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Two-dimensional crystallization of rabbit C-reactive protein monomeric subunits

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C-reactive protein (CRP) is one of the most characteristic acute-phase proteins. Modified CRP is the monomeric form of native CRP and has recently been suggested to exist under physiological conditions. In the current work, CRP subunits were separated from stock CRP solution by size-exclusion chromatography. Two forms of two-dimensional crystals composed of monomeric CRP were obtained on negatively charged lipid monolayers: a previously reported form, MI, and a new form, MII. A projection map at 2.0 nm resolution of the two-dimensional MII crystals was obtained. The formation of the two forms of two-dimensional crystal exhibited a dependence on pH. At pH values of less than 5.5 the subunits assembled in MI packing, while at pH values greater than 6.5 they assembled in MII packing. When using modified CRP prepared by acidic denaturation, only MI crystals could be formed. The fact that CRP subunits produced by dissociation or denaturation could form highly ordered two-dimensional crystals indicates that they have a certain homogeneous structure, which supports the previous suggestion of the existence of modified CRP *in vivo*.

1. Introduction

C-reactive protein (CRP) is one of the most characteristic acute-phase proteins and is found in many vertebrates (Steel & Whitehead, 1994; Tillett & Francis, 1930). In response to cell damage, tissue injury or inflammation, the concentration of CRP in serum can rise to 300 $\mu g\ ml^{-1}$, about 1000 times its normal concentration, within 24 h. In 1965, Gotschlich reported that affinity-chromatographically purified CRP from serum was primarily in the pentameric state (Gotschlich & Edelman, 1965). This oligomeric state of so-called 'native CRP' was further supported by Osmand *et al.* (1977), who reported that CRP in solution is composed of five identical 23 kDa subunits in a planar pentameric arrangement and classified as a pentraxin. Each subunit of CRP has a calcium-dependent specific binding site for phosphorylcholine (PC) and both the monoclonal marking technique (Roux *et al.*, 1983) and structural analysis (Shrive *et al.*, 1996; Thompson *et al.*, 1999) revealed all the PC-binding sites to be on the same face of the pentameric disc. Based on the specific binding property of CRP with PC, affinity chromatography has been extensively used in the purification of the protein from serums of different animals (Nunomura, 1992). In the past several decades, most of the biochemical and physiological properties of native CRP have been determined based on studies of the pentameric protein.

However, other conformational types of CRP have been reported. In 1983, Potempa and coworkers reported that in the presence of EDTA, CRP treated with urea or acid runs faster in native gel electrophoresis and is less soluble than native CRP (Potempa *et al.*, 1983). The structural composition of such modified CRP is monomeric, having non-proteolytic conformational changes (Potempa *et al.*, 1983, 1987; James *et al.*, 1981; Fairbanks, 1984). Monomeric or modified CRP exhibited physiological functions that are distinct from those of native CRP. It was found that the modified form has different antigenic binding sites (termed 'neo-CRP') from native CRP and loses its PC-binding activity (Potempa *et al.*, 1987). Such neo-CRP associates with the free subunit of CRP and has been detected in various tissues throughout the body (Bray *et al.*, 1987, 1988; Samberg *et al.*, 1988; Rees *et al.*, 1989; Shields, 1993). Recently, the immunohistochemical localization of modified CRP antigen in normal vascular tissue suggested the stable existence of modified CRP *in vivo* (Diehl *et al.*, 2000). The dissociation of CRP has also been observed by electron microscopy (Sui *et al.*, 1996; Wang & Sui, 2001). After dissociation, the subunits reveal new epitopes that play different physiological roles to those

of the pentamers (Potempa *et al.*, 1987). The epitopes may arise either from the exposure of new sites on the surfaces of the subunits or from conformational changes of the subunits after dissociation. Therefore, the structural characterization of CRP subunits under the modification conditions is of significance.

In the present work, we studied the two-dimensional crystallization of CRP subunits produced by dissociation of the pentameric form. A new type of two-dimensional crystal formed by the monomers of CRP was obtained. The result that the CRP monomer subunits could form highly ordered two-dimensional crystals indicates that they have a degree of homogeneous structure, which supports the previous suggestion for the existence of modified CRP *in vivo*.

