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Computational Analysis of C-Reactive Protein for Assessment of Molecular Dynamics and Interaction Properties

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Abstract

Serum C-reactive protein (CRP) is used as a marker of inflammation in several diseases including autoimmune disease and cardiovascular disease. CRP, a member of the pentraxin family, is comprised of five identical subunits. CRP has diverse ligand-binding properties which depend upon different structural states of CRP. However, little is known about the molecular dynamics and interaction properties of CRP. In this study, we used SAPS, SCRATCH protein predictor, PDBsum, ConSurf, ProtScale, Drawhca, ASAView, SCide and SRide server and performed comprehensive analyses of molecular dynamics, protein–protein and residue–residue interactions of CRP. We used 1GNH.pdb file for the crystal structure of human CRP which generated two pentamers (ABCDE and FGHIJ). The number of residues involved in residue–residue interactions between A–B, B–C, C–D, D–E, F–G, G–H, H–I, I–J, A–E and F–J subunits were 12, 11, 10, 11, 12, 11, 10, 11, 10 and 10, respectively. Fifteen antiparallel β sheets were involved in β -sheet topology, and five β hairpins were involved in forming the secondary structure. Analysis of hydrophobic segment distribution revealed deviations in surface hydrophobicity at different cavities present in CRP. Approximately 33 % of all residues were involved in the stabilization centers. We show that the bioinformatics tools can provide a rapid method to predict molecular dynamics and interaction properties of CRP. Our prediction of molecular dynamics and interaction properties of CRP combined with the modeling data based on the known 3D structure of CRP is helpful in designing stable forms of CRP mutants for structure–function studies of CRP and may facilitate in silico drug design for therapeutic targeting of CRP.

Keywords

C-reactive protein (CRP); Autoimmune and cardiovascular disease; Molecular dynamics; Interaction properties; In silico analysis

Introduction

Human C-reactive protein (CRP), a member of the pentraxin family, is a host-defense protein conserved throughout evolution [1–3]. The serum level of CRP rises in inflammatory diseases [4]. A significant prognostic association has been noted between increased levels of serum CRP and cardiovascular disease [5]. CRP consists of five identical non-covalently associated 23,028-Da subunits arranged as a cyclic pentamer [6, 7]. The major ligand-binding specificity of native pentameric CRP is for phosphocholine-containing substances [8]. When pentameric structure of CRP is slightly altered by acidic pH treatment, then CRP can also bind to complement factor H and atherogenic lipoproteins [9–11]. Under certain conditions, pentameric CRP dissociates into monomers and monomeric CRP displays a variety of different ligand-binding activities [12–17]. CRP is also a possible drug target for myocardial infarction, a disease in which phosphocholine-bound CRP-mediated complement activation worsens the condition [18]. Understanding the molecular dynamics and interaction properties of CRP may provide useful information about the structure–function relationships of CRP and may assist in further development of drugs for therapeutic targeting of CRP.

Molecular dynamics and interaction properties of proteins play significant roles in maintaining their stability and, therefore, in defining their functions [19, 20]. Several factors are associated with the molecular dynamics and interaction properties of proteins, such as the nature of the amino acid composition, topology of the disulfide bonds, topology of the secondary structure, conserved residues, hydrogen bonds, interaction between protein subunits, hydrophobic segments, charge distribution, stabilization centers, stabilizing residues and solvent accessibility. The amino acid composition of a protein is informative about the fundamental and complex traits and changes in amino acid usages and about the amino acid patterns around disulfide bonds [21, 22]. Disulfide bonds form conformational boundary, induce structural stability and influence protein folding and functionality [23–27]. From the topology of the secondary structure, we derive information about α helices, β sheets, random coil regions, protein folding patterns, protein stability patterns and C-terminal and N-terminal ends [28]. Hydrogen bonds play a vital role in protein folding, protein structure and molecular recognition and protein stability [29]. Investigation of the positions of conserved residues is also of structural and functional importance [30, 31]. Studying the interactions between the subunits of a protein also informs us about the stability pattern and helps to identify interaction prototype between the subunits [32, 33]. Another important factor for protein stability is hydrophobic interactions, and it is believed that the hydrophobic interactions are major contributors of stabilizing the native structure of proteins in an aqueous environment [34–36]. An additional factor is charge density which is a fundamental parameter for protein stability [37]. It has been shown that the surface charge affects the stability of proteins and is one of the requirements for tailoring proteins with increased thermal stability [38]. Stabilization center of a protein, related to stabilization center residues, provides a well balanced, stable and functional protein [39, 40]. The common stabilization center residues are found at covered positions and have either hydrophobic or aromatic side chains, but some polar or charged residues also play significant roles in the stabilization [40]. Solvent accessibility, a factor associated with the protein stability, is the ratio of the solvent accessible surface area of a residue in 3D structures to that in an extended tripeptide conformation and specifies the arrangement and packing of residues [41]. Little information is available related to CRP about the molecular dynamics and interaction properties associated with its stability.

