

The sequence of human retinal S-antigen reveals similarities with α -transducin

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The complete amino acid sequence of human retinal S-antigen (48 kDa protein), a retinal protein involved in the visual process has been determined by cDNA sequencing. The largest cDNA was 1590 base pairs (bp) and it contained an entire coding sequence. The similarity of nucleotide sequence between the human and bovine is approximately 80%. The predicted amino acid sequence indicates that human S-antigen has 405 residues and its molecular mass is 45050 Da. The amino acid sequence homology between human and bovine is 81%. There is no overall sequence similarity between S-antigen and other proteins listed in the National Biomedical Research Foundation (NBRF) protein data base. However, local regions of sequence homology with α -transducin ($T\alpha$) are apparent including the putative rhodopsin binding and phosphoryl binding sites. In addition, human S-antigen has sequences identical to bovine uveitopathogenic sites, indicating that some types of human uveitis may in part be related to the animal model of experimental autoimmune uveitis (EAU).

S-antigen; 48-kDa protein; Human cDNA; Amino acid sequence; Uveitis; Visual process

1. INTRODUCTION

Vision begins with the conversion of light energy into an electrical impulse (phototransduction) [1,2]. This is accomplished by the outer segments of the photoreceptor cells of the retina. Initial absorption of light converts rhodopsin into an active form (R^*) which interacts with and activates hundreds of transducin (Td) molecules [3–5]. The activated Td interacts with phosphodiesterase (PDE), each molecule of which hydrolyzes thousands of molecules of cyclic guanosine monophosphate (cGMP) [5–7]. The decrease of

cGMP concentration blocks the entry of sodium ions by closing Na^+ channels [8–10]. Thus, light energy is amplified to generate a membrane potential. This electrical signal is processed into a neuronal signal by other retinal cells. The rhodopsin bleached by light is then phosphorylated by rhodopsin kinase at multiple serines and threonines near the carboxyl terminus [11]. S-antigen binds to the R^* and R^*P to play a negative regulatory role in the visual process [12–14]. As a contrasting hypothesis, S-antigen does not bind to R^*P but directly inactivates PDE [15,16]. Many similar proteins have been shown to be involved in the visual system, the adenylyl cyclase system and hormone receptor signal transduction [17]. Therefore, it is possible that the adenylyl cyclase system has a protein similar to S-antigen which plays a role in negative regulation. However, only in the photoreceptor cells in retina and pinealocytes in pineal gland, has S-antigen been detected by immunocytochemistry [18].

S-antigen is also a highly antigenic protein

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Abbreviations: NBRF, National Biomedical Research Foundation; EAU, experimental autoimmune uveitis; R^* , photoactivated rhodopsin; R^*P , phosphorylated photoactivated rhodopsin; CD, circular dichroism; Td, transducin; PDE, phosphodiesterase; cGMP, cyclic guanosine monophosphate

responsible for the induction of experimental autoimmune uveitis (EAU) [19,20]. Recently the structure of bovine S-antigen [21–23] and sites responsible for EAU have been characterized [24]. In order to understand the functional role of S-antigen in phototransduction and in the induction of human uveitis, it is important to determine the structure of human S-antigen. Here, we report the amino acid sequence of human S-antigen and discuss its role in the visual process and in human disease.

2. MATERIALS AND METHODS

Human retinal library (generous gift from Dr J. Nathans, Genentech) was screened with ^{32}P -labelled 5' noncoding sequence of a bovine S-antigen cDNA probe (331 bp long) as described [22,23]. Initially, 1×10^5 plaques were screened giving 1×10^3 positive plaques. Most of the clones seem to have nearly full-size cDNA insert. Nine cDNA inserts were subcloned into bluescribe plasmid (Stratagene, San Diego, CA) and amplified.

Restriction enzyme analysis was performed with *EcoRI*, *BamHI*, *HaeIII*, *BglII*, *KpnI*, *SacI*, *XhoI* and *PstI* enzymes (International Biotechnologies Inc., New Haven, CT). DNA sequences were obtained by the dideoxy chain termination method [25] modified for [^{35}S]ATP (Amersham Inc., Arlington Heights, IL) [21]. Specific oligonucleotide primer probes (15 or 20 mer) were made on an automated DNA synthesizer (Model 380B, Applied Biosystem, Inc., Foster City, CA).

