Vol. 148, No. 1, 1987 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 14, 1987 Pages 308-313

SERUM AMYLOID P COMPONENT IS THE MAJOR CALCIUM-DEPENDENT SPECIFIC DNA BINDING PROTEIN OF THE SERUM

M.B. Pepys and P.J.G. Butler

MRC Acute Phase Protein Research Group, Immunological Medicine Unit, Department of Medicine, Royal Postgraduate Medical School, London W12 0HS and MRC Laboratory of Molecular Biology, Cambridge CB2 2QH

Received August 31, 1987

SUMMARY: Serum amyloid P component (SAP), a member of the highly conserved pentraxin family of plasma proteins, was found to be the only protein in whole normal or acute phase serum which underwent specific calcium-dependent binding to either single or double-stranded DNA immobilised on gel. Isolated purified SAP also bound to long chromatin, to H1-stripped chromatin and to native DNA in solution at physiological ionic strength. Pure SAP which had been immobilised on gel, specifically bound nucleosome core particles from solution. These observations strongly suggest that SAP may bind to extracellular chromatin and DNA in vivo and that this may be its physiological role.

Chromatin and DNA from the nuclei of cells dying in vivo or nuclear material shed during normal maturation of erythrocyte precursors are presumably digested within phagocytic cells but it is not known how they are handled before phagocytosis occurs. The mechanisms involved are important in view of the potential biological activity of the genetic material. Furthermore, in certain autoallergic diseases, particularly systemic lupus erythematosus, autoantibodies to DNA and other nuclear constituents are produced and are responsible for the pathogenesis of serious inflammatory tissue damage (1).

The pentraxins are a unique family of pentameric plasma proteins, which in man comprise C-reactive protein (CRP), the classical acute phase protein, and serum amyloid P component (SAP), an almost universal constituent of amyloid deposits (2). They have been very stably conserved with respect to both structure and calcium-dependent ligand binding specificity throughout vertebrate evolution (3). A homologue of vertebrate CRP also exists in the arachnid, Limulus polyphemus, the horseshoe crab (4).

SAP is a normal plasma glycoprotein of unknown function which circulates at a concentration of around 20-40 mg/l, and is not a

0006-291X/87 $1.50

significant acute phase reactant in most species (2). It is a non-fibrillar constituent of amyloid deposits and localises there due to its calcium-dependent binding specificity for ligands exposed in all different types of amyloid fibrils (5). However, it is not likely that SAP has been conserved solely to participate in the pathological process of amyloidosis and the identity of physiological ligands is therefore of interest.

MATERIALS AND METHODS

Binding of SAP from whole serum to immobilised DNA. Denatured single stranded calf thymus DNA (Sigma) was immobilised by trapping in agarose gel (6) and by coupling to CNBr-Sepharose (7), producing gels containing 3.9 mg DNA/ml and 0.47 mg DNA/ml respectively. The gels were thoroughly washed to remove any DNA not firmly attached and were then equilibrated with TC buffer (10 mM Tris, 140 mM NaCl, 2 mM Ca^{++}, pH 8.0). The volumes of gel shown in Table I were mixed with either pooled normal human serum or acute phase human serum and incubated with continuous mixing for 30 min at 21°C. Gels prepared and treated identically except for omission of the DNA were used as controls. After centrifugation the supernatants were removed, the beads were thoroughly washed with TC and calcium-dependently bound protein was then eluted with 3 washes of TE buffer (10 mM Tris, 10 mM EDTA, 140 mM NaCl, pH 8.0), each equal in volume to the volume of the beads. The three eluates from each tube were pooled and the concentrations of SAP and CRP in the sera, the supernatants and the eluates were measured by electroimmuno-assay (8). Minor binding of SAP to control beads was due to the known calcium-dependent affinity of SAP for agarose (9).

Binding of nucleosome core particles by immobilised pentraxins. Pentraxins, in isolated pure form (10,11), were coupled at 1 mg/ml of gel to CNBr-activated Sepharose 4B (Pharmacia). Control beads from the same lot were also treated identically, residual active groups being blocked with ethanolamine, but without any protein being coupled. The gels were all equilibrated with TC buffer at pH 8.0 containing 10 mM Tris, 2 mM Ca^{++} and either 20 mM NaCl or 140 ml NaCl to provide final ionic strengths of 34 and 154 mM respectively. 1 ml volumes of packed beads bearing each protein and of control beads were incubated for 30 min at 21°C with 1 ml volumes of chicken erythrocyte nucleosome core particles (12) in solution in the same TC buffer as the beads. The mixtures were then centrifuged, the supernatant removed and the beads washed twice with 2 ml volumes of buffer at the appropriate concentration; the washes were retained. Cores bound to the beads in a calcium-dependent fashion were then eluted by washing twice with 1.1 ml volumes of TE buffer (10 mM Tris, 140 mM NaCl, 10 mM EDTA, pH 8.0). The concentrations of cores offered and in the supernatant, washes and eluates were estimated by measuring the A_{260} of suitable dilutions in 0.1 M NaOH, and using a value for $E^{0.1\%}_{1\ cm} = 10$.

