig. 5B). Both SDS and CTAB can have full promotive effect on mCRP reduction,

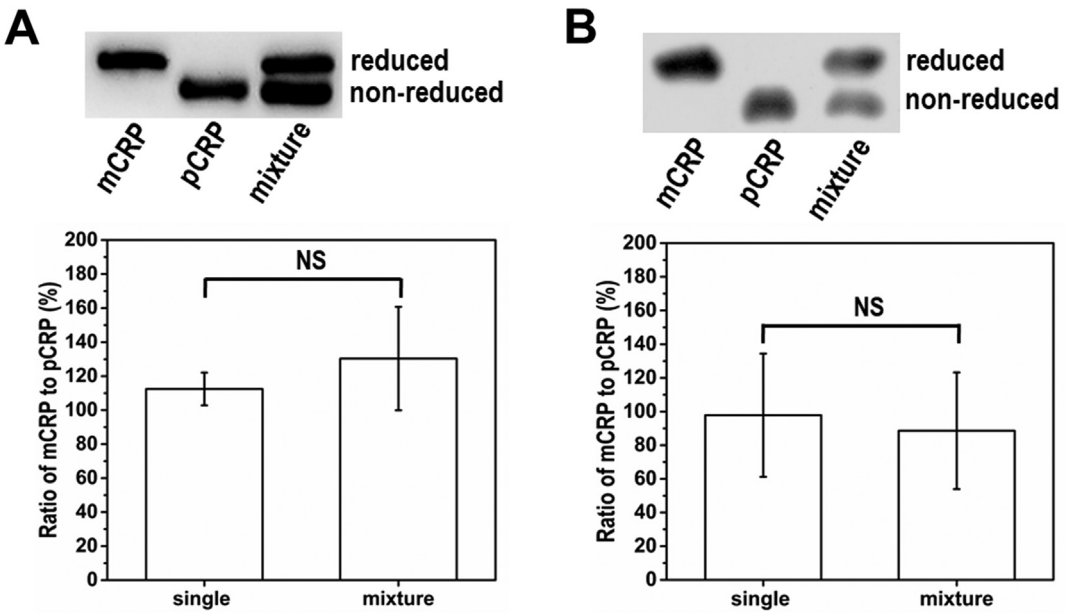

Fig. 6. Verifying the applicability of the optimal reduction condition for mixture of mCRP and pCRP. Adding mCRP, pCRP and their mixture into TBS-Ca buffer **A)** or equal volume of serum **B)** to make the final concentration is 20μg/ml. Then they were incubated with 30 mM DTT containing 0.05% CTAB for 1 h at 37 °C, and then blocked with 200 mM NEM for 30 min at 37 °C. Representative western blot results are shown. The results are presented as means ± SE, n = 3. "single" in the column means the ratio of gray scale value of reduced mCRP (first lane) to that of non-reduced pCRP (second lane). "mixture" in the column means the ratio of gray scale value of reduced band in mixture (third lane) to that of non-reduced band in mixture (third lane). NS, no significant difference.

but CTAB is a superior choice (Fig. 5C and D). This is primarily because SDS has a small promotive effect on pCRP reduction, potentially attributable to the depolymerization of a part of pCRP under the action of SDS. Thus, our findings suggest that a suitable detergent is necessary to promote mCRP reduction, particularly one that allows for mCRP and pCRP to be complete distinguishable from each other, indirectly allowing for accurate quantitative analysis of mCRP and pCRP.

3.5. Verify the applicability of the optimal reduction condition for mixture of mCRP and pCRP

From the above exploration, we constructed the optimal reduction conditions of mCRP in system containing its individual component. Then we repeated the above procedure in a more complex system through mixing mCRP with pCRP in TBS-Ca buffer and serum. This allowed us to verify the applicability of the optimal mCRP reduction conditions established under individual component. From the results (Fig. 6), the ratio of reduced mCRP to non-reduced pCRP between single and mixture have no significant difference either in buffer ($P = .605$) or in serum ($P = .863$). For the mixture system, this mean that mCRP and pCRP can be effectively discriminated from each other and there is no interference between them. This will further facilitate their respective quantitative analysis. The results also indicate that the method could reliably detect mCRP and pCRP in physiological samples in a single assay.

