Molecular cloning, nucleotide sequence heterozygosity and regulation of rabbit serum amyloid A cDNA

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Serum amyloid A (SAA), a glycoprotein produced by the liver, is induced by infection, inflammation and neoplasia (1). The levels of SAA mRNA and protein can increase up to 500-fold during the acute-phase response (2). Here we present the nucleotide sequences for two cDNA clones encoding different molecular species of rabbit SAA. A partial SAA cDNA clone (SA7-3) cloned from a λ gt11 rabbit liver cDNA library (3) (from Dr.P.Guzelian, U. of Va.) was 32 P-labeled and used to isolate a full length SAA cDNA clone (SA8-1) from a λ gt11 rabbit liver cDNA library from Clontech, Inc. Both clones were transferred into pUC118 and sequenced using the dideoxy sequencing method (4). Clone SA8-1 contains a putative leader sequence of 57 bases (underlined), 303 nucleotides coding for the mature protein and 108 nucleotides of 3' untranslated sequence followed by a poly-A tail. Comparison of the sequence of the two clones shows 3 base substitutions (in bold) in the translated region, only one of which changes the amino acid residue (Ala to Asp), and 4 substitutions and 2 short deletions in the 3' untranslated region. Comparison¹ of the deduced amino acid sequence of rabbit SAA with that of other mammals shows a 75% identity with three known forms of mouse SAA, and 84% and 74% identity with mink and human SAA, respectively.

RNA isolated from the livers of a control (saline injected) and a turpentine-treated rabbit 24 hours after treatment was electrophoresed on a 1% agarose gel and hybridized with 32 P-labeled SA8-1 (6). Densitometric analysis of the Northern blots showed the levels of SAA mRNA were elevated approximately 75-fold over the very low levels in the control (data not shown), consistent with reports on other mammals undergoing an acute-phase response.

The poly-A mRNA isolated from the liver of the treated animal will be useful for construction of a λ gt11 cDNA library containing sequences enriched in acute-phase responsive proteins. Whether the variant SAA clones presented here represent 2 non-allelic SAA genes remains to be elucidated.

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CGCACCCCC CAAGTGGCCG CTACGGGGGC GCACAGCGTG CTCACCAAAA ACCGACACC ATG AAG CTC 9
CTC TCA GGC CTG CTT CTC TGC TCC TTG GTC CTG GGA GTC AGC GGC CAA GGC TGG TTC TCC TTC 72
Leu Ser Gly Leu Leu Leu Cys Ser Leu Val Leu Gly Val Ser Gly Gln Gly Trp Phe Ser Phe
ATC GGT GAG GCT GTG AGA GGG GCA GGG GAC ATG TGG AGA GCC TAC TCC GAC ATG AGA GAA GCC 135
Ile Gly Glu Ala Val Arg Gly Ala Gly Asp Met Trp Arg Ala Tyr Ser Asp Met Arg Glu Ala
AAT TAC ATA AAT GCA GAC AAG TAC TTC CAC GCT CGG GGC AAC TAT GAC GCT GGC CAA AGG GGC 198
Asn Tyr Ile Asn Ala Asp Lys Tyr Phe His Ala Arg Gly Asn Tyr Asp Ala Ala Gln Arg Gly
C TTC CAC GCT CGG GGC AAC TAT GAC GCT GGC CAA AGG GGC
CCT GGG GGT GTC TGG GCT GCC AAG GTC ATC AGT GAC GTC AGA GAG GAC CTT CAG AGA CTC ATG 261
Pro Gly Gly Val Trp Ala Ala Lys Val Ile Ser Asp Val Arg Glu Asp Leu Gln Arg Leu Met
CCT GGG GGT GTC TGG GCT GCC AAG GTC ATC AGT GAC GTC AGA GAG GAC CTT CAG AGA CTC ATG
GGC CAC GGA GCA GAG GAC TGG ATG GAC GGC CAG GCT GCC AAC GAA TGG GGC CGC AGT GGC AAA 324
Gly His Gly Ala Glu Asp Ser Met Asp Ala Gln Ala Ala Asn Glu Trp Gly Arg Ser Gly Lys
GGC CAC GGA GCA GAG GAC TGG ATG GAC GAC CAG GCT GCC AAC GAA TGG GGC CGC AGT GGC AAA
Asp
GAC CCC AAC CAC TTC CGA CCC AAG GGC CTG CCC GAC AAA TAC TGA GCTCCCTGCT CTGCTGCCCC 389
Asp Pro Asn His Phe Arg Pro Lys Gly Leu Pro Asp Lys Tyr
GAC CCC AAC CAC TTC CGA CCC AAG GGC CTA CCC GAC AAA TAC TGA GCTCCCTACT CTGCTGCCCC
TGAGGACTGG GACCCAGAGT GCTGAGTCCC GTGTGCGTGA AGCTGGTGCC GGGCACACAA CTGGTGCTTA ATAAATGGCT
TGAGGACT-- GACCCAGAGT ACTGAGTCCC GTGTGCGTGA AGCTGGTGCC GGGCACACAA CTGGTGCTTA ATAAATGGCT
ATAAGACTGA AAAAAAAAAA....
ATAAGACTGA AAAAAAAAAA....

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Figure 1. Nucleotide and derived amino acid sequence of two cDNA clones of rabbit serum amyloid A (SAA) protein. The sequence of clone SA8-1 is given above that of SA7-3. The putative amino acid signal sequence and the consensus sequence for poly-adenylation in the untranslated 3' DNA sequence prior to the poly-A tail are underlined. The initiation and termination codons are double underlined. The sequences of SA8-1 and SA7-3 will appear in the EMBL/GenBank/DBJ Nucleotide Sequence Databases under accession numbers X16428 and X16427, respectively.

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¹Analyses were carried out with the 'FASTA' program and PIR (Protein Identification Resource) amino acid sequence data base obtained from Dr W.R.Pearson