The aim of this study was to highlight the molecular dynamics and interaction properties related to the stability of CRP. We performed a rapid structural bioinformatics analysis of CRP to obtain a better model of the amino acid composition, topology of the disulfide bond,

secondary structure topology mapping, conserved residues, interactions between protein subunits and residues, hydrophobic segments, charge distribution, stabilization centers, stabilizing residues and solvent accessibility. Our results provide significant insights into the structural information associated with the stability of CRP which may enhance our understanding of the structure–function relationships of CRP.

Materials and Methods

Collection of Data

We collected data on the human CRP gene from the National Center for Biotechnology Information (NCBI) [42]. The amino acid sequence of CRP, in FASTA format, was collected with the accession number from the NCBI database and further analyzed. The PDB file of CRP (1GNH.pdb) was extracted from the protein data bank (www.rcsb.org) for further analysis [43].

Amino Acid Composition, Disulfide Bond Topology, Secondary Structure Topology Mapping and Conserved Residues

We used the statistical analysis of protein sequences (SAPS) as a tool to analyze the amino acid prototype and protein sequence properties, such as amino acid composition, high-scoring hydrophobic segments and tandem and periodic repeats [44]. To explore the secondary structure aspects, such as disulfide bond topology prediction, we used “SCRATCH protein predictor” which has different tools for structural proteomics computational analysis [45].

To predict the pictorial view of the secondary structure and to understand the conserved residues, we used PDBsum server which is a database of graphical summaries of the secondary structure elements of proteins in the Protein Data Bank [46, 47]. Using this tool, we developed a topology diagram of CRP to show the conserved residues and to understand the impact and connectivity of the helices and strands in CRP. Finally, the PDBsum server, which uses Gail Hutchinson’s HERA algorithm, was also used to generate hydrogen bond plots to identify the hydrogen bonds in the secondary structure [48, 49].

Assembly of CRP Subunits, Interactions Between Subunits and Interactions Between Residues

We used PDBsum server to understand the interactions between and assembly of the five subunits of CRP. This server helps to analyze the interfaces between the subunits and summarizes the interactions across any selected interface. This server also provides information about the residues that actually interact across the interface [48].

Hydrophobic Segment Prediction

For hydrophobic segment prediction, we used ConSurf server, SAPS tool, ProtScale and Drawhca server. ConSurf server allows visualization of the hydrophobic and hydrophilic surfaces properties using the concept of molecular hydrophobicity potential (MHP) [50]. ConSurf server provides the structure which can be visualized using PyMOL. The SAPS tool was used to calculate high-scoring hydrophobic segments. ProtScale, a general resource for showing amino acid propensity scales, was used to analyze hydrophobicity plots using a sliding window algorithm [51]. Drawhca server was used to perform hydrophobic cluster analysis (HCA) [52].

Solvent Accessibility Prediction

The solvent accessibility, the locus of the center of the solvent molecule, rolls over the van der Waals surface of the protein which can be calculated using the “rolling ball” algorithm [53]. We predicted the solvent accessibility and generated a graphical plot of the solvent accessibility by using ASAView [54].

Charge Distribution Prediction

Charge distribution was obtained using ConSurf server and SAPS tool. To visualize charge distribution, we use Con-Surf server and this server use PyMOL interface for visualization the result [50, 55]. SAPS tool uses algorithms for SAPS (statistical analysis of protein sequences) to predict the distribution of charges, high-scoring positive charge segment, high-scoring negative charge segment, high-scoring mixed charge segment and high-scoring uncharged segments.

Stabilization Centers and Stabilizing Residues Prediction

Stabilization centers were identified and analyzed by using SCide server [56], and the stabilizing residues were identified by using SRide server [57]. We also calculated the stabilization center elements in the sequence. Stabilizing residues were calculated by using the surrounding hydrophobicity, long-range order, stabilization center and conservation score as described previously [58]. We used a conservation score of 6 as the cutoff value to identify the stabilizing residues.

Results and Discussion

Information about human CRP and its gene was derived from the NCBI data bank (Table S1).