3. RESULTS AND DISCUSSION

Several positive plaques for human S-antigen cDNAs were obtained from a human retina cDNA library by hybridization with bovine S-antigen cDNA probes [21–23]. Nine of them contained the full coding sequence of S-antigen and had the same restriction sites for *BglII*, *EcoRI*, *KpnI*, *SacI* and *XhoI* (not shown). Three cDNAs out of nine clones had different *HaeIII* sites in the coding sequence indicating that S-antigen may be polymorphic, since the cDNA library was constructed from multiple retinas [26]. The largest cDNA insert, 1590 bp was sequenced (fig.1). This cDNA insert has a poly-A tail at the 3' end but lacks 100–150 bp at the 5' end. The bovine and mouse mRNA size is approximately 1600–1700 nucleotides (nt). The sequence similarity between human and bovine DNA is approximately 80% (fig.2). However, similarity in the non-coding sequence is less than 30%, while in the coding sequence it is 86%. Both human and bovine cDNAs have a long 5' noncoding sequence (300–400 nt)

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GGGATCTAGCGAGGATGCCCGCTACAAATCCCCACATCAGCTAGCCAGGAGCCTCAGC
10      20      30      40      50      60
GCTGCCCGCTTCCAGGCTCATCTGGCAAGACCGTACCAGCTTGCTCAGAACAGGGCTGGCT
70      80      90      100     110     120
ATTTCATCATCTCAGAGCATAGAGACCGCTCTCCTTGCCACCGCGCCCTGCCAGCTGGTTG
130     140     150     160     170     180
                                     M A A S G K T 7
GTGACAATCACAAGGTGTAGAAGTTGCCAGGACAGATAACATGGCAGCGAGCGGAAGA
190     200     210     220     230     240
S K S E P N H V I F K K I S R D K S V T 27
CCAGCAAGTCCGAACCGAACATGTTATCTTCAAGAAGATCTCCGGGACAAATCGGTGA
250     260     270     280     290     300
I Y L G N R D Y I D H V S Q V Q P V D G 47
CCATTCACCTGGGGAAGAGAGACTACATAGACCATCTGACCAAGTCAGCCTGTGGATG
310     320     330     340     350     360
V V L V D P D L V K G K K V Y V T L T C 67
GTCTCCTGTGGTGTGATCTGATCTTGTGAAGGGAAGAAAGTGTATGTCACTCTGAAC
370     380     390     400     410     420
A F R Y G Q E D V D V I G L T F R R D L 87
GGCCCTTCGGCTATGCGCAAGAGGACGTTGACGTGATCGGCTTGACCTTCGGCAGGGACC
430     440     450     460     470     480
Y F S R V Q V Y P P V G A A S T P T K L 107
TGTACTTCTCCCGGTCAGGTGTATCCTCTGCGGGCGGAGCAGCCCAACAAAC
490     500     510     520     530     540
Q E S L L K K L G S N T Y P F L L T F P 127
TGAAGACAGAGCTGCTTAAAGCTGGGGAGCAACACGATCCGCTTTCGCTGAGCTTTC
550     560     570     580     590     600
D Y L P C S V M L Q P A P Q D S G K S C 147
CTGACTACTTGGCTGTTCAGTGTGTGAGCGACGTCACAAAGATTGAGGCAAGTCTCT
610     620     630     640     650     660
G V D F E V K A F A T D S T D A E E D K 167
CTGGCTTGACTTTGAGCTCAAAGCATTCGCCACAGACAGCAGCCGATGCCGAAAGAGGAC
670     680     690     700     710     720
I P K K S S V R Y L I R S V Q H A P L E 187
AAATCCCAAGAGAGCTCCGTGGATATCTGATCCGTATGTACAGCATGCCCGCACTTG
730     740     750     760     770     780
M G P Q P R A E A T W Q F F M S D K P L 207
ACATCGCTCCCGACCGCCGACGTCAGCGGACCTCCGACCTTCTCATCTCTCACAGCCGC
790     800     810     820     830     840
H L A V S L N R E I Y F H G E P I P V T 227