RESULTS

Calcium-dependent binding of SAP to DNA and chromatin. SAP present either in normal serum or in acute phase serum, containing

Table I. Calcium-dependent binding of SAP from human serum to immobilised DNA

Expt. 1	Beads (1.0 ml)	Normal Serum (ml)	SAP offered (μg)	SAP bound (μg)
DNA-Sepharose	(0.47 mg DNA)	10	180	108
Control Sepharose		10	180	9
DNA-Sepharose	(0.47 mg DNA)	50	900	207
Control Sepharose		50	900	18

Expt. 2	Beads (0.1 ml)	Acute phase serum (ml)	SAP offered (μg)	SAP bound (μg)	CRP offered (μg)	CRP bound (μg)
DNA-agarose	(0.39 mg DNA)	5	230	56	850	0
Control agarose		5	230	19	850	0

Results comparable to those above were also obtained in experiments with native, double-stranded calf thymus DNA adsorbed onto cellulose (Pharmacia).

high levels of CRP, bound specifically in a calcium-dependent fashion to both native and denatured DNA immobilised on cellulose (13), in agarose gel, or covalently coupled to Sepharose (Table I, Fig. 1). This binding took place from the physiological milieu of undiluted serum at physiological ionic strength. Furthermore, SAP was the only serum protein which became bound and was then eluted in significant quantities from DNA beads compared to

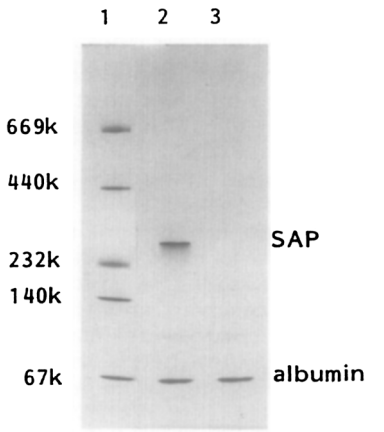

Figure 1. Gradient (4-30%) polyacrylamide gel electrophoresis (Pharmacia) of proteins eluted with EDTA after incubation of DNA-Sepharose and control Sepharose beads with normal human serum. Samples were run without denaturation. Lane 1, marker proteins of known molecular weight as shown; lane 2, TE eluate from DNA-Sepharose after incubation with normal human serum; lane 3, TE eluate from control Sepharose after incubation with normal human serum. The protein band in lane 3 is albumin, a carry over from the serum where it is present at 40 mg/ml, compared with the SAP concentration of 18 μg/ml.

Table II. Calcium-dependent binding of nucleosome core particles by immobilised pentraxins

Immobilised protein	Ionic strength of TC buffer (mM)	Cores offered (μg)	Cores not bound (μg)	Cores eluted with TE (μg)	Total Cores recovered (μg)
Human SAP	154	205	158	45	203
Human CRP	154	205	197	3	200
Limulus CRP	154	205	201	3	204
None	154	205	200	0	200
Human SAP	34	194	52	132	184
Human CRP	34	194	173	17	190
Limulus CRP	34	194	192	0	192
None	34	194	185	0	186

control beads lacking DNA (Fig. 1). In most experiments no specific binding of CRP from acute phase serum to DNA was detected, despite the quantity of CRP offered having been up to 5 fold greater than that of SAP by weight and 10 fold by molarity, but occasionally trace binding did occur.

When the same experiments were repeated using sera of various animals other than man, specific calcium-dependent binding of SAP to DNA, comparable to that of human SAP, was detected in all species tested: mouse, rat, guinea pig, cow and plaice (Pleuronectes platessa L.). In contrast, CRP from the rat, rabbit, plaice and Limulus, as well as hamster female protein, did not bind to DNA, thus resembling the behaviour of human CRP.

When isolated, purified human CRP or SAP were incubated in solution at physiological ionic strength (154 mM) with native long chromatin, with H1-stripped chromatin or with native DNA (prepared from chicken erythrocytes (12,14), the SAP showed major calcium-dependent binding to all these ligands whereas CRP bound only in trace amounts.

Calcium-dependent binding of nucleosome core particles by immobilised SAP. The markedly different chromatin binding by human SAP and CRP in whole serum was confirmed in experiments in which the pentraxins were immobilised on Sepharose and tested for their ability to bind core particles in solution (Table 2). Human SAP clearly bound a substantial quantity of cores whilst both human CRP and Limulus CRP, bound only trace amounts. Binding by human proteins was enhanced at low ionic strength but the activity of CRP remained very small compared to that of SAP.