2. Materials and methods

2.1. Reagents and chemicals

The phospholipids 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), 1,2-dipalmitoyl-*sn*-glycero-3-phosphoserine (DPPS), 1,2-dimyrsitoyl-*sn*-glycero-3-phosphoserine (DMPS), 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), egg-phosphocholine (egg-PC), lyso-phosphocholine (lyso-PC) and phosphorylcholine chloride (PC), EDTA, ultra-pure urea, Sepharose 4B, phosphorylethanolamine-Sepharose 4B and sheep anti-human CRP antiserum were purchased from Sigma Chemical Co. (St Louis, MO, USA). Deionized water was purchased from the Micro-Electronic Institute of Tsinghua University. All the other reagents were of analytical grade and were purchased locally.

2.2. Purification of rabbit CRP

Rabbit C-reactive protein was purified from the acute-phase serum of rabbits by affinity chromatography on phosphorylethanolamine-Sepharose 4B according to a published procedure (Sui *et al.*, 1996). The purity of the protein is confirmed to be over 99% by SDS–PAGE with silver staining. The functional activity of the purified CRP was examined by immunoprecipitation with C-polysaccharide and with sheep anti-human CRP antiserum. The purified CRP was stored as stock CRP solution in calcium-free TBS (0.02 *M* Tris–HCl, 0.15 *M* NaCl, 0.02% sodium azide pH 7.8) at 277 K.

2.2.1. Analysis and isolation of CRP subunits from stock CRP solution using an AKTA purifier. It has been reported that isolated native CRP preparation stored in buffer lacking calcium or containing chelator resulted in the formation of monomeric CRP (Motie *et al.*, 1996). In the present study, we also found monomeric CRP stock solutions lacking calcium. The following is the procedure to detect and isolate monomeric CRP from stock CRP solution.

For analysis, 200 µl of CRP at a concentration of 0.2 mg ml^{-1} in TBS was applied to an AKTA Purifier with a Superdex 200 column (Pharmacia, Uppsala, Sweden). For isolation, we applied 2 ml CRP (fresh or stock) solution at a concentration of 1 mg ml^{-1} in TBS onto the AKTA Purifier using a HiLoad 26/60 Superdex 200 column (Pharmacia, Uppsala, Sweden). The elution buffer was the same as the storage buffer. The flow rates for analysis and isolation were 0.5 and 1.0 ml min^{-1}, respectively.

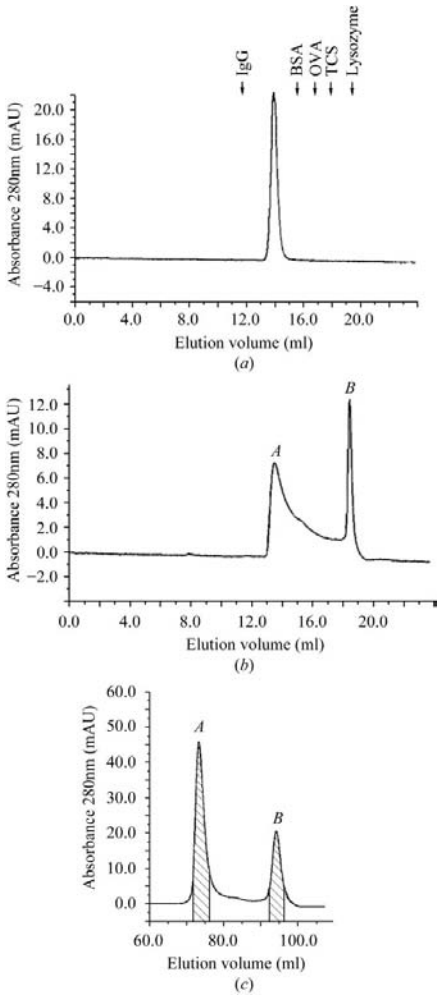

Figure 1
Size-exclusion chromatography for the analysis and preparation of CRP. The column was equilibrated with 0.02 *M* Tris–HCl, 0.15 *M* NaCl pH 7.8 and 0.02% sodium azide at room temperature. The flow rate was 0.5 ml min^{-1} for analysis and 1.0 ml min^{-1} for preparation. Control proteins used: IgG (MW 150 kDa), bovine serum albumin (BSA; MW 68 kDa), ovalbumin (OVA; MW 45 kDa), trichosanthin (TCS; MW 26 kDa), lysozyme (MW 14 kDa). (*a*) Superdex 200 chromatograph of fresh CRP, which has only one peak at 13.77 indicating a MW of 120 kDa. (*b*) Superdex 200 chromatograph of CRP stored in buffer for two weeks showing the resolution of first the 120 kDa component and then the 22 kDa component. (*c*) HiLoad 26/60 Superdex 200 column chromatograph of the isolation of monomeric CRP from stored CRP; the collection ranges of components *A* and *B* are shaded in the profile.

