Biochem. J. (1984) **221**, 903–906
Printed in Great Britain

Amino acid sequence homology between rat and human C-reactive protein

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(*Received 7 February 1984/Accepted 1 June 1984*)

The rat serum protein that undergoes Ca^{2+}-dependent binding to pneumococcal C-polysaccharide and to phosphocholine residues, and that is evidently a member of the pentraxin family of proteins by virtue of its appearance under the electron microscope, has been variously designated as rat C-reactive protein (CRP) [de Beer, Baltz, Munn, Feinstein, Taylor, Bruton, Clamp & Pepys (1982) *Immunology* **45**, 55–70], 'phosphoryl choline-binding protein' [Nagpurkar & Mookerjea (1981) *J. Biol. Chem.* **256**, 7440–7448] and rat serum amyloid P component (SAP) [Pontet, D'Asnieres, Gache, Escaig & Engler (1981) *Biochim. Biophys. Acta* **671**, 202–210]. The partial amino acid sequence (45 residues) towards the *C*-terminus of this protein was determined, and it showed 71.7% identity with the known sequence of human CRP but only 54.3% identity with human SAP. Since human CRP and SAP are themselves approximately 50% homologous, the level of identity between the rat protein and human SAP is evidence only of membership of the pentraxin family. In contrast, the much greater resemblance to human CRP confirms that the rat C-polysaccharide-binding/phosphocholine-binding protein is in fact rat CRP.

The pentraxins are a family of plasma proteins composed of polypeptide subunits arranged with cyclic pentameric symmetry in a disc-like configuration (Osmand *et al.*, 1977; Pepys & Baltz, 1983). The two pentraxins known in man are C-reactive protein (CRP) and serum amyloid P component (SAP). Despite a number of differences, including complete lack of antigenic cross-reactivity (Baltz *et al.*, 1982), they share 50% strict residue-for-residue identity of amino acid sequence (Oliveira *et al.*, 1979; Anderson & Mole, 1982) and the capacity for Ca^{2+}-dependent ligand binding, albeit to different ligands (Pepys *et al.*, 1977*a*). CRP binds best to phosphocholine residues (Volanakis & Kaplan, 1971), for example in pneumococcal C-polysaccharide (CPS), whereas SAP specifically recognizes the pyruvate acetal of galactose as it exists in agarose (Pepys *et al.*, 1977*b*; Hind *et al.*, 1984). On the basis of these binding reactivities, it has been possible to separate CRP-like and SAP-like pentraxins from serum of many species (Pepys *et al.*, 1978; Baltz *et al.*, 1982; Pepys & Baltz, 1983). The available sequences of the phosphocholine-binding proteins generally resemble human CRP more closely, whereas those of agarose-binding proteins are generally closer to human SAP (Pepys *et al.*, 1982; Taylor, 1983).

We have previously described the isolation of two distinct pentraxins in the rat, one binding to CPS and the other to agarose but not CPS, and on the basis of these specificities we designated the former as rat CRP and the latter as rat SAP (de Beer *et al.*, 1982). Nagpurkar & Mookerjea (1981) independently isolated a rat serum protein that they called 'phosphoryl choline-binding protein (PCBP)', and Pontet *et al.* (1982) isolated a phosphocholine-binding pentraxin from rat serum that they called rat 'SAP'. We report here below that the proteins isolated in these three laboratories are immunochemically identical and share the same polypeptide chain composition, and furthermore that the amino acid sequence of a 45-residue portion of our rat CRP shows about 70% identity with human CRP.

Abbreviations used: CRP, C-reactive protein; SAP, serum amyloid P component; CPS, pneumococcal C-polysaccharide.

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Materials and methods

Rat CRP was isolated in pure form from normal rat serum by Ca^{2+}-dependent affinity chromatography on CPS–Sepharose, solid-phase immunoabsorption with anti-(rat SAP) antibody and gel filtration as previously described (de Beer *et al.*, 1982). The protein was reduced and carboxymethylated before digestion of 2.5 mg dissolved at 1 mg/ml in 70% (w/v) formic acid with a 100-fold molar excess of CNBr over the expected number of methionine residues. After 24h at room temperature the products of digestion were separated on four I-125 columns, each 0.78cm internal diam. × 30cm long (Waters Associates), connected in series to a model 440 high-pressure-liquid-chromatography system containing a U6K injection system (Waters Associates). The eluent was 15% (v/v) propan-1-ol in 20% (v/v) acetic acid (Anderson & Mole, 1982). The effluent was monitored at 280 nm, and eluted peptides were manually collected in accordance with the absorbance profile. Two fractions apparently containing single fragments, as shown by sodium dodecyl sulphate/5–20%-(w/v)-gradient-polyacrylamide-gel electrophoresis, were designated A and B and were sequenced by using a Beckman model 890C automated Sequenator equipped with a cold-trap. The 0.1M-Quadrol programme (Beckman no. 121078) as modified by J. E. M. was used and the phenylthiohydantoin derivatives were identified by high-pressure-liquid-chromatography methods as previously described (Anderson & Mole, 1982).