DISCUSSION

We have shown here that SAP is the single major calcium-dependent, DNA binding protein in whole normal serum or in acute phase serum, that the reaction with DNA and with chromatin occurs in a physiological milieu and that it is conserved across species. These observations argue strongly in favour of the SAP-chromatin and SAP-DNA interactions being an important biological phenomenon.

In contrast CRP in whole acute phase serum did not bind to immobilised DNA, isolated CRP did not bind significantly to soluble chromatin or DNA, and nucleosome core particles in solution at physiological ionic strength were not bound by immobilised CRP. However, isolated human CRP (results not shown) and rabbit CRP (R. Buck, R. Thompson and J.O. Thomas, personal communication) co-precipitated with core particles in solution at low, subphysiological ionic strength in the presence of 2 mM Ca^{++}. This may explain, in part, the previous report of an avid interaction between CRP and chromatin (15) which we failed to confirm here.

Experiments currently in progress indicate that there is a stoiochiometric relationship in the binding of human SAP to core particles in solution and these studies, together with the resolution of the crystal structure of SAP which is also in progress, (16,17) should help to elucidate the molecular basis of the SAP-chromatin interaction. Conserved amino acid sequences in the pentraxins, which are likely to participate directly in ligand binding, have recently been identified by our group (18,19). The sequences include residue differences which may be responsible for varying ligand specificities between SAP and CRP. Furthermore, and of particular interest in view of the present experimental observations of DNA binding by SAP, these same sequences show hitherto unrecognised homology with histones H1 and H4 (18,19). Meanwhile, whole animal and cell culture experiments are in progress to establish the functional effects of the binding of SAP in terms of in vivo handling of DNA and chromatin.

ACKNOWLEDGEMENTS

This work was supported in part by MRC Programme Grant G979/51 to M.B.P. We thank Glenys Tennent, Ruth Gallimore and Dr Iffat Sardharwalla for assistance with experiments and Beth Sontrop for expert secretarial assistance.

REFERENCES

1. Hughes, G.R.V. (Ed.) (1982) Systemic lupus erythematosus, Clin. Rheum. Dis. W.B. Saunders, London.
2. Pepys, M.B. and Baltz, M.L. (1983) Adv. Immunol. 34, 141-212.
3. Baltz, M.L., de Beer, F.C., Feinstein, A., Munn, E.A., Milstein, C.P., Fletcher, T.C., March, J.F., Taylor J., Bruton, C., Clamp, J.R., Davies, A.J.S. and Pepys, M.B. (1982) Ann. N.Y. Acad. Sci. 389, 49-75.
4. Nguyen, N.Y., Suzuki, A., Boykins, R.A. and Liu, T.-Y. (1986) J. Biol. Chem. 261, 10456-10465.
5. Baltz, M.L., Caspi, D., Evans, D.J., Rowe, I.F., Hind, C.R.K., and Pepys, M.B. (1986) Clin. Exp. Immunol. 66, 691-700.
6. Schaller, H., Nusslein, C., Bonhoeffer, F.J., Kurtz, C. and Nietzschmann, I. (1972) Eur. J. Biochem. 26, 474-481.
7. Poonian, M.S., Schlabach, A.J. and Weissbach, A. (1971) Biochemistry 10, 424-427.
8. Pepys, M.B., Dash, A.C., Markham, R.E., Thomas, H.C., Williams, B.D. and Petrie, A. (1978) Clin. Exp. Immunol. 32, 119-124.
9. Pepys, M.B., Dash, A.C., Munn, E.A., Feinstein, A., Skinner, M., Cohen, A.S., Gewurz, H., Osmand, A.P., and Painter, R.H. (1977) Lancet i, 1029-1031.
10. de Beer, F.C. and Pepys, M.B. (1982) J. Immunol. Methods 50, 17-31.
11. Robey, F.A. and Liu, T.-Y. (1981) J. Biol. Chem. 256, 969-975.
12. Lutter, L.C. (1978) J. Mol. Biol. 124, 391-420.
13. Alberts, B. and Herrick, G. (1971) Methods Enzymol. 21D, 198-217.
14. Bates, D.L., Butler, P.J.G., Pearson, E.L. and Thomas, J.O. (1981) Eur. J. Biochem. 119, 469-476.
15. Robey, F.A., Jones, K.D., Tanaka, T. and Liu, T.-Y. (1984) J. Biol. Chem. 259, 7311-3716.
16. Oliva, G., O'Hara, B.P., Wood, S., Pepys, M.B. and Blundell, T. (1986) In Protides of the Biological Fluids Colloquium XXXIV (H. Peeters, Ed.), pp. 371-374. Pergamon Press, Oxford.
17. Oliva, G., O'Hara, B.P., Wood, S., Pepys, M.B. and Blundell, T.L. (1987) J. Mol. Biol. (submitted for publication).
18. Turnell, W.G. (1987) J. Mol. Biol. (submitted for publication).
19. Travers, A.A. and Turnell, W.G. (1987) J. Mol. Biol. (submitted for publication).