2.2.2. Two-dimensional crystallization. The lipid-layer approach to form two-dimensional protein crystals is a widely used technique (Uzgiris & Kornberg, 1983). In the present work, we adopted the same method as in our previous work (Sui *et al.*, 1996; Qin *et al.*, 1995; Wang & Sui, 1999). Droplets (~15 µl) of calcium-free rabbit CRP solutions with different protein concentrations, subphase compositions and pH values were placed in Teflon wells. The surfaces of the droplets were coated with 0.5–1 µl of lipid mixture solutions of varying composition in chloroform/methanol [3:1(*v*/*v*)]. Incubation was carried out in a humid atmosphere at different temperatures and for different periods of time.

2.2.3. Electron microscopy and image processing. After incubation in the well for an appropriate time period, the lipid monolayers with attached proteins were picked up on carbon-coated grids. Briefly, the grids were placed horizontally onto the film at the air–water interface and picked up after they reached the monolayers. After blotting off the residual solution on the grids, they were negatively stained with uranyl acetate [1%(*w*/*v*)] for 1–2 min.

The negatively stained samples were examined in a Philips CM120 transmission electron microscope under a 100 kV accelerating voltage at a magnification of about 50 000. Interesting areas of the grids were recorded on Kodak SQ-163 films. The best images were selected both by eye and by optical diffraction and were digitized at a step size of 25 µm pixel^{-1} using an AGFA Duoscan camera system. The digitized images were processed using the *ICE* (Hardt *et al.*, 1996) and *MRC* (Crowther *et al.*, 1996) image-processing packages.

3. Results and discussion

3.1. CRP stored in buffer shows spontaneous disassociation

Previous studies reported that native CRP may undergo dissociation in solutions lacking calcium (Gotschlich & Edelman, 1965; Potempa *et al.*, 1983; Myles *et al.*, 1990). In the present work, we use size-exclusion chromatography to examine the oligomeric

state of CRP in calcium-free solution. Fresh CRP just purified from serum of rabbit and CRP stored for variable time intervals at 277 K in the presence of EDTA or the absence of calcium were analyzed by size-exclusion chromatography. The results indicated that the dissociation of CRP occurred spontaneously and was obvious when storage was for more than 3 d. The size-exclusion chromatographs for fresh and stored CRP show one main component (MW $\simeq$ 120 kDa) from the fresh CRP solution (Fig. 1*a*) and a second peak (*B* at MW 22 kDa) from the stored CRP solution (Fig. 1*b*). Since the molecular weight of pentameric CRP is about 120 kDa, the 22 kDa peak of Fig. 1(*b*) indicates dissociation to monomeric CRP during storage. The peak representing pentameric CRP in Fig. 1(*a*) is very sharp, indicating a tight homogeneous quaternary structure of fresh CRP, in contrast to the broader corresponding peak for pentameric CRP in Fig. 1(*b*). The monomer peak and broadening of the pentamer peak reflect the dissociation of CRP during storage.

The subunits in the pentameric CRP primarily interact through salt bridges between adjacent subunits (Shrive *et al.*, 1996). The results revealed previously and in the current work suggested that these interactions can be weakened and broken under certain conditions, such as at lower pH values and in the absence of calcium. From the chromatography of stored CRP, several minor components could also be found between peaks *A* and *B* (Fig. 1*b*), implying the existence of CRP dissociation intermediates. These intermediates do not have distinctive elution peaks with exact molecular weights of lower oligomeric forms, indicating different degrees of relaxation in the quaternary structures of CRP during the process of dissociation. On the other hand, the obvious sharp peak *B* indicates that the final products of the dissociation process are homogeneous and stable CRP monomeric subunits.

3.2. CRP subunits isolated from stored solution can form two types of two-dimensional crystal, MI and MII

According to the above analysis, the stock CRP solution contains at least two components, *A* and *B* (pentameric and monomeric CRP, respectively). Using size-exclusion chromatography, the components *A* and *B* were isolated (Fig. 1*c*). Two-dimensional crystallization trials of monomeric CRP were performed under various conditions. With the subphase pH in the region of 4.0–5.5 and lipid mixtures (eggPC, lysoPC and DMPS at a molar ratio of 5:1:1), two-dimensional crystals were obtained after incubation at room temperature for about 36 h as previously reported (Sui *et al.*, 1996). Here, we refer to this two-dimensional crystal as MI, with unit-cell parameters $a = 74$, $b = 67$ Å, $\gamma = 95.5^\circ$.