Samples of rat phosphocholine-binding protein and antiserum thereto were kindly provided by Professor S. Mookerjea and samples of so-called 'rat SAP' and antiserum to it were kindly provided by Dr. M. Pontet. These proteins were compared with our own preparations of rat CRP in sodium dodecyl sulphate/polyacrylamide-gel electrophoresis (Laemmli, 1970).

Results

The samples of 'rat PCBP' (Mookerjea) and 'rat SAP' (Pontet) gave profiles identical with that of our own rat CRP in sodium dodecyl sulphate/polyacrylamide-gel electrophoresis, as reported previously (de Beer *et al.*, 1982). Antisera from the three laboratories gave immunoprecipitation lines of complete identity with each other against whole rat serum and against each of the isolated proteins.

The Sequenator yields obtained with fractions A and B from CNBr-cleaved rat CRP are shown in Table 1. Although only a single band was observed when fraction A was run in sodium dodecyl sulphate/polyacrylamide-gel electrophoresis, it clearly contained two peptides, indicated by the presence of two phenylthiohydantoin derivatives per Sequenator cycle. There is considerable over-

Table 1. *Sequenator yields of CNBr-cleavage fragments of rat CRP*

Amino acid residues are indicated in terms of the one-letter notation [see *Biochem. J.* (1984) **219**, 367]; X, unidentified residue; N, sum of values for aspartic acid and asparagine; Q, sum of values for glutamic acid and glutamine.

	Fraction A							Fraction B					
Cycle			Recovery (nmol)	Cycle			Recovery (nmol)	Cycle		Recovery (nmol)	Cycle		Recovery (nmol)
1	V,	W	(5.9), (2.3)	21	F,	P	(2.0), (2.2)	1	W	(9.7)	21	P	(1.6)
2	F,	D	(3.3), (1.4)	22	I,	N	(1.6), (0.5)	2	D	(4.3)	22	N	(2.3)
3	S,	F	(0.2), (1.4)	23	K,	V	(2.5), (0.9)	3	F	(5.3)	23	V	(2.4)
4	P,	V	(2.6), (1.7)	24	P,	L	(1.5), (0.6)	4	V	(5.9)	24	L	(2.6)
5	N,	L	(2.5), (1.5)	25	Q,	N	(0.8), (0.4)	5	L	(6.1)	25	N	(2.2)
6	V,	S	(3.9), (0.1)	26	L,	(W)	(1.1), (–)	6	–	(–)	26	W	(3.8)
7	L,	P	(3.4), (1.2)	27	(W),	R*	(0.8)	7	P	(6.1)	27	R*	
8	N,	E	(2.3), (1.2)	28	P,	A	(0.9), (0.5)	8	E	(3.3)	28	A	(1.6)
9	(W),	Q	(4.2), (0.7)	29		L	(1.1)	9	Q	(5.2)	29	L	(2.5)
10	R,*	I	(1.2)	30		F	(0.7)	10	I	(6.5)	30	F	(3.0)
11	A,	N	(2.7), (0.6)	31		Y	(0.4)	11	N	(2.9)	31	Y	(1.4)
12	L,	A	(2.2), (1.5)					12	A	(3.2)	32	E	(2.4)
13	(F),	V	(2.7), (1.1)					13	V	(2.8)	33	T	(0.5)
14	Y,	X	(2.9), ( )					14	Y	(2.2)	34	H*	
15	E,	V	(2.2), (1.1)					15	V	(3.2)	35	G	(2.4)
16	T,	G	(0.4), (1.0)					16	G	(1.9)	36	D	(1.0)
17	H,*	R*						17	R*		37	V	(2.4)
18	G,	V	(1.8), (1.1)					18	V	(5.5)	38	F	(1.4)
19	D,	(F)	(0.5), (0.9)					19	F	(5.5)	39	I	(1.9)
20	V,	S	(1.9), (0.1)					20	S	(0.02)	40	K	(1.7)

* Qualitative identification on high-pressure liquid chromatography.

