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Rat serum amyloid P component

Analysis of cDNA sequence and gene expression

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cDNA clones for rat serum amyloid P component (SAP) were isolated, and the derived amino acid sequence for pre-SAP was determined from the complete nucleotide sequence. Rat SAP is encoded by ~ 1 kb of mRNA, and the mature SAP protein is predicted to be 208 amino acids long. An increase in hepatic mRNA levels for rat SAP was found after administration of lipopolysaccharide, and SAP mRNA levels in livers of unstimulated male rats were lower than in hepatic RNA from female rats.

INTRODUCTION

Serum amyloid P component (SAP) is a pentraxin, so named because of the discoid pentagonal conformation assumed in the circulation. This pentameric symmetry is shared with another circulating pentraxin, C-reactive protein (CRP). Pentraxins are synthesized principally in hepatocytes, and the serum concentrations of SAP and CRP differentially alter during the course of systemic inflammation in many animals (Pepys *et al.*, 1982). In man, CRP is a major acute-phase plasma protein, with marked increases in its serum concentration documented within hours of the onset of tissue injury or necrosis (Kushner *et al.*, 1978). However, the serum concentration of SAP in man does not alter significantly during the course of inflammation. Although human SAP and CRP share the basic discoid arrangement of monomeric subunits, several differences, including ligand-binding specificities and glycosylation patterns, have been documented (Pepys *et al.*, 1982). In addition, human SAP is found in normal glomerular basement membranes and in the microfibrillar areas of elastic tissue surrounding blood vessels (Dyck *et al.*, 1980; Breathnach *et al.*, 1981).

Amyloid may be deposited in many organs and is composed of fibrillary elements with characteristic staining properties. Amyloid fibrils may be derived from immunoglobulin light chains, calcitonin-like peptides, pre-albumin variants, β_2-microglobulin or serum amyloid A (SAA) (Husby & Sletten, 1986). SAP is immunochemically detectable in amyloid deposits involving most of these fibril precursors (Pepys *et al.*, 1982). SAA is a major acute-phase reactant in many species, and cleavage of a *C*-terminal peptide of SAA may result in deposition of amyloid A fibrils in reactive amyloidosis arising during states of chronic inflammation. Intra- and inter-specific variability in susceptibility to reactive amyloidosis has been described.

Reactive amyloidosis occurs very infrequently in rats, and SAA is not found in the high-density lipoprotein fraction of rat serum (Baltz *et al.*, 1987). SAP, however, has been purified from rat serum, and the approximate molecular mass of monomeric rat SAP is 24.5 kDa. Ultrastructural differences between rat SAP and SAP moieties from other amyloid-prone species have been noted and may also be a factor in the resistance of rats to formation of amyloid (de Beer *et al.*, 1982). In order to permit a comparison of rat SAP with similar proteins in other species, the current study describes the derived primary structure for rat SAP and compares these data with sequences from other species. In addition, the hepatic expression of rat SAP mRNA has been studied.

MATERIALS AND METHODS

Isolation and characterization of rat SAP cDNA clones

Recombinant plaques (1×10^6) of a Sprague–Dawley rat hepatic cDNA library (λZAPII), generously provided by Dr. Jonathan Gitlin (Department of Pediatrics, Washington University School of Medicine, St Louis, MO, U.S.A.), were screened on nitrocellulose membranes using a [α-^{32}P]dCTP-labelled Syrian-hamster female-protein cDNA probe, pFP1 (Dowton *et al.*, 1985). This probe reflects the coding sequence for hamster female protein, which is now known to be SAP in that species. The probe was labelled by nick-translation to a specific radioactivity of 1×10^8 c.p.m./μg, and filter preparation, pre-hybridization and hybridization was performed as described by Sambrook *et al.* (1989). Post-hybridization washing was performed for 30 min at 55 °C in 15 mM-NaCl/1.5 mM-sodium citrate/0.1 % SDS. Positive hybridization signals were identified by autoradiography using Kodak XAR-5 film at −70 °C for 24 h. Ten positive clones were identified.

After plaque purification, phagemids containing rat SAP cDNA inserts were released by auto-excision using helper phage R408, and the plasmid DNA was isolated (Clewell & Helsinki, 1969). Insert size was determined by agarose-gel electrophoresis of the plasmids after endonuclease digestion. Two clones, prSAP1 and prSAP8, were selected for further studies.