When the pH was adjusted to 6.5–8.0, a new form of two-dimensional crystal was observed at room temperature after 24 h incubation, which we refer to here as MII. Fig. 2(*a*) shows an electron micrograph of the negatively stained MII two-dimensional crystal on an eggPC/lysoPC/DMPS (5:1:1) monolayer. Fig. 2(*b*) shows the computed diffraction pattern of the MII crystal. The unit-cell parameters are $a = 206\,(1)$, $b = 161\,(1)$ Å, $\gamma = 90\,(1)^\circ$. The (10, 0) reflection spot visible by eye corresponds to 20.6 Å resolution. A symmetry analysis of the MII two-dimensional crystal revealed that the MII crystal belongs to the *p2gg* layer group with an average phase residual of 16°. Such symmetry implies that the adsorption and self-assembly of CRP subunits onto the monolayer surface occurs on both sides of the molecule. The projection density map with *p2gg* symmetry is shown in Fig. 2(*c*), in which three monomers were arranged in a triangular manner and four such triangles make up one unit cell.

From the above experiments, it is obvious that the two-dimensional crystallization of CRP subunits shows some degree of pH-dependence. In order to test this, further experiments were performed. The CRP subunits were crystallized under relatively mild conditions and both forms of the two-dimensional crystals MI and MII were observed (data not shown). Neither fresh CRP nor isolated pentameric CRP could form two-dimensional crystals of CRP subunits on lipid monolayers under the above conditions. This means that the two-dimensional crystals of the CRP subunits on monolayers are composed of the CRP monomeric subunits in solution adsorbed onto the membranes and not composed of subunits formed by lateral dissociation of pentamers after adsorption onto the lipid layer.

Although the two types of crystals have different lattice parameters and symmetry properties, a similar point can be reached by comparison of their two-dimensional arrangement. Fig. 3 shows the noise-filtered images of MI and MII, from which we can observe that the monomers in both crystals are arranged in quite a similar manner: the crystals are assembled of CRP monomers in a 'T'-like arrangement, each 'T'-like unit

