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ANALYSIS OF C-REACTIVE PROTEIN FORM DIFFERENT SPECIES USING COMPUTATIONAL ANALYSIS AND MOLECULAR DYNAMICS

Biological Science

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ABSTRACT

C-reactive protein, an acute phase protein, is pentameric molecule containing 5 identical subunit of ~25 kDa each in human. C-reactive protein is a reported biomarker of inflammation of human and many other species. Using bioinformatics tools and sequence analysis algorithms, comparative study was done between human and other species for CRP sequences. The sequence homology study with human will help to select the right approach for purification of CRP from other species to make diagnostic reagent. This would also provide the idea of selecting host for antibody generation which is complementary part of antigen in immune assay.

KEYWORDS

CRP, acute phase protein, inflammatory biomarker, CRP purification.

1.INTRODUCTION

C-reactive protein (CRP) is classic acute-phase protein and exquisitely sensitive objective marker of inflammation, tissue damage, and infection in several conditions [1]. C- reactive protein (CRP) was discovered in the serum of patients with acute inflammation that reacted with the C- (capsular) polysaccharide of pneumococcus and hence called CRP [1]. The concentration of CRP in plasma increases during inflammatory states and in several clinical malfunctions including cancer stages. CRP binds to specific molecular configurations in specific pattern that are typically exposed during cell death and found on the surfaces of pathogens. The concentration of CRP drastically elicit within hours after tissue injury or infection in human which suggests that it contributes to defense mechanism of patients, in the innate immune response [2]. A monomeric form of human CRP contains 224 amino acid residues with molar mass of ~25 KDa and its pentameric form contains 5 identical monomeric unit which form annular pentameric disc in shape [3,4]. In its active pentameric conformation, CRP used to bind to phosphocholine (PCh) in a Ca^{2+}-dependent interaction which are present on the surface of damaged cells and microbes in inflammation condition [5].

In this study, we have done the homology study of human CRP with CRP of other domestic animals and experimental animals with the help of bioinformatics analysis. The whole amino acid sequences of CRP from different species were taken for bioinformatics analysis.

2.COLLECTION OF DATA

2.1 Data collection

We have retrieved different sequences of CRP from UniProt and National Center for Biotechnology Information (NCBI) [6]. Protein sequences of CRP in fasta format were collected with an accession number. Other details including length of protein (i.e. amino acid composition), composition of signal sequence and mature peptide, presence of calcium binding domain and position of disulfide bonds are collected from data base.

2.2 Phylogenetic analysis

Phylogenetic analysis was done by Clustal 2.1 tools. Various species for CRP sequence in fasta format was taken as input data. Along with *homo sapiens* sequence (P02741), amino acid sequences of CRP of various domestic pet animals and experimental animals were selected for study. Domestic pet animals included *Canis lupus familiaris* (E2R5J0), *Felis catus* (XP_003999711.3), *Sus scrofa* (O19062), *Bos taurus* (NP_001137569.1), *Equus caballus* (XP_001504452.1), *Ovis aries* (XP_027821246.1), and *Capra hircus* (XP_017901842.1). Experimental animals included *Mus musculus* (P14847), *Rattus norvegicus* (P48199), *Gallus gallus* (*Q2EJU6), Cavia porcellus* (P49254), *Oryctolagus cuniculus* (P02742), *Macaca mulatta* (XP_001117250.2) and *Pan troglodytes* (H2Q0D1).

2.3 Purification approach of CRP using computational analysis

CRP has Ca^{2+}-dependent affinity for phosphocholine (PCh) hence in most of the cases purification of human CRP was attempted using PCh-affinity chromatograph along with ion exchange chromatography and gel filtration chromatography [7]. On the basis of homology study and other molecular properties, same strategy can be adopted for the purification of CRP form different species.

2.4 Antibody development approach for CRP using computational analysis

Serum concentration of CRP is determined as biomarker of inflammation [8]. For the development of immuno assay of CRP, antibodies are required along with CRP antigen. By homology study, we can select the species to get high titer of antibody by immunization of CRP antigen.