Complete nucleotide sequences for inserts of prSAP1 and prSAP8 were determined on both strands using the dideoxynucleotide-chain-termination method and Sequenase (Sanger *et al.*, 1977). DNA sequence analyses were assisted by the Microgenie program (Beckman Instruments).

Analysis of expression of rat SAP mRNA

For studies of SAP-gene expression in non-stimulated rats, RNA was isolated from seven female and seven male animals.

Abbreviations used: SAP, serum amyloid P component; CRP, C-reactive protein; SAA, serum amyloid A; LPS, lipopolysaccharide.

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The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases.

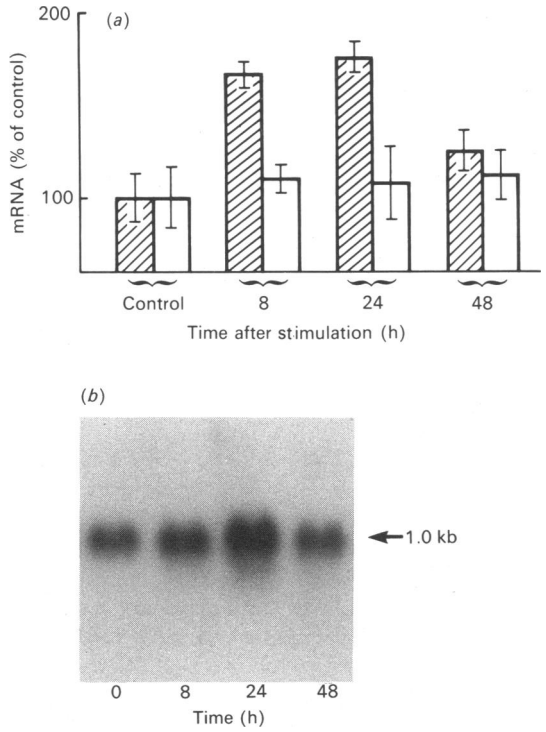

Fig. 1. (*a*) Expression of rat SAP (▨) and actin (□) mRNA in liver during inflammation and (*b*) representative autoradiogram of RNA blot hybridized with radiolabelled prSAP1 demonstrating 1 kb mRNA

Hepatic RNA was isolated from four adult female rats at the following time points after stimulation with lipopolysaccharide: 0, 8, 24 and 48 h. Results are displayed as a percentage of the control signal determined by densitometry of the autoradiograph. (*b*) RNA samples are one set from the experiment shown in (*a*).

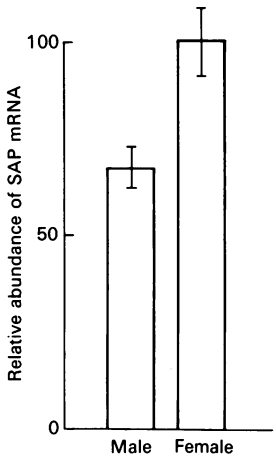

Fig. 2. Gender difference in expression of rat SAP mRNA

Hepatic RNA was isolated from seven male and seven female rats, and for each sample three separate aliquots (5, 10 and 15 μg) were subjected to RNA blot analysis. Results are displayed as a percentage of the maximal signal, determined by densitometry of autoradiographs.

Littermates were used, and animals were individually caged for 96 h before they were killed. Hepatic RNA was also isolated from rats at various time points after induction of an acute-phase response by administration of 200 μg of lipopolysaccharide (LPS; from *Escherichia coli* serotype 0111:B4)/kg. Four female rats were used for each time point.

Total hepatic cellular RNA was isolated by guanidinium isothiocyanate extraction and ultracentrifugation over a 5.7 M-CsCl cushion in an SW50.1 rotor. RNA analyses were performed by blot hybridization of rat RNA after agarose/formaldehyde-gel electrophoresis and transfer to a nylon membrane (Sambrook *et al.*, 1989). Pre-hybridization and hybridization with linearized prSAP1 plasmid, radiolabelled by nick-translation, were performed for 12 h each at 65 °C in 0.75 M-NaCl/0.075 M-sodium citrate/5 × Denhardt's solution/0.1 % pyrophosphate/denatured salmon sperm DNA (100 μg/ml). Hybridizing signals were revealed by autoradiography. The uniformity of RNA application to the gel was checked by observing the ethidium bromide-stained RNA gel under u.v. light. In addition, to study the effect of LPS administration on mRNA populations in general, the RNA blots were stripped and rehybridized with a cross-reacting actin cDNA probe, pAC269 (Schwartz *et al.*, 1980). Data for expression of SAP and actin mRNA in rat livers after administration of LPS is shown in Fig. 1.