4. Discussion

Accumulating evidence demonstrated that mCRP is the main conformation of CRP in inflammatory tissue and mediate the development and regulation of inflammation. Although pCRP has been regarded as a diagnostic and prognostic marker for inflammatory disease, the tight correlation of mCRP and inflammation indicate that mCRP appears to be a more specific marker than pCRP. However, the correlation between pCRP and mCRP, and their respective strength as disease marker remain ill-identified. As mCRP can bind to a variety of serum proteins or lipids (Habersberger et al., 2012; Li et al., 2016), there may be an obstacle to the binding of antibodies against the corresponding epitopes of mCRP, leading to underestimation of its quantity. Additionally, cross-reactivity between pCRP and mCRP is found when using clinical ELISA kit or pCRP exceeds a certain concentration. These deficiencies indicate that the present ELISA-based quantification of CRPs may not be able to solve these problems and a more accurate quantitative assay is urgently needed.

Wang et al. (Wang et al., 2011) showed the distinctive sensitivities of mCRP and pCRP to reducing agents. The difference may be a simple strategy to distinguish and quantify them in one assay. However, we found that only a part of mCRP was reduced after incubation with DTT, even though increasing the dosage of reductant and prolonging the incubation time. Accordingly, the critical aspect of this strategy is to find an optimal condition to maximize the reduction of mCRP but not affecting pCRP. We further explored the effect of different reducing agent, blocking agent and detergent. The results showed that reduction ratio of mCRP cannot be affected by types of reducing and blocking agent, and detergent is necessary to promote the reduction of mCRP. This is probably because mCRP be inclined to form aggregation due to its higher hydrophobicity, thus hindering the contact between disulfide bond and reductant.

Through the optimization of the above conditions, we successfully established the conditions which can realize the complete reduction of mCRP without affecting pCRP, and separated them from each other, relevant for further quantitative analysis of them in one assay. Although western blot can easily analyze the optimal reducing conditions and measure the relative ratio between mCRP and pCRP, a more accurate quantitative assay (e.g., mass spectrometry) may be a superior choice. Such sensitivity difference of mCRP and pCRP to reducing agents may be used as a basic principle to develop more accurate quantitative methods in the future, which is meaningful for better study clinical correlation between them and reveal their respective strength as inflammatory markers.

5. Conclusions

This method takes advantage of the different sensitivity between

pCRP and mCRP to reductive agent. Through the optimization of the above conditions, we successfully separated the mCRP from the pCRP in buffer and serum systems, relevant for further quantitative analysis of the pCRP and mCRP in clinical samples. Our newly described method may avoid the problems of present ELISA assay, and measure them in one assay.

Author contributions

Z. Yao, L. Zhang and H. Wu designed the research. Z. Yao, L. Ji performed the research and analyzed the data. Z. Yao, Y. Zhang drafted the article, L. Potempa, I. Rajab revised it critically for important intellectual content. J. Lv, S. Liu edited figures. All authors reviewed the results and approved the final version of the manuscript.

Declarations of interest

The authors have no financial conflicts of interest.

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References

Albert, C.M., Ma, J., Rifai, N., Stampfer, M.J., Ridker, P.M., 2002. Prospective study of C-reactive protein, homocysteine, and plasma lipid levels as predictors of sudden cardiac death. Circulation 105, 2595–2599.

Allin, K.H., Nordestgaard, B.G., Zacho, J., Tybjaerghansen, A., Bojesen, S.E., 2010. C-reactive protein and the risk of cancer: a mendelian randomization study. J. Natl. Cancer Inst. 102, 202–206.

Braig, D., Nero, T.L., Koch, H.G., Kaiser, B., Wang, X., Thiele, J.R., Morton, C.J., Zeller, J., Kiefer, J., Potempa, L.A., et al., 2017. Transitional changes in the CRP structure lead to the exposure of proinflammatory binding sites. Nat. Commun. 8, 14188.

Eisenhardt, S.U., Habersberger, J., Murphy, A., Chen, Y.C., Woollard, K.J., Bassler, N., Qian, H., von Zur Muhlen, C., Hagemeyer, C.E., Ahrens, I., et al., 2009. Dissociation of pentameric to monomeric C-reactive protein on activated platelets localizes inflammation to atherosclerotic plaques. Circ. Res. 105, 128–137.