3.RESULT

3.1 Result of phylogenetic analysis

"CLUSTAL 2.1 Multiple Sequence Alignments" application tool was used for the phylogenetic analysis. All the species has 224-230 amino acid composition however *Homo sapiens*, *Bos taurus*, *Macaca* mulatta, *Pan troglodytes, Ovis aries, Capra hircus* have 224 amino acid sequence (fig. 1A) with signal sequence at amino terminus. Human CRP has highest sequence homology with *Pan troglodytes* (99.10%) followed by *Macaca mulatta* (91.07) (fig 1B). Since all three species are classified as primates and in evolution, sequence of CRP was almost conserved, so *Macaca mulatta* and *Pan troglodytes* can be used for therapeutic target of CRP inflammation. Although CRP of *Canis lupus familiaris* (dog) have only 57 % of sequence homology with human, however it has similar molecular properties as human including 1 disulfide bond, presence of two Ca^{2+} per molecule. Hence dog has recommended as animal model for study of the mechanism of CRP expression and structure-function relationship.

3.2 Purification approach of CRP using computational analysis

Citizens of European Union and United States are much sensitive for diagnostics of their pets including dog, cat, cow and horse [9]. In case of inflammation condition in these animals, level of CRP is high as same as human [10]. As mentioned CRP has Ca^{2+}-dependent affinity for phosphocholine (PCh) and in homology study with human CRP sequence, homology with cat, dog, cow and horse are 64.57 %, 57.58 %, 68.75 % and 64.12 % respectively. Isoelectric point (pI) of CRP protein from human, dog, cat, cow and horse are 5.45, 5.49, 4.81, 6.43 and 5.75 respectively which are in restricted acidic range. Since all the species including human, dog, cat, cow and horse having Ca^{2+}-dependent affinity for phosphocholine (PCh), PCh column can be used as first step of purification of CRP from these species. Because of homology proportion and restricted acidic pI range of these species, anion exchange column (Diethylaminoethyl cellulose-DEAE and UnoSphere Q at pH 7-9 can be used as polishing step for purification of CRP after PCh column.

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Sequence 1: sp|P02741|CRP_HUMAN          224 aa
Sequence 2: sp|P14847|CRP_MOUSE          225 aa
Sequence 3: sp|P48199|CRP_RAT            230 aa
Sequence 4: sp|P49254|CRP_GUINEA         225 aa
Sequence 5: sp|P02742|CRP_RABIT          225 aa
Sequence 6: sp|O19062|CRP_PIG            222 aa
Sequence 7: tr|E2R5J0|CRP_DOG            248 aa
Sequence 8: NP_001137569.1_Cow           224 aa
Sequence 9: XP_001117250.2_Rhesus Monkey 224 aa
Sequence 10: XP_001504452.1_Horse        223 aa
Sequence 11: sp|H2Q0D1|CRP_ chimpanzee   224 aa
Sequence 12: XP_027821246.1_Sheep        224 aa
Sequence 13: XP_017901842.1_Goat         224 aa
Sequence 14: Q2EJU6_CHICK                227 aa
Sequence 15: XP_003999711.3_Cat          223 aa
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(A)