TGCACCTTGGCTCTCTCTCAACAGAGAGATCTTTCCATGGGGAGCCCATCCCTGTGA
850     860     870     880     890     900
V T V T N N T E K T V K K I K A C V E Q 247
CCGTGACTGTCAACAATAACACAGAGAGACCGTGAAGAGATTAAAGCATCGCTGGAAC
910     920     930     940     950     960
V A N V V L Y S S D Y Y V K P V A M E E 267
AGGTGCCCAATGTGGTTCTCTACTCGAGTATTATACGTCAGGCGCGTGGCTATGGAGG
970     980     990     1000    1010    1020
A Q E K V P P N S T L T K T L T L L P L 287
AAGCCCAAGAAAAGTGGCAGCAACAGCACTTTGAGCAAGACCTTGAGCGTCTCCGCT
1030    1040    1050    1060    1070    1080
L A N N R E R R G I A L D G K I K H E D 307
TGCTGGCTAACAAATCGAGAAAGCAGAGGATTCGCCCTGATGGGAAATCAAGCAGAGG
1090    1100    1110    1120    1130    1140
T N L A S S T I I K E G I D R T V L G I 327
ACACAAACCTTGGCTCCAGCAGCATCATTAAAGAGGGCATAGACCGGACCGTCTGGGAA
1150    1160    1170    1180    1190    1200
L V V S Y Q I K V K L T V S G F L G E L T 347
TCTGGTGTCTTACAGATCAAGGTGAAGTCAAGTGTGACGCTTTCTGGGAGAGCTCA
1210    1220    1230    1240    1250    1260
S S E V A T E V P F R L M H P Q P E D P 367
CCTCGAGTGAAGTCCGCACTGAGGTCCCATTCGGCTCATGACCCCTCAGCGTGGAGGC
1270    1280    1290    1300    1310    1320
A K E S I Q D A N L V F E E F A R H N L 387
CAGCTAAGGAAAGTATTGAGTGCATAATTAGTTTTCGAGGAGTTTCTGCCATAATC
1330    1340    1350    1360    1370    1380
K D A G E A E E G K R D K N D A D E 405
TGAAAGATCGAGGAGAGCTGAGGAGGGGAAGAGAGACAAGAATGACGCTGATGAGTAA
1390    1400    1410    1420    1430    1440
GATGTGGGCTCAGGATGCCGGAATAACCTGTAGTACCAAGTGAACGAGCAAAAGCGCC
1450    1460    1470    1480    1490    1500
ACAGTTAGTCTTTGGAGTTATGCTGCGTATGAAAGGATGAGTCTTCTTCGAGAAATA
1510    1520    1530    1540    1550    1560
AACCTTGTCTTCTCGCGTGAATAAAAAA
1570    1580    1590

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Fig.1. The sequence of cDNA clone for human S-antigen. The upper line shows predicted amino acid sequence and lower line shows nucleotide sequence. Numbers on the right indicate amino acid residues and subscripts of nucleotides indicate nucleotide residues from 5' end. The underlined nucleotides preceding the S-antigen sequences denote the first methionine codon and in frame termination codon.

	Phosphoryl Binding Site												Guanine Binding Site					
Consensus Sequence	Gly	x	x	x	x	Gly	Lys		Asp	x	x	Gly		Asn	Lys	x	Asp	
Human S-Ag	Ala	Pro	Gln	Asp	Ser	Gly	Lys	(139-145)	Asp	Val	Ile	Gly	(77-80)	Ser	Lys	Ser	Glu	(8-11)
	Gly	Ile	Ala	Leu	Asp	Gly	Lys	(296-302)										
Bovine S-Ag	Ala	Pro	Gln	Asp	Val	Gly	Lys	(135-141)	Asp	Val	Met	Gly	(73-76)	Asn	Lys	Pro	Ala	(4-7)
	Gly	Ile	Ala	Leu	Asp	Gly	Lys	(292-298)										

Fig.2. Homologies of human and bovine S-antigen to the consensus sequence of GTP/GDP binding sites. The numbers in parentheses represent the amino acid residues. Consensus spacing of consensus sequences are either 40–80 or 130–170 amino acid residues between the first and second elements and 40–80 amino acid residues between the second and third sequence elements. X indicates any amino acid residues.

and a relatively short 3' noncoding sequence (~150 nt).