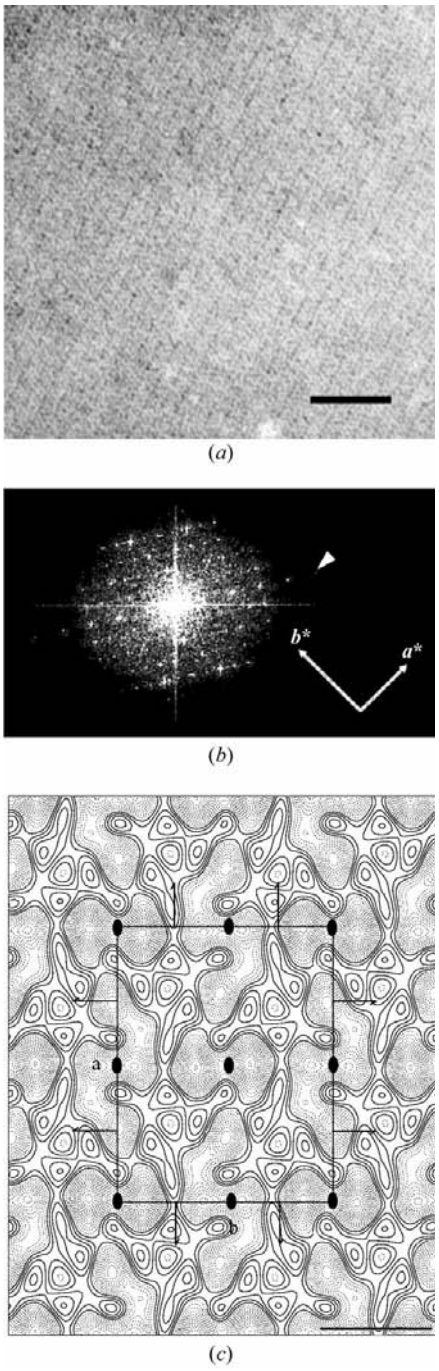

Figure 2
A MII monomeric two-dimensional crystal formed on egg-PC/lyso-PC/DMPS (in a molar ratio of 5:1:1) monolayers by non-specific adsorption. In the preparation of two-dimensional crystals, the subphase contained 0.1 mg ml^{-1} CRP in TBS pH 7.0 and the incubations were performed at 293 K for about 36 h. (*a*) Electron micrograph of one of the MII two-dimensional crystals. (*b*) Fourier-transformed spectrum of the image of the crystals after unbending using the *CCUNBEND* program (MRC). The (10, 0) reflection in the spectrum, representing about 2.0 nm resolution, is indicated by the arrow. (*c*) Calculated average projection map from seven images of different MII two-dimensional crystals at a resolution of 2.0 nm. One unit cell is enclosed by the rectangle. The twofolds and the screw axis are indicated. The scale bar in (*a*) represents 50 nm, while that in (*c*) represents 10 nm.

consisting of two entities. The size and electronic density of the entities in MII are quite similar (~60 Å in the long axis and ~35 Å in the short axis) to that of previously reported MI crystal (Sui *et al.*, 1996) and are consistent with the known atomic structure of the CRP monomer. Thus, we suggest that the basic entities in MI and MII two-dimensional crystals are CRP monomers. In the MI crystal form, each 'T'-like structural unit is like a brick with which the whole two-dimensional array is assembled. In the MII crystal form, the unit cell consists of four triangular structures and each triangle consists of three monomers. An intriguing assembly fashion is that in which two adjacent monomers in each triangular structure assemble in a 'T'-like manner, thus forming a triangular ring-like structure. The included angle between two monomers of the 'T'-like structure in MI is about 75°, while that in MII is about 60°, indicating that the interactions between monomers in MI and MII are not identical. In addition, there is a 10% difference in the calculated surface densities of monomeric CRP between the MI and MII structures (4.04 and 3.62 per 10^{-4} Å^{-2}, respectively). Potempa *et al.* (1983) reported that the pI of modified CRP is about 5.5, so the charge on the monomer will vary as the pH changes around the pI. The dependence of subunit assembly upon pH may reflect to a certain extent the difference in the surface electronic potential or even in the conformation of CRP subunits.

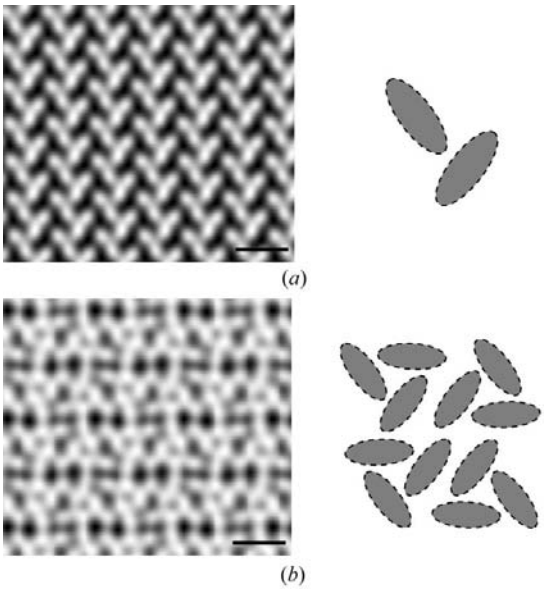

Figure 3
The noise-filtered image of MI (*a*) and MII (*b*) crystals. The resolutions of the images used to produce (*a*) and (*b*) are about 2.3 and 2.1 nm, respectively. The scale bars in (*a*) and (*b*) represent 10 nm. (*a*) and (*b*) show that the monomers in both MI and MII crystals use quite a similar manner of assembly: monomers assemble the whole crystal in a 'T'-like arrangement. The difference is that in MI crystal the unit cell is composed of two monomers, while the unit cell of MII crystal is composed of four triangular structures and each triangle is made up of three monomers. In order to show a clearer view, a sketch map of the unit cell is placed beside the filtered image, in which the shaded ellipses represent monomers.

3.3. Only the MI crystal form is obtained using modified CRP prepared by acidic denaturation

Modified CRP with monomeric form exhibits physiological functions that are distinct from those of native CRP. Modified CRP can be prepared by acidic denaturation by adjusting the native CRP solution pH to 2.0 with HCl in the presence of EDTA and incubating at ambient temperature for 1 min prior to neutralization with NaOH (Potempa *et al.*, 1986, 1987, 1988, 1991, 1996; Fairbanks, 1984; Fiedel *et al.*, 1982).