For analysis of SAP induction after LPS administration ($n = 4$) and comparisons of relative amounts of SAP mRNA between males ($n = 7$) and females ($n = 7$), 5, 20 and 15 μg RNA samples were loaded in contiguous lanes of the gel. Autoradiographic signals were analysed by densitometry using a Beckman DU64 spectrophotometer. The densitometrically determined area under each curve was divided by the amount of RNA loaded in the corresponding lane. The means ± S.E.M. for these integrations were determined and are displayed in Figs. 1 and 2. For the study of SAP and mRNA accumulation after LPS administration, the ordinate displays the densitometry data expressed as a percentage of the mean control sample signal (Fig. 1*a*). For the analysis of gender difference in SAP expression, the ordinate displays the densitometry data arbitrarily expressed as a percentage of the maximal mean signal (female) (Fig. 2).

RESULTS AND DISCUSSION

In man, SAP is identical with the amyloid P component isolated from amyloid deposits (Prelli *et al.*, 1985). Although amyloid P is derived from circulating SAP, the role of SAP during the acute-phase response or during the induction of amyloidogenesis has not been clearly defined (Baltz *et al.*, 1986). Similarly, the mechanisms of interaction between SAP and amyloid-fibril proteins have yet to be elucidated. SAP binds avidly to DNA, and a role for pentraxins in the removal of nucleic acids at sites of tissue necrosis has been postulated (Pepys & Butler, 1987).

Although conservation of SAP amino acid sequences has been demonstrated among various species, marked differences in the expression of SAP genes occur. Murine SAP is a major acute-phase reactant, whereas in man the serum concentration of SAP does not significantly alter after an inflammatory stimulus (Pepys *et al.*, 1978, 1979). In guinea pigs, SAP is not a major acute-phase plasma protein, but serum concentrations of SAP have been reported to be elevated 2.5-fold after marked stimulation (Maudsley *et al.*, 1986). No sex difference has been observed in the serum levels of rat SAP, and a 2-fold increase in the serum concentration of rat SAP after inflammation has been demonstrated in previous studies (de Beer *et al.*, 1982; Nakada *et al.*, 1986). The current study has focused upon the structure and expression of mRNA encoding rat SAP.

Isolation and characterization of rat SAP cDNA clones

Ten cDNA clones were isolated by cross-hybridization with a Syrian-hamster female-protein cDNA probe (Dowton *et al.*,

1985). The two clones selected for detailed sequence analyses contained inserts of 931 bp (prSAP1) and 218 bp (prSAP8) respectively. The nucleotide sequence for prSAP8 was identical with the corresponding region at the 3′ end of prSAP1, but stopped before the polyadenylation signal. The amino acid sequence of the rat SAP primary translation product, which was derived from prSAP1, contained 228 amino acids, including a 20-residue signal peptide. prSAP1 also contained 82 bp of 5′ untranslated sequence and 165 nucleotides in the 3′ untranslated region (Fig. 3). A polyadenylation signal (AAATAAAAA) was present 23 nucleotides before the poly(A) tail at the 3′ end of prSAP1. Rat SAP shares considerable sequence identity with pentraxins of other species, including man, mouse and hamster (Dowton *et al.*, 1985; Mantzouranis *et al.*, 1985; Ishikawa *et al.*, 1987). Maximal amino-acid-sequence identity (79%) is found between rat and murine SAP. The sizes of mature SAP monomers are predicted to be different in various species, with SAP from rat and hamster (Syrian and Armenian) containing 208 and 212 amino acid residues respectively (Dowton & Waggoner, 1989), whereas the monomers of this protein from mouse and man are smaller: 204 amino acids for human SAP, and 203 residues for murine SAP (Mantzouranis *et al.*, 1985; Ishikawa *et al.*, 1987). These differences are largely explicable by amino-acid-sequence differences at the *C*-terminus of the protein (Fig. 4*a*). Fig. 4(*b*) depicts the nucleotide sequence data from cDNA clones described for the corresponding region in each species and shows that shorter mature monomers of human and murine SAP arise because of a shift of frame due to the absence of a single cytosine residue, generating a termination codon (TGA) at an earlier site in the transcript sequence in man and mouse compared with hamster. Although rat SAP is predicted to be four amino acids shorter than hamster SAP, three of four *C*-terminal residues are identical (Fig. 4*a*). Comparison of the rat and hamster SAP cDNA sequences in this region reveals a 12 bp deletion ending four codons before the termination codon (Fig. 4*b*).