Habersberger, J., Strang, F., Scheichl, A., Htun, N., Bassler, N., Merivirta, R.M., Diehl, P., Krippner, G., Meikle, P., Eisenhardt, S.U., et al., 2012. Circulating microparticles generate and transport monomeric C-reactive protein in patients with myocardial infarction. Cardiovasc. Res. 96, 64–72.

Hirschfield, G.M., Herbert, J., Kahan, M.C., Pepys, M.B., 2003. Human C-reactive protein does not protect against acute lipopolysaccharide challenge in mice. J. Immunol. 171, 6046–6051.

Ji, S.R., Wu, Y., Potempa, L.A., Liang, Y.H., Zhao, J., 2006. Effect of modified C-reactive protein on complement activation: a possible complement regulatory role of modified or monomeric C-reactive protein in atherosclerotic lesions. Arterioscler. Thromb. Vasc. Biol. 26, 935–941.

Ji, S.R., Wu, Y., Zhu, L., Potempa, L.A., Sheng, F.L., Lu, W., Zhao, J., 2007. Cell membranes and liposomes dissociate C-reactive protein (CRP) to form a new, biologically active structural intermediate: mCRP(m). FASEB J. 21, 284–294.

Ji, S.R., Ma, L., Bai, C.J., Shi, J.M., Li, H.Y., Potempa, L.A., Filep, J.G., Zhao, J., Wu, Y., 2009. Monomeric C-reactive protein activates endothelial cells via interaction with lipid raft microdomains. FASEB J. 23, 1806–1816.

Kovacs, A., Tornvall, P., Nilsson, R., Tegner, J., Hamsten, A., Bjorkegren, J., 2007. Human C-reactive protein slows atherosclerosis development in a mouse model with human-like hypercholesterolemia. Proc. Natl. Acad. Sci. U. S. A. 104, 13768–13773.

Li, H.Y., Wang, J., Meng, F., Jia, Z.K., Su, Y., Bai, Q.F., Lv, L.L., Ma, F.R., Potempa, L.A., Yan, Y.B., et al., 2016. An intrinsically disordered motif mediates diverse actions of monomeric C-reactive protein. J. Biol. Chem. 291, 8795–8804.

Li, Q.Y., Li, H.Y., Fu, G., Yu, F., Wu, Y., Zhao, M.H., 2017. Autoantibodies against C-reactive protein influence complement activation and clinical course in lupus nephritis. J. Am. Soc. Nephrol. 28, 3044–3054.

Mihlan, M., Blom, A.M., Kupreishvili, K., Lauer, N., Stelzner, K., Bergström, F., Niessen, H.W., Zipfel, P.F., 2011. Monomeric C-reactive protein modulates classic complement activation on necrotic cells. FASEB J. 25, 4198–4210.

Mold, C., Rodriguez, W., Rodic-Polic, B., Du Clos, T.W., 2002. C-reactive protein mediates protection from lipopolysaccharide through interactions with Fc gamma R. J. Immunol. 169, 7019–7025.

Molins, B., Pena, E., de la Torre, R., Badimon, L., 2011. Monomeric C-reactive protein is prothrombotic and dissociates from circulating pentameric C-reactive protein on adhered activated platelets under flow. Cardiovasc. Res. 92, 328–337.

Paul, A., Ko, K.W.S., Li, L., Yechoor, V., Mccrory, M.A., Szalai, A.J., Chan, L., 2004. C-reactive protein accelerates the progression of atherosclerosis in apolipoprotein E-deficient mice. Circulation 109, 647–655.

Pepys, M.B., Hirschfield, G.M., 2003. C-reactive protein: a critical update. J. Clin. Invest. 111, 1805–1812.

Potempa, L.A., Yao, Z.Y., Ji, S.R., Filep, J.G., Wu, Y., 2015. Solubilization and purification of recombinant modified C-reactive protein from inclusion bodies using reversible anhydride modification. Biophys Rep 1, 18–33.

Ridker, P.M., Cushman, M., Stampfer, M.J., Tracy, R.P., Hennekens, C.H., 1997. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. New Engl J Med 336, 973–979.

Ridker, P.M., Rifai, N., Rose, L.M., Buring, J.E., Cook, N.R., 2002. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. New Engl J Med 347, 1557–1565.

