Isolation and Purification of C-Reactive Protein From Acute-Phase Human Serum By Lecithin Ligand

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ABSTRACT

C-reactive protein (CRP) was isolated from acute-phase human serum by calcium-dependent lecithin-ligand.

Addition of lecithin to whole serum in the presence of calcium ions resulted in the binding of (CRP) to lecithin with formation of floccules. Subsequent treatment of the $CaCl_2$—distilled water washed lecithin—CRP complexes following suspension in citrate—saline with chloroform resulted in the isolation of essentially purified CRP preparations. The major serum component precipitating with CRP was immunoglobulin M,(IgM).

Purity ranged from 53 to 94% of CRP from original pooled acute phase serum samples. Preparations were obtained following purification on Sephadex G-200 gel filtration chromatography.

Key words: Purification , Albumins , Blood serum and Lecithin

عزل وتنقية بروتين سي التفاعلي من مصل الدم البشري في المرحلة الحادة باستخدام عضيدة الليسيثي

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الخلاصة

تم عزل بروتين سي التفاعلي من مصل الدم البشري في المرحلة الحادة باستخدام عضيدة اللسيثين المعتمدة على ايونات الكالسيوم. إن إضافة الليسيثين إلى مصل الدم بوجود ايونات الكالسيوم يؤدي إلى ارتباط بروتين سي التفاعلي مع اللسيثين ليكون معقد يظهربشكل نتف. بعد ذلك يتم غسل المعقد بمحلول كلوريد الكالسيوم $CaCl^2$ المذاب بالماء المقطر ثم يعلق بدارئ الستراتالملحي مع الكلوروفورم مما أدى إلى عزل بروتين سي التفاعلي المنقى بصورة أساسية.لقد وجد ان البروتين الأكثر ترسبامع بروتين سي التفاعلي في هذه الطريقة هو الامينوكلوبيولين صنف M (IgM) وتراوحت نقاوة بروتين سي التفاعليمابين 53–94% من مجموعه الأصلي في نماذج مصل الدم. ولزيادة التنقية تم تمرير الناتج على عمود من مادة هلام السيفادكس درجة 200.

الكلمات المفتاحية: تنقية ، البومين ، مصل الدم والليستين

Introduction

C-reactive protein (CRP) is a trace protein in the circulation of healthy subjects, with a median concentration of 1 mg/L. As the prototypical member of the acute phase proteins, however, concentration can increase 100-fold or more in response to injury, infection, or inflammation. Acute phase phenomena may also accompany chronic inflammatory disorders. Moderately increased plasma CRP concentrations are found in smokers and under conditions of atherosclerosis, psychological stress, diabetes, and obesity, and in the elderly. (Cao *et al*,2003 ; Danesh *et al* ,2004).

CRP levels in serum or plasma have been used as an indicator of inflammation and infection. Recent evidence also suggests that CRP levels may have value in identifying individuals at greater risk of a heart attack (Ballou and Kushner,1992). CRP the prototypical acute-phase protein, is produced by liver hepatocytes and regulated by cytokines, particularly interleukin-6 (Pepys,1995; Ramadori and Christ,1999) and tumor necrosis factor. (Butterweck, *et al*. 2003).

Circulating concentrations of CRP indicate inflammatory activity, and the recent development of highly sensitive CRP assays (Riafi *et al*. 1999 ; Roberts *et al*. 2000 ; Wilkins *et al*.1998) has led to the discovery that slight increases in CRP (>1–2 mg/L) are indicative of low-grade inflammatory processes that may be related to the pathophysiology of cardiovascular disease. Cogent data suggest that it is produced in the atherosclerotic lesion (especially by smooth muscle cells and macrophages (Jalal *et al*. 2004).

Though the role of CRP in pathological processes was not clear its ability to complex in presence of $Ca^{2+}$to variety ligands can be demonstrated directly by allowing CRP, provided either by whole acute phase human or animal serum, or in isolated purified form, to contact ligands of human, animal, microbial or parasitic origin, or ligands derived by chemical synthesis. Examples of such ligands include native and modified forms of natural or synthetic lipids, ligands include native (De Beer *et al*. 1982),(Pepys *et al*. 1985) and modified plasma lipoproteins, damaged cell membranes. (Narkates and Volanakis 1982) a number of different phospholipids and related compounds (Volanakis and Wirtz 1979), and small nuclear ribonucleoprotein particles. (Du Clos 1989 ; Pepys *et al*. 1994) and lecithin(Yoshitsugi *et al*. 1974).

Although there now are several methods to isolate and purified CRP we have used a procedure utilizing lecithin ligand in the presence of Ca2+ for the isolation of CRP and sephadex G-200 column for the purification from human serum.

The aim of the project

The aim of this project was to isolate and purify CRP as first step for preparing a diagnosis kit for CRP .

Materials and Methods

Source of acute phase sera. Serum samples of individuals with a tuberculosis (TB) were obtain from Abin-Alkhteeb hospital in (Al-Tweatha - Baghdad) Serum samples were pooled and stored in the refrigerator with 0.1% sodium azide (final concentration) added as preservative until ready for fractionation.