Expression of hepatic SAP mRNA

Although considerably similarity of primary structure of rat SAP has been shown with other animals, the expression of SAP genes in each of these species is quite different. Murine SAP is an acute-phase reactant, and the expression of SAP in mice is genetically determined, with differences between normal levels in various inbred strains of mice (Pepys *et al.*, 1979). In man, the serum concentrations of SAP do not alter appreciably after an inflammatory stimulus (Pepys *et al.*, 1978). In the Syrian hamster, expression of the SAP counterpart, female protein, is dually regulated by the mediators of acute inflammation as well as sex steroids (Coe & Ross, 1983). In the closely related Armenian hamster, SAP is an acute-phase reactant in both sexes, and the direction of response is not gender-dependent (Dowton & Waggoner, 1989).

These studies demonstrate that the level of hepatic SAP mRNA increases after stimulation with LPS in proportion to the reported increases in serum concentration (Nakada *et al.*, 1986) (Fig. 1). The regulation of expression is shown to occur before translation, which is characteristic for several pentraxin genes from other species (Dowton *et al.*, 1985; Goldberger *et al.*, 1987). In addition, the resting SAP mRNA levels are higher in female rats than in male animals (Fig. 2). These mRNA data, coupled with the reported absence of a gender difference in serum SAP

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                                          ATCTGAAGAGCATAGGGGGCCACCACACTTTCGTCCCATACCCAAGCAACAGCTGCTGCTGCTGTCATACCCTGGACCAAGC   82

-20                                     -10                                      1                                  10
ATG GAC AAG CTG CTG CTT TGG ATG TCT GTC TTC ACC AGC CTT CTT TCA GAA GCC TTT GCT CAG ACA GAC CTC AAT CAG AAG GTA TTT GTG   172
 M   D   K   L   L   L   W   M   S   V   F   T   S   L   L   S   E   A   F   A   Q   T   D   L   N   Q   K   V   F   V

                                    20                                      30                                      40
TTC CCC AGA GAA TCT GAA ACT GAT TAT GTG AAG CTG ATC CCA TGG CTA GAA AAA CCG CTG CAG AAT TTT ACA CTG TGT TTC CGA GCC TAC   262
 F   P   R   E   S   E   T   D   Y   V   K   L   I   P   W   L   E   K   P   L   Q   N   F   T   L   C   F   R   A   Y

                                    50                                      60                                      70
AGT GAC CTT TCC CGC TCT CAG AGT CTT TTC TCC TAC AGT GTC AAC AGC AGA GAC AAT GAG CTA CTA ATT TAT AAA GCA AAG TTG GAA CAA   352
 S   D   L   S   R   S   Q   S   L   F   S   Y   S   V   N   S   R   D   N   E   L   L   I   Y   K   A   K   L   E   Q

                                    80                                      90                                      100
TAT AGC CTA TAC ATT GGA AAT TCA AAA GTC ACA GTC CGT GGT TTA GAA GAA TTC CCT TCT CCA ATA CAT TTC TGT ACC AGC TGG GAG TCC   442
 Y   S   L   Y   I   G   N   S   K   V   T   V   R   G   L   E   E   F   P   S   P   I   H   F   C   T   S   W   E   S

                                    110                                     120                                     130
TCC TCT GGT ATT GCT GAA TTT TGG GTC AAT GGA AAG CCT TGG GTA AAA AAG GGT TTG CAG AAG GGA TAC ACT GTG AAA TCC TCA CCC AGT   532
 S   S   G   I   A   E   F   W   V   N   G   K   P   W   V   K   K   G   L   Q   K   G   Y   T   V   K   S   S   P   S

                                    140                                     150                                     160
ATT GTC CTG GGA CAG GAG CAG GAT ACG TAT GGA GGA GGG TTT GAT AAG ACA CAG TCC TTT GTG GGA GAG ATT GCA GAT TTG TAC ATG TGG   622
 I   V   L   G   Q   E   Q   D   T   Y   G   G   G   F   D   K   T   Q   S   F   V   G   E   I   A   D   L   Y   M   W