The effect of acidic denaturation on the two-dimensional crystallization of the proteins was examined. Two different samples were treated by acidic denaturation: native CRP and CRP monomeric subunits separated from stock CRP. After acidic denaturation, two-dimensional crystallization trials revealed that for both samples only MI crystals could be formed. As mentioned above, however, CRP subunits produced by spontaneous disassociation could form both MI and MII crystals. Such a difference in crystallization behavior suggests that the CRP subunits prepared in different ways may have some difference in structure or conformation. Since low pH could induce a change in the tertiary conformation of monomeric CRP and more hydrophobic exposure (Wu *et al.*, 2002), we suggest that the acidic denaturation condition (pH 2.0) may induce certain irreversible change in the structure or conformation of CRP subunits, leading to the preferred MI packing.

Although many studies have been carried out on modified CRP, it still remains uncertain whether modified CRP is a natural protein existing in the body. The fact that CRP subunits produced by dissociation or denaturation could form highly ordered two-dimensional crystals indicates they have a homogeneous structure, which supports previous evidence for the existence of the modified CRP *in vivo*. Shields (1993) suggested that the modified form of CRP is a consequence of the local environment change at the inflammatory site (such as low pH, oxygen radicals, enzymatic action *etc.*). We also found that using a relatively mild acidic buffer (pH 6.0), the dissociation rate of native CRP rapidly increases (data not shown). Cell damage or tissue injury may induce a local mild acidic micro-environment (Tannock & Rotin, 1989; Cohen, 1994). Hence it is the dissociation (but not denaturation) condition that may occur *in vivo* and we propose that the CRP subunits produced by dissociation may be a more natural form than the modified CRP produced by denaturation.

3.4. Two-dimensional crystallization of CRP subunits on a lipid layer is induced by electrostatic interaction

As well as on the lipid mixture of eggPC, lysoPC and DMPS mentioned above, MI or MII crystals also can be formed on monolayers composed of DPPC and DMPS (molar ratio of 5:1) under the same conditions (data not shown). Unlike the formation of pentameric CRP two-dimensional crystals (Wang & Sui, 1999, 2001), lysoPC is not essential for two-dimensional crystallization of CRP subunits. The absence of calcium in the incubation system further suggests that there is no specific binding during the formation of the two-dimensional crystals. On the other hand, on lipid monolayers composed of only pure DOPC, pure DPPC or pure egg-PC, no monomeric CRP two-dimensional crystals could be observed, indicating negatively charged PS is necessary for the formation of two-dimensional crystals. The inhibition effect of ionic strength showed that when the concentration of NaCl was higher than 150 m*M*, the quality of the two-dimensional crystals was inversely proportional to the concentration of NaCl. When the ionic concentration was higher than 300 m*M*, no two-dimensional crystals could be obtained. These results evidently suggested that the two-dimensional crystallization of CRP monomeric subunits on the negatively charged lipid monolayer was induced by electrostatic interactions.

The lipid mixture we adopted to obtain the two-dimensional crystals of monomeric CRP is composed of PC and PS. Here, PS is the essential component for the formation of the two-dimensional crystals. The asymmetry of the cell membrane shows that the negatively charged lipids such as PS are mostly distributed in the inner membranes.

When the cells are necrotic or damaged, the PS in the inner leaflet of the cell membrane can be exposed to the matrix, which may induce modified CRP non-specifically to react with membrane and lead to monomer-specific functions.

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References

Bray, R. A., Samberg, N. L., Gewurz, H., Potempa, L. A. & Landay, A. L. (1988). *J. Immunol.* **140**, 4271–4278.

Bray, B. A., Samberg, N. L., Landay, A. L., Gewurz, H. & Potempa, L. A. (1987). *Clin. Immunol. Newsl.* **8**, 137–140.

Cohen, M. S. (1994). *Clin. Infect. Dis.* **18**, S170–S179.

Crowther, R. A., Henderson, R. & Smith, J. M. (1996). *J. Struct. Biol.* **116**, 9–16.

Diehl, E. E., Haines, G. K. III, Radosevich, J. A. & Potempa, L. A. (2000). *Am. J. Med. Sci.* **319**, 79–83.

Fairbanks, T. R. (1984). PhD dissertation. Rush University, Chicago, Illinois, USA.