Lecithin was used as obtained commercially without further purification. Calcium chloride was obtained from BDH company.

CRP interaction with lecithin and its isolation according to (Yoshitsugi *et al*. 1974) as follow 120 ml of pooled sera was mixed with 3 g of solid lecithin and

stirred with a magnetic stirrer until the appearance of grossly lipemic serum then to this lipemic mixture was added 180 ml of 0.02M CaCl2 in distilled water,pH of 5.0, the total mixture then placed in a dialysis membrane and dialyzed overnight at 4 against 1 liter of o.o2 M CaCl2. The dialysate was changed 3 times during this period . The heavy flocculent precipitate were collected by centrifugation at 2500 rpm for 30 min and the precipitates washed 3 times with 30 ml of cold 0.02 M CaCl2. The precipitate was then suspended in 60 ml of 0.05 M citrate saline pH (7.4). This appeared as lipemic homogeneous suspension. To this was added 120 ml of chloroform and the mixture stirred at room temperature for 1hr. The milky mixture was centrifuged at 2500 rpm for 30 min and the clear aqueous upper layer removed and dialyzed against 3 changes of 3 liter aliquots of 0.02 M CaCl2 at 4c and the precipitate collected by centrifugation at 2500 rpm for 15 min. The precipitate was washed 3 times with 0.02 M CaCl2 and redissolved in 0.05 M citrate -0.15 M NaCl solution. The clear protein solution was examined for presence of CRP.

The solution was passed at 100 ml/h over 2 cm x 85 cm column of sephdex G-200 equilibrated with phosphate buffer saline pH (7.4).

The column was washed with phosphate buffer saline until the A280 of the effluent was zero.

Results and Discussion

Human serum samples with high titers of CRP were found to contain high concentrations of CRP (Pepys, 1995) Serum samples from human with a wide variety of clinical conditions have been mixure appeared homogeneous giving examined in order to gain idea of the CRP response. Results were presented in table 1. in clinically normal man, CRP if present at all in serum was in a concentration less than that clearly detectable(Nilsson and Hanson1962).

The data presented show strong evidence for binding of C-reactive protein to lecithin in presence of Ca^{2+} (Yoshitsugi, *et al*., 1974). Table 2 summarized the percentage of purity of CRP from various pooled serum samples following precipitation with lecithin in presence of Ca^{2+}.The total protein content was measured by A_{280} nm. Active CRP obtained range from 53-92% of purity.

Highly purified CRP can be obtained following sephadex G-200 filtration (Fig.1).

The major co-precipitating protein was IgM and minor proteins undetectable immunologically in other preparations was IgA as detected by radial immunodiffusion. (Fig.2). The occurrence of IgM protein in CRP preparations posses an interesting possibility in that in some of the original pools of acute phase serum, antibodies to phospholipids as found in cells membranes may have been present (Yoshitsugi *et al*., 1974).

We have confirmed that the method was very efficient, readily providing large yields of substantially purified CRP.

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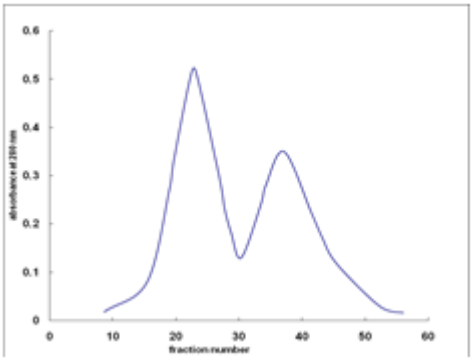

Fig (1) Gel filtration chromogram of human CRP on Sephadex G-200

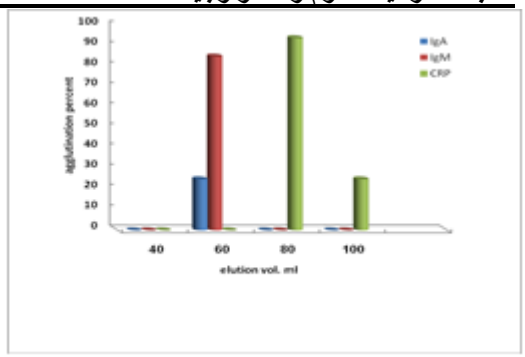

Fig. (2) Radial immunodiffusion and latex- agglutination of the fractions

Table (1) C-reactive Protein Levels in Human Serum

Clinical conditions	No. CRP positive / No. examined	Comment
Serum of healthy peoples	1/30	Weak positive
Serum of patients with variety diseases	20/20	Titer 2 to 256
Serum of patients with TB	20/20	Titer 128 to 512

Table (2) Purity of Human C-reactive Protein from

sample	Vol. ml	Total protein mg	CRP content mg	Purity of CRP %
Pooled serum	120	ND*	64	**ND**
After dialysis	60	60	32	**53**
After gel filtration	25	17	16	**94**

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