                                    170                                     180                                     190
GAC AGT GTG CTG ACC CCA GAG AAC ATT CAT TCT GTG GAC AGA GGT TTC CCA CCC AAT CCT AAT ATT TTG GAT TGG CGG GCC CTG AAT TAT   712
 D   S   V   L   T   P   E   N   I   H   S   V   D   R   G   F   P   P   N   P   N   I   L   D   W   R   A   L   N   Y

                                    200
GAA ATA AAT GGT TAT GTA GTC ATC AAG CCC CGT ATG TGG GAC AAC AAA AGC TCA TGAAAATGAGATGATCAGGGTGTTCGAGGTCAAGGTGGCAGAACTCA   813
 E   I   N   G   Y   V   V   I   K   P   R   M   W   D   N   K   S   S

CTCTATCTGGAGTTTTTCTTCTTTATGAATGTCCTGTCTAAATATCTGCCAAATAAAAATCCTATCAAATTCTAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA   931
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Fig. 3. Rat SAP cDNA (prSAP1) nucleotide sequence

The derived amino acid sequence for rat SAP is shown below the nucleotide sequence. Nucleotide sequence for the shorter cDNA clone, prSAP8, extended from nucleotide 630 to 848 in the sequence of prSAP1. Numbers in the right margin refer to the last nucleotide in each row. Derived amino acids are numbered above the codons, with the putative first residue of mature rat SAP numbered 1. The polyadenylation signal is underlined.

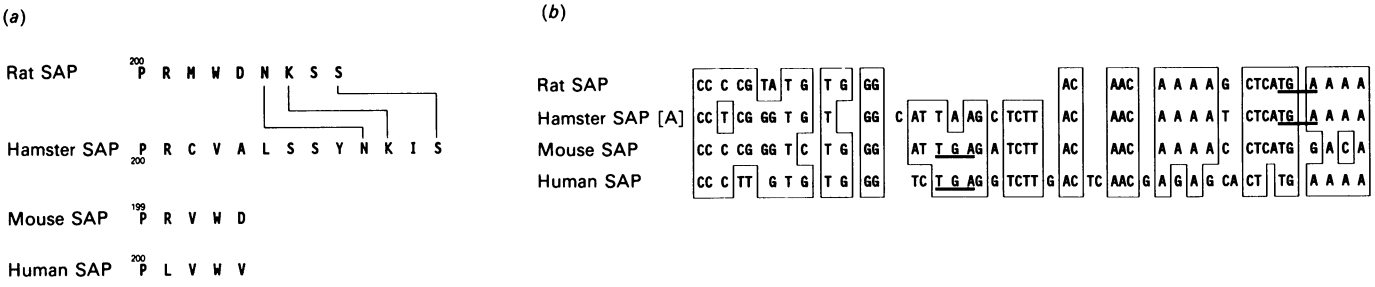

Fig. 4. *C*-Terminal amino acid sequences for rat, hamster, mouse and human SAP and (*b*) nucleotide sequences corresponding to amino acids shown in (*a*)

(*a*) Lines drawn from rat to hamster sequences highlight the similarity of terminal amino acid residues. The numbers indicate the amino acid position in the mature SAP monomer of each species. (*b*) Boxes indicate identical bases. The depicted alignment maximizes base identity between species. Termination codons are underlined. [A] refers to Armenian hamster.

levels in rats, suggest that translational or post-translational processing may be differentially regulated in female and male rats. Alternatively, the half-life of SAP may be longer in female rats. The importance or mechanism of this gender difference is unexplained. However, gender-dependent variations in the concentration of serum SAP from several species have been described. In humans, females have slightly, but significantly, lower plasma SAP concentrations than males (Pepys *et al.*, 1978). The expression of the SAP homologue in the Syrian hamster, the female protein, is known to be sex-limited. Female Syrian hamsters have high serum concentrations in the resting state, which decrease with administration of an inflammatory stimulus, whereas in males a 6-fold elevation of female protein in the serum occurs during inflammation (Coe & Ross, 1983). The magnitude of increase of rat SAP mRNA levels above baseline in males and females after administration of equivalent doses of LPS was found to be similar (results not shown).

Conclusion

The determination of the derived amino acid sequence for rat RAP provided the basis for future investigation of protein structures which permit assembly of pentraxin molecules. The availability of similar data for several species will not only permit studies of SAP protein structure but also may stimulate investigation of the mechanisms of differential predisposition to amyloid formation.

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