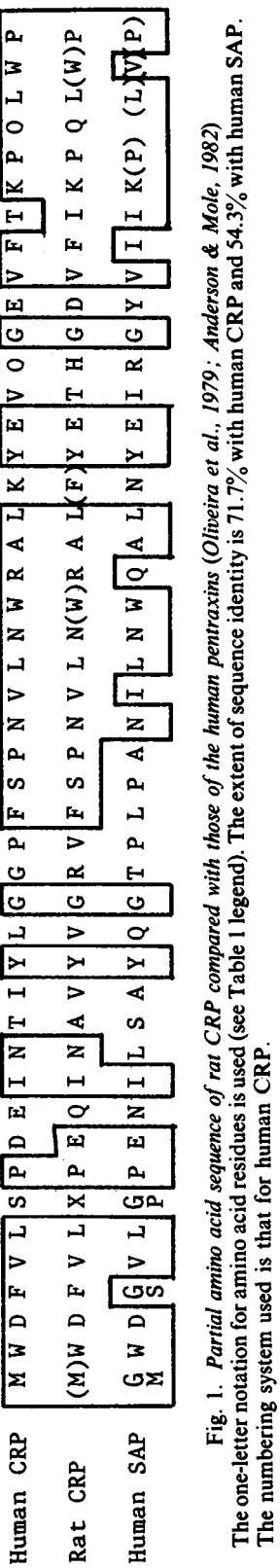

Fig. 1. *Partial amino acid sequence of rat CRP compared with those of the human pentraxins (Oliveira et al., 1979; Anderson & Mole, 1982)*
The one-letter notation for amino acid residues is used (see Table 1 legend). The extent of sequence identity is 71.7% with human CRP and 54.3% with human SAP. The numbering system used is that for human CRP.

lap between these two sequences, enabling a continuous sequence of 45 residues to be assigned. The amino acid sequence obtained from fraction B confirms this assignment, and the sequence obtained is aligned in Fig. 1 with the known sequences of the *C*-terminal regions of human CRP (Oliveira *et al.*, 1979) and SAP (Anderson & Mole, 1982). The alignment demonstrates that the available sequence of the protein that we have called rat CRP has 71.7% identity with human CRP and only 54.3% identity with human SAP.

Discussion

In our earlier work (de Beer *et al.*, 1982) we isolated and characterized two distinct, immunologically non-cross-reactive, pentraxins from rat serum. One underwent Ca^{2+}-dependent binding to CPS and was therefore designated as CRP. It also bound to a lesser extent to agarose, albeit more than the weak agarose-binding previously reported for human CRP (Pepys *et al.*, 1977*a*). Unlike human CRP, the rat protein did not precipitate CPS or agglutinate CPS-coated erythrocytes, it was glycosylated and its normal serum concentration (about 300mg/l) was over 300 times greater than that of human CRP. Nevertheless its amino acid composition was remarkably similar to that of human CRP. The other rat pentraxin bound only to agarose and was therefore designated as SAP. In view of our experience with pentraxins in many species and the appreciable differences that exist between them, despite their sharing of major amino acid sequence homology and Ca^{2+}-dependent ligand specificity, we considered that the nomenclature that we adopted for the two distinct different rat pentraxins correctly represented homology with their human counterparts.

On the other hand, Nagpurkar & Mookerjea (1981) considered whether their so-called 'phosphoryl choline-binding protein (PCBP)' might be CRP, but rejected this because they failed to demonstrate the Ca^{2+}-dependence of its binding, because they detected no immunochemical cross-reactivity with human CRP and because they could not isolate a comparable protein from normal human serum. The latter is not surprising, since the concentration of CRP in normal human serum is less than 1mg/ml, and immunochemical cross-reactivity among the pentraxins of different species is exceptional and is seen only with a small minority of antisera. In subsequent publications they have reported on the Ca^{2+}-dependence of binding, but, ignoring the pentraxin appearance under the electron microscope, have continued to distinguish their 'PCBP' from CRP of other species (Nagpurkar *et al.*, 1983; Connelly *et al.*, 1983).

The designation by Pontet *et al.* (1982) of the rat pentraxin that they isolated as SAP was remarkable, considering that they showed clearly its phosphocholine-binding property, the primary identifying characteristic of a CRP. Furthermore, they did not report any attempt to isolate an agarose-binding but non-phosphocholine-binding pentraxin, in other words a true SAP counterpart.

We show here that the proteins and their respective antisera obtained from the laboratories of Mookerjea and Pontet are identical with our own, and all three groups are therefore clearly dealing with the same protein. Identification of this molecule as rat CRP is now confirmed by the very much greater degree of amino acid sequence homology with human CRP than with human SAP.

This work was supported in part by Medical Research Council Programme Grant G979/51 to M. B. P. We thank Professor S. Mookerjea and Dr. M. Pontet for kindly exchanging samples of their proteins and antisera for comparison.

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