Fiedel, B. A., Simpson, R. M. & Gewurz, H. (1982). *Immunology*, **45**, 439–447.

Gotschlich, E. C. & Edelman, G. M. (1965). *Proc. Natl Acad. Sci. USA*, **54**, 558–566.

Hardt, S., Wang, B. & Schmid, M. F. (1996). *J. Struct. Biol.* **116**, 68–70.

James, K., Hansen, B. & Gewurz, H. (1981). *J. Immunol.* **127**, 2545–2552.

Motie, M., Brockmeier, S. & Potempa, L. A. (1996). *J. Immunol.* **156**, 4435–4441.

Myles, D. A. A., Rule, S. A., Delucas, L. J., Babu, Y. S., Xu, Y., Volanakis, J. E., Bugg, C. E., Bailey, S. & Greenhough, T. J. (1990). *J. Mol. Biol.* **216**, 491–496.

Nunomura, W. (1992). *Zool. Sci.* **9**, 499–513.

Osmand, A. P., Friedenson, B., Gewurz, H., Painter, R. H., Hofmann, T. & Shelton, E. (1977). *Proc. Natl Acad. Sci. USA*, **74**, 739–743.

Potempa, L. A., Gewurz, H., Harris, J. E. & Braun, D. P. (1986). *Protides Biol. Fluids*, **34**, 287–290.

Potempa, L. A., Maldonado, B. A., Laurent, P., Zemel, E. S. & Gewurz, H. (1983). *Mol. Immunol.* **20**, 1165–1175.

Potempa, L. A., Motie, M., Anderson, B., Klein, E. & Baurmeister, U. (1991). *Clin. Mater.* **11**, 105–117.

Potempa, L. A., Motie, M., Wright, K. E., Crump, B. L., Radosevich, J. A., Sakai, N., Hua, L. G., Tanaka, K., Kojima, E. & Tsuboi, A. (1996). *Exp. Hematol.* **24**, 258–264.

Potempa, L. A., Siegel, J. N., Fiedel, B. A., Potempa, R. T. & Gewurz, H. (1987). *Mol. Immunol.* **24**, 531–541.

Potempa, L. A., Zeller, J. M., Fiedel, B. A., Kinoshita, C. M. & Gewurz, H. (1988). *Inflammation*, **12**, 391–405.

Qin, H., Liu, Z. & Sui, S. F. (1995). *Biophys. J.* **68**, 2493–2496.

Rees, R. F., Siegel, J. N., Lint, T. F. & Gewurz, H. (1989). *Clin. Res.* **37**, 559A.

Roux, K. H., Kilpatrick, J. M., Volanakis, J. E. & Kearny, J. F. (1983). *J. Immunol.* **131**, 2411–2415.

Samberg, N. L., Bray, R. A., Gewurz, H., Landay, A. L. & Potempa, L. A. (1988). *Cell. Immunol.* **116**, 86–98.

Shields, M. J. (1993). *Immunol. Res.* **12**, 37–47.

Shrive, A. K., Cheetham, G. M. T., Holden, D., Myles, D. A. A., Turnell, W. G., Volanakis, J. E., Pepys, M. B., Bloomer, A. C. & Greenhough, T. J. (1996). *Nature Struct. Biol.* **3**, 346–354.

Steel, D. M. & Whitehead, A. S. (1994). *Immunol. Today*, **15**, 81–88.

Sui, S. F., Liu, Z., Li, W., Xiao, C. D., Wang, S. X., Gao, Q. F. & Zhou, Q. Z. (1996). *FEBS Lett.* **388**, 103–111.

Tannock, I. F. & Rotin, D. (1989). *Cancer Res.* **49**, 4373–4384.

Thompson, D., Pypes, M. B. & Wood, S. P. (1999). *Struct. Fold. Des.* **7**, 169–177.

Tillett, W. S. & Francis, T. (1930). *J. Exp. Med.* **52**, 561–571.

Uzgiris, E. E. & Kornberg, R. D. (1983). *Nature (London)*, **301**, 125–129.

Wang, H. W. & Sui, S. F. (1999). *J. Struct. Biol.* **127**, 283–286.

Wang, H. W. & Sui, S. F. (2001). *J. Struct. Biol.* **134**, 46–55.

Wu, Y., Ji, S. R., Wang, H. W. & Sui, S. F. (2002). *Biochemistry (Moscow)*, **67**, 1377–1382.