Nature of the Amino Acid Composition, Disulfide Bond Topology, Secondary Structure with Conserved Residues, β Sheet Topology and Hydrogen Bond Pattern in β Sheet Topology

The amino acid compositional analysis, high-scoring hydrophobic segments and spacings of C are shown in Table S2. The prediction results of the amino acid composition of CRP are shown in Fig. S1. As shown, the composition of Ser is highest (10.2 %) among all other amino acids. The lowest composition was observed for Cys (1 %), Met (1 %) and His (1 %). There are two half Cys residues (Cys36 and Cys97) which form an intra-disulfide bond and which may provide the stability to individual CRP subunits. Intra-disulfide bond acts as a conserved switch that controls the structure and functions of CRP. Reduction in the intra-disulfide bond may help in loss of pentameric symmetry [59] and thus the dissociation of pentameric CRP (pCRP). It has been also noted that reduction in the disulfide bridge resulted in a more active mCRP form and Cys play critical role for reduction. It is also found that sequence in the region of the Cys as well plays a significant role in reduction associated functional alterations and also regulated cholesterol binding. The cholesterol-binding sequence is located in the region of the Cys36 residues (for e.g., aa 35–aa 47) and Cys97 residues that mediates mCRP stimulation cells (specially of endothelial cells) through communication with lipid rafts [60, 61]. Unlocking the cholesterol-binding sequence probably dependable for translating reduction-induced structural changes to form significant mCRP [60]. Conversely, highest Ser composition informs us that this residue plays an important role secondary structure formation and to form the ligand-binding site in this protein. In D(1) dopamine receptor agonists, three conserved serine residues play an important role in agonist binding and receptor activation [62].

Figure 1a shows a model for the secondary structure elements: α helices, helix–helix interactions, β sheets, β hairpins, β bulges, β strands, β turns and γ turns. This model shows five β hairpins which are involved in the secondary structure formation. From conserved residue analysis, ranked from least conserved to highest conserved, fourteen residues were found to be highly conserved. Highly conserved residues may play a significant role in the formation of ligand-binding site as well as ligand binding [63]. The conserved domains of CRP have been reported previously [59]. Figure 1b shows the β sheet topology with directions and connectivity of CRP helices and strands. We found that about fifteen β sheets were involved in the formation of β sheet topology. As reported previously, the β sheets are antiparallel [6, 64]. Presently, understanding of the dissociation of pentameric CRP (pCRP) to monomeric CRP (mCRP) is possible therapeutic target for inflammatory reactions. The dissociation process occurs along with the loss of largely β -sheet in secondary structure with relative add to α -helix [65]. Figure 1c shows the hydrogen bond pattern in β sheet topology. One to two intra-hydrogen bonds were found between the β sheet residues. More residues were involved in forming the inter-hydrogen bonds between the β sheets. There may be a possibility that modification of inter-hydrogen bonds between the β sheets may help in dissociation process β -sheet in secondary structure which will help in dissociation of pentameric CRP (pCRP) to monomeric CRP (mCRP). Hydrogen bonds play vital role in the formation of 3D structures and stabilization especially osmolyte-induced protein stabilization [66]. Three-dimensional structures in proteins, hydrogen bonds can be formed among backbone groups or in helices and sheets or among side chains such as serine or threonine O–H groups and carbonyl carbons of side chains ($-\text{C}=\text{O}$). In the case of the β -sheet, the hydrogen bridges are created between the chains. In CRP, hydrogen bonds are necessary to maintain pentameric structure of CRP by joining the neighboring subunits. Hydrogen bonds are also significant for ligand-binding and protomer assembly in all pentraxins [67]. Computational biological analysis and molecular dynamics analyses have suggested that the osmolytes stabilize proteins by modifying the hydrogen bonds in the protein hydration layer [68].

Subunit Assembly, Interactions Between Subunits and Interactions Between Amino Acids

Figure 2a shows interactions across protein–protein interfaces, and Fig. 2b shows a model of the interaction between the subunits. The model shows that the hydrogen bonds and non-bonded contacts are responsible for the interaction. Our model is showing the number of interactions across two interfaces as well as details of the individual residue–residue interactions across these interfaces (Fig. 3). Our work supports the result of Thompson et al. [7] where they found that hydrogen bonds along with van der Waals forces and salt bridges help in the formation of pentameric structure of native CRP. We used 1GNH.pdb file for the crystal structure of CRP which generates two pentamers (ABCDE and FGHIJ) regardless of the software used. The number of residues involved in residue–residue interactions between A–B, B–C, C–D, D–E, F–G, G–H, H–I, I–J, A–E and F–J monomers were 12, 11, 10, 11, 12, 11, 10, 11, 10 and 10, respectively. These protein–protein interactions which stabilize the native structure of proteins appear to be a novel way for transforming the activities of proteins. Specific inhibitors of CRP can be designed based on the protein–protein interaction data [69].