Robey, F.A., Jones, K.D., Steinberg, A.D., 1985. C-reactive protein mediates the solubilization of nuclear DNA by complement in vitro. J. Exp. Med. 161, 1344–1356.

Rost, N.S., Wolf, P.A., Kase, C.S., Kellyhayes, M., Silbershatz, H., Massaro, J.M., Dagostino, R.B., Franzblau, C., Wilson, P.W.F., 2001. Plasma concentration of C-reactive protein and risk of ischemic stroke and transient ischemic attack the Framingham study. Stroke 32, 2575–2579.

Sakkinen, P.A., Abbott, R.D., Curb, J.D., Rodriguez, B.L., Yano, K., Tracy, R.P., 2002. C-reactive protein and myocardial infarction. J. Clin. Epidemiol. 55, 445–451.

Schwedler, S.B., 2003. Tubular staining of modified C-reactive protein in diabetic chronic kidney disease. Nephrol. Dial. Transplant. 18, 2300–2307.

Shrive, A.K., Gheetham, G.M., Holden, D., Myles, D.A., Turnell, W.G., Volanakis, J.E., Pepys, M.B., Bloomer, A.C., Greenhough, T.J., 1996. Three dimensional structure of human C-reactive protein. Nat. Struct. Mol. Biol. 3, 346–354.

Strang, F., Scheichl, A., Chen, Y.C., Wang, X., Htun, N.M., Bassler, N., Eisenhardt, S.U., Habersberger, J., Peter, K., 2012. Amyloid plaques dissociate pentameric to monomeric C-reactive protein: a novel pathomechanism driving cortical inflammation in Alzheimer's disease? Brain Pathol. 22, 337–346.

Tan, Y., Yu, F., Yang, H., Chen, M., Fang, Q., Zhao, M.H., 2008. Autoantibodies against monomeric C-reactive protein in sera from patients with lupus nephritis are associated with disease activity and renal tubulointerstitial lesions. Hum. Immunol. 69, 840–844.

Taylor, K.E., van den Berg, C.W., 2007. Structural and functional comparison of native pentameric, denatured monomeric and biotinylated C-reactive protein. Immunology 120, 404–411.

Thiele, J.R., Habersberger, J., Braig, D., Schmidt, Y., Goerendt, K., Maurer, V., Bannasch, H., Scheichl, A., Woollard, K.J., von Dobschütz, E., 2014. Dissociation of pentameric to monomeric C-reactive protein localizes and aggravates inflammation in vivo proof of a powerful proinflammatory mechanism and a new anti-inflammatory strategy. Circulation 130, 35–50.

Thiele, J.R., Zeller, J., Bannasch, H., Stark, G.B., Peter, K., Eisenhardt, S.U., 2015. Targeting C-reactive protein in inflammatory disease by preventing conformational changes. Mediat. Inflamm. 2015, 372432.

Wang, M.Y., Ji, S.R., Bai, C.J., El Kebir, D., Li, H.Y., Shi, J.M., Zhu, W., Costantino, S., Zhou, H.H., Potempa, L.A., et al., 2011. A redox switch in C-reactive protein modulates activation of endothelial cells. FASEB J. 25, 3186–3196.

Williams, D.K., Muddiman, D.C., 2009. Absolute quantification of C-reactive protein in human plasma derived from patients with epithelial ovarian cancer utilizing protein cleavage isotope dilution mass spectrometry. J. Proteome Res. 8, 1085–1090.

Ying, S.-C., Gewurz, H., Kinoshita, C., Potempa, L., Siegel, J., 1989. Identification and partial characterization of multiple native and neoantigenic epitopes of human C-reactive protein by using monoclonal antibodies. J. Immunol. 143, 221–228.

Zacho, J., Tybjaerghansen, A., Jensen, J.S., Grande, P., Sillesen, H., Nordestgaard, B.G., 2008. Genetically elevated C-reactive protein and ischemic vascular disease. New Engl J Med 359, 1897–1908.

Zhang, L., Li, H.Y., Li, W., Shen, Z.Y., Wang, Y.D., Ji, S.R., Wu, Y., 2018. An ELISA assay for quantifying monomeric C-reactive protein in plasma. Front. Immunol. 9, 511.