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Sequences (1:2)  Aligned. Score: 68.75
Sequences (1:3)  Aligned. Score: 63.8393
Sequences (1:4)  Aligned. Score: 72.3214
Sequences (1:5)  Aligned. Score: 73.2143
Sequences (1:6)  Aligned. Score: 64.4144
Sequences (1:7)  Aligned. Score: 57.5893
Sequences (1:8)  Aligned. Score: 68.75
Sequences (1:9)  Aligned. Score: 91.0714
Sequences (1:10) Aligned. Score: 64.1256
Sequences (1:11) Aligned. Score: 99.1071
Sequences (1:12) Aligned. Score: 47.3214
Sequences (1:13) Aligned. Score: 67.8571
Sequences (1:14) Aligned. Score: 45.0893
Sequences (1:15) Aligned. Score: 64.574
```

(B)

Figure 1: Multiple sequence alignment (A) amino acid composition of CRP, (B) Pairwise alignments alignment with Human

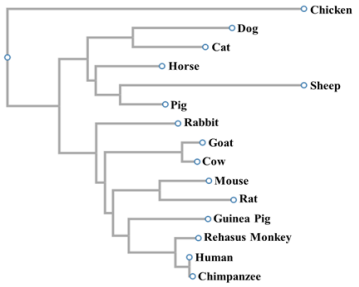

Figure 2: Phylogenetic tree construction of CRP using Clustal 2.1 software.

3.3 Antibody development approach for CRP using computational analysis

For the development of immuno assay of CRP detection, either form of antibody i.e. polyclonal antibody (pAb) or monoclonal antibody (mAb) is required. In case of CRP, polyclonal antibody is preferred to make nephelometry and turbidimetry kit [11]. As phylogenetic study, chicken would be a good option for polyclonal antibody development against human CRP protein, since it is far away from human in phylogenetic tree (fig. 2). However, for pAb generation sheep and goat would be preferred option because of availability and ease of regulations. Since mouse and rabbit are most usable option for mAb development [12] and both species shows enough distance in phylogenetic tree, it would give good titer against human, dog, cat and horse CRP antigens if attempted for development of monoclonal antibody.

4.DISCUSSION

C-reactive protein was the first acute phase protein described and it has still importance as inflammatory marker in human for various clinical conditions including trauma, autoimmune disease, infection necrosis and carcinomas [13-15]. In dog, CRP is increased with turpentine oil injection with 14 days of recovery course, which indicates the direct correlation of CRP concentration with progression of inflammation [16]. CRP concentration is also increased in acute pancreatitis [17], inflammatory bowel disease [18] and gastric mucosal injury [19] in dog. In cat and horse, SAA is the main detectable protein in inflammation [20]. Correlation of CRP level in cat and cow with inflammation and malignancies were not established [21]. In stress condition including transportation, nutritional stress, slippery floors, social isolation, concentration of SAA is increased [22-24]. However CRP concentration is elevated after 12 hr of stress condition and come down to base line by 36 hr which suggest that CRP should also be considered as important biomarker to assess the level of stress in a dairy herd [25]. In adult horse, the serum CRP concentration is increased in 24 hr after inflammatory stimuli, and reached at peak value within 3 to 5 days. After treatment, CRP level in serum decreased to base line concentration in next 2 to 3 weeks. This indicates the direct correlation of CRP concentration with inflammation in adult horse and it is one of the acute-phase reactive protein [26]. So CPR is one of the acute phase protein present in inflammation condition in human as well as in many animal species including dog and horse. By homology study we have concluded that same approach can be used for the purification of CRP from horse and dog to make diagnostic reagent i.e. control and calibrator. This study also provides the information regarding the selection of host for the generation of polyclonal antibody against human, horse and dog. Chimpanzee and Rhesus macaque shows highest homology with human sequence hence these two species can be used to study for therapeutic application against CRP inflammation in human.

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REFERENCES

1. Gewurz H, Mold C, Siegel J, Fiedel B. C-reactive protein and the acute phase response. Adv Intern Med. 1982; 27:345-72.
2. Black S, Kushner I and Samols D. C-reactive Protein. J Bio Chem. 2004; 279 (47), 48487–48490.
3. http://www.genecards.org/cgi-bin/carddisp.pl?gene=CRP