Hydrophobicity

Mapping of the hydrophobic segments is shown in Fig. 4. Analysis of hydrophobic segment distribution reveals deviations in surface hydrophobicity at different cavities present in CRP (Fig. 4a). We also analyzed high-scoring hydrophobic segments (Fig. 4b), hydrophobicity plot of the amino acid sequence (Fig. 4c) and hydrophobic cluster analysis (HCA) (Fig. 4d). The structure showed large hydrophobic and hydrophilic potential patches; however, significant amount hydrophobic patches are present on the surface area. We also detected

numerous small and big hydrophobic patches dispersed on the surface and hydrophobic grooves that might constitute the interaction sites responsible for the stability of CRP. Hydrophobicity plot analysis showed that CRP is hydrophobic in nature and contains segments of low complexity and high-predicted flexibility (Fig. 4c). HCA provided a 2D representation of the sequences where sets of adjacent hydrophobic residues in the patterns were encircled and termed hydrophobic clusters. More than 33 hydrophobic clusters were found in CRP (Fig. 4d).

The packing of residues in the hydrophobic area is essential for the stability and native properties of a protein. Hydrophobic interaction is one of the most important factors for protein folding and stability [70–72]. Hydrophobic effect plays a major task in the stability of native protein structures, and it is well accepted since long. However, experimental studies propose that this explanation is suitable, and theoretical work using model systems and realistic protein structures also confirms such observations. However, the detail is not clear about at the molecular level—how the aqueous solvent contributes to protein structural stability as well as the role and behavior of water near proteins [73]. However, our detected hydrophobic patches of CRP may be the most important balancing factor for the stability of CRP. On the other hand, hydrophobic pocket also plays an important role in the Ca^{2+} -dependent binding of CRP to phosphocholine [6]. In fact, phosphocholine-binding site is containing of two synchronized calcium ions adjoining to a hydrophobic pocket. Phe-66 and Glu-81 are the two major residues for phosphocholine binding. Phe-66 offers hydrophobic interactions with the methyl groups of phosphocholine, while Glu-81 placed on the opposite side of the hydrophobic pocket interacts with the positively charged choline nitrogen [74–76]. It was also noted that for interaction between CRP and FcR, two hydrophobic regions formed by the α helix (Glu 169–Leu 176) and adjacent loop (Gly 177–Asn 186) on the A face of CRP provide the important area of contact between CRP and FcR [77].

Solvent Accessibility

Chain-wise computation of solvent accessibility plot and graphical model of solvent accessibility are shown in Fig. 5. As shown, more than one residue contributes to the hydrophobic or electrostatic charge property of CRP and their distribution pattern. It was noted that the carboxyl-terminal half include two loops that are solvent exposed in crystal structure and varied in conformation [78]. However, the distribution of higher solvent accessible residues also presents a view of distribution of charged, hydrophobic or polar residues in different ranges of solvent accessibility. This will be helpful for further analysis of relative number and nature of contacts in other protein structures.

Charge Distribution

The electrostatic surfaces of CRP were clearly dissimilar, showing large positive and negative potential patches (Fig. S2A). To further quantify the number of positively charged, negatively charged and uncharged residues in a CRP subunit, we analyzed each segment (Fig. S2B). We found that 18 positively charged and 22 negatively charged residues provided the charge stability of CRP. Uncharged residues were more in number than the charged residues. We also analyzed high-scoring positive segments, high-scoring negative charge segments, high-scoring mixed charge segments and high-scoring uncharged segments (Fig. S3). The charge residues are responsible for ligand binding. One model was proposed for C1q binding with CRP. This model proposed mainly positively charged C1q head interacts with the mostly negatively charged central pore of the CRP pentamer [79]. C1q binding is occurred along with by slight conformational modification in the CRP structure [80]. Nevertheless, protein stability involves charge distribution of folding state [75], and the charged segments (both negatively charged and positively charged) provide the charge stability of CRP.