The initiation codon at base 222 is in a sequence which conforms well to the Kozak consensus sequence (TAACATG compared with CANCATG) [27]. The open reading frame between 222 and 1436 appears to contain the entire coding sequence for S-antigen, a protein consisting of 405 amino acids with a calculated molecular mass of 45050 Da. The sequence identity at the protein level between human and bovine is 81%.

Another small open reading frame between 15 and 200 which could encode 60 amino acid residues is observed and it has no similarity with any sequence in the NBRF database. Bovine S-antigen cDNA has a similar open reading frame in the 5' noncoding sequence. There is also no significant nucleotide or amino acid sequence similarity between the small open reading frames in human and bovine 5' noncoding sequences. Such short open reading frames in the 5' noncoding region have been reported in other cDNAs [28,29] and they appear to have influence on their translation [30].

Although the function of S-antigen in the phototransduction process is not well understood, a possible relationship with GTP binding proteins exists. S-antigen and GTP-binding proteins have some interesting local sequence similarities. GTP binding proteins including proto-oncogenes referred to as ras proteins, bacterial elongation factors (EF-Tu and EF-G), α -tubulin, adenylate kinase, signal-transducing G-proteins and $T\alpha$ contain highly conserved functional sequences [31]. The

striking feature is at the GTP binding site, including a consensus sequence composed of three elements, Gly/Ala-X-X-X-X-Gly-Lys, Asp-X-X-Gly and Asn-Lys-X-Asp with conserved spacing. Human and bovine S-antigens have four such sequences as shown in fig.2, but in the reverse orientation. They have phosphoryl binding consensus sites Ala-X-X-X-X-Gly-Lys and Asp-X-X-Gly with the right consensus spacing. In addition, both S-antigens have Gly-X-X-X-X-Gly-Lys elements at 295–301 (human) and 292–298 (bovine) without consensus spacing. These three sites may form binding pockets for phosphates [32].

In contrast to the phosphoryl binding sites, there is no obvious candidate for guanine binding sites in the S-antigen sequence [31]. The closest homology in bovine is An-Lys-Pro-Ala with the right spacing. Thus, S-antigen may not bind to GTP. When Asp of Asn-Lys-X-Asp is replaced by Asn as Asn-Lys-X-Asn in the ras mutant protein, the affinity for GTP is lowered by a factor of 100 [33]. Addi-

QDANLVFEE...FARHNLKDAGEA	(373-393)	Human	} S-Ag
QDENFVFEE...FARONLKDAGEY	(372-392)	Bovine	
QNVKVFEDA VTDIIIKENLKD CGLF	(326-350)	T α - Transducin	
<div>ADP-Ribosylation Site</div> <div>↑</div>			

Fig.3. Homologies of human, mouse and bovine S-antigen sequences to $T\alpha$ sequence. Dark boxes indicate the identical amino acid residues and half shade boxes indicate conserved amino acid residues. Bovine sequence was published [21–23]. S-antigen was not ADP-ribosylated by pertussis toxin [23]. The numbers in parentheses represent the amino acid residues.

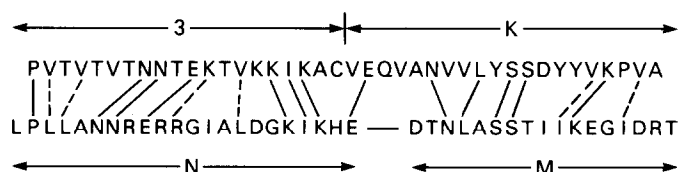


Fig. 4. Sequence similarity of human S