4. Wolford JK, Gruber JD, Ossowski VM, Vozarova B, Antonio Tataranni P, Bogardus C, Hanson RL. A C-reactive protein promoter polymorphism is associated with type 2 diabetes mellitus in Pima Indians. Mol Genet Metab. 2003; 78(2):136-44.
5. Volanakis JE, Kaplan MH. Specificity of C-reactive protein for choline phosphate residues of pneumococcal C-polysaccharide. Proc Soc Exp Biol Med. 1971; 136:612–614.
6. Wheeler D L, Barrett T, Benson DA, Bryant S H, Canese K, Chetvernin V., et al. Database resources of the National Center for Biotechnology Information. Nucleic Acids Res. 2007; 35, D5–D12.
7. Thirumalai A, Singh SK, Hammond DJ Jr, Gang TB, Ngwa DN, Pathak A, Agrawal A. Purification of recombinant C-reactive protein mutants. J Immunol Methods. 2017; 443:26-32.
8. Kushner I. Acute phase proteins. In: BD Rose, editor, UPTODATE. Waltham, MA, 2006.
9. Garofalo L, Mariacher A, Fanelli R, Fico R, Lorenzini R. Hindering the illegal trade in dog and cat furs through a DNA-based protocol for species identification. Peer J. 2018; 6, 1-17.
10. Cray C, Zaias J, Altman NH. Acute Phase Response in Animals: A Review. Comp Med. 2009; 59(6): 517–526.
11. Kaur P, Qureshi I, Khadke P. C - reactive protein: an inflammatory biomarker present in multiple disease pathogenesis. GJRA, 2019; 8(10), 19-22.
12. Weber J., Peng H, Rader C. From rabbit antibody repertoires to rabbit monoclonal antibodies, Exp Mol Med. 2017; 49(3):1-12.
13. Abernethy JT, Avery OT. The occurrence during acute infections of a protein not normally present in the blood. I. Distribution of the reactive protein in patients' sera and the effect of calicum on the flocculation reaction with C polysaccaride of Pneumococcus. J Exp Med. 1941; 73:173–182.
14. Pepys MB, Hirschfield GM. C-reactive protein: a critical update. J Clin Invest. 2003; 111:1805–1812.
15. Tillett WS, Francis T. Serological reactions in pneumonia with a non-protein somatic fraction of Pneumococcus. J Exp Med. 1930; 52:561–571.
16. Hayashi S, Jinbo T, Iguchi K, Shimizu M, Shimada T, Nomura M, Ishida Y, Yamamoto S. A comparison of the concentrations of C-reactive protein and α1-acid glycoprotein in the serum of young and adult dogs with acute inflammation. Vet Res Commun. 2001; 25:117–126.
17. Mansfield CS, James FE, Robertson ID. Development of a clinical severity index for dogs with acute pancreatitis. J Am Vet Med Assoc. 2008; 233:936–944.
18. Jergens AE, Schreiner CA, Frank DE, Niyo Y, Ahrens FE, Eckersall PD, Benson TJ, Evans R. A scoring index for disease activity in canine inflammatory bowel disease. J Vet Intern Med. 2003; 17:291–297.
19. Bayramli G, Ulutas B. Acute phase protein response in dogs with experimentally induced gastric mucosal injury. Vet Clin Pathol. 2008; 37:312–316.
20. Kajikawa T, Furuta A, Onishi T, Tajima T, Sugii S. Changes in concentrations of serum amyloid A protein, α1-acid glycoprotein, haptoglobin, and C-reactive protein in feline sera due to induced inflammation and surgery. Vet Immunol Immunopathol. 1999; 68:91–98.
21. Shida T, Kuribayashi T, Seita T, Maruo T, Yamamoto S. Characterization of C-reactive protein (CRP), α-acid glycoprotein (AAG), Derum amyloid A (SAA) in Dogs and Cat with Malignant cancer. Intern J Appl res Vet Med. 2011; 9 (4), 376-381.
22. Lomborg SR, Nielsen LR, Heegaard PM, Jacobsen S. Acute phase proteins in cattle after exposure to complex stress. Vet Res Commun. 2008; 32:575–582.
23. Arthington JD, Eichert SD, Kunkle WE, Martin FG. Effect of transportation and commingling on the acute-phase protein response, growth, and feed intake of newly weaned beef calves. J Anim Sci. 2003; 81:1120–1125.
24. Saco Y, Fina M, Gimenez M, Pato R, Piedrafita J, Bassols A. Evaluation of serum cortisol, metabolic parameters, acute phase proteins and faecal corticosterone as

indicators of stress in cows. Vet J. 2008; 177:439–441.
25. Lee WC, Hsiao HC, Wu YL, Lin JH, Lee YP, Fung HP, et al. Serum C-reactive protein in dairy herds, Can J Vet Res. 2003; 67(2): 102–107.
26. Yamashita K, Fujinaga T, Okumura M, Takiguchi M, Tsunoda N, Mizuno S. Serum C-reactive protein (CRP) in horses: the effect of aging, sex, delivery and inflammations on its concentration. J Vet Med Sci. 1991; 53(6):1019-24.