Stabilization Centers and Stabilizing Residues

Results for computing the stabilization centers in CRP are shown in Fig. 6. We found that 33 % of all residues were involved with the stabilization centers. We inferred that these residues might contribute additional stability to CRP. From the prediction of stabilizing residues, we found that seven stabilizing residues were available in CRP (Table 1). Among the stabilizing residues, highest conservation score (9) was observed in Thr34 and Trp162 and their surrounding hydrophobicity (H_p) were 25.57 and 21.04, respectively. Highest surrounding hydrophobicity (H_p) was observed in Ser50 (26.0). However, from predicted stabilizing residues, we assumed that these residues might provide additional stability to CRP.

Computer prediction and modeling of protein structures play a dominant role in stabilizing the native structures [81–83]. Identification of residues which are accountable for the stabilization center especially stabilization of the structure would lead to better understanding of the mechanism of stabilization. This information will also be important for protein engineering and to unfold the mechanism of the structure–function relationships [84].

Conclusions

Stability of proteins is related to the molecular dynamics and interaction properties of the protein [36, 85]. Our data on the molecular dynamics and interaction properties of CRP suggest that CRP is an extremely stable protein in its native form. Our study is an effort to understand the interaction properties and molecular dynamics pattern of CRP. Our computational analysis establishes that CRP has highly diverse structural topology with electrostatic and hydrophobic surfaces. With the understanding of different structural properties of CRP, future research can open up the mechanism of the structure–function relationships of CRP. This study also highlighted a rapid method to calculate the molecular dynamics and interaction properties of CRP. Our prediction of molecular dynamics and interaction properties and models represent a rational molecular platform for initiating in silico drug design studies to the designing of new inhibitors of CRP, to guide site-directed mutagenesis and protein engineering of CRP to design more stable forms of CRP mutants for understanding the structure–function relationships of CRP.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Secondary structure topology, conserved residues, β sheet topology and hydrogen bond pattern of CRP. **a** Secondary structural elements (β sheets, β hairpins, β bulges, β turns, strands, helices, helix-helix interactions, γ turns and conserved residues). Highly conserved residues are marked as (*asterisk*). The amino acid sequence is colored red or blue depending on CATH structural domain 1 or 2, respectively. Catalytic residues are *boxed*. Red dots on top of the residues indicate residues that interact with ligands. **b** Topology diagram illustrating the β strands, represented by the *large arrows*, join up, side-by-side, to shape the domain's central β sheet. The figure also illustrates the relative locations of the α helices (*the red cylinders*). The *small arrows* specify the directionality of the protein chain, from the

N- to the C-terminus. **c** Intra- and inter-hydrogen bond pattern between residues in β sheet topology (Color figure online)

Fig. 2.
3D structural model of CRP containing five subunits and interactions between the subunits.
a 3D structural model which contains five subunits. **b** Schematic diagram showing the interactions between the subunits (Color figure online)

Fig. 3.

A model showing the number of interactions across the interfaces and the individual residue-residue interactions across the interfaces along with involved residues. **a** Interaction between A–B interface **b** Interaction between B–C interface **c** Interaction between C–D interface **d** Interaction between D–E interface **e** Interaction between F–G interface **f** Interaction between G–H interface **g** Interaction between H–I interface **h** Interaction between I–J interface **i** Interaction between A–E interface **j** Interaction between F–J interface (Color figure online)

Fig. 4. Hydrophobic segment analysis of CRP. **a** Hydrophobic segments in the pentameric structure of CRP. Hydrophobic patches are shown separately. Hydrophobic atoms are shown in *gray color* **b** High-scoring hydrophobic segments **c** Hydrophobicity plot of the amino acid sequence **d** Hydrophobic cluster analysis (Color figure online)

Fig. 5. Solvent accessibility of CRP. **a** Relative solvent accessibility plots in the original order of residues **b** Graphical model of solvent accessibility of CRP. *Blue color* represents positively charged residues, *red color* represents negatively charged residues, *green color* represents polar uncharged residues and *yellow/Cys gray color* represents hydrophobic residues (Color figure online)

Fig. 6. Stabilization center prediction of CRP. **a** Stabilization center in a CRP subunit. Location of stabilization center elements in the sequence is marked with *vertical line*, while other positions are marked with *horizontal line* **b** Total number of residues associated with stabilization center (Color figure online)

Table 1

Stabilizing residues of CRP

Residue	Conservation score	Surrounding hydrophobicity (H_p)	LRO
Phe33	6	21.97	0.03883
Thr34	9	25.57	0.04854
Ser50	8	26.00	0.05340
Ile51	6	24.95	0.03883
Phe52	8	25.87	0.02913
Asn160	8	23.63	0.03883
Trp162	9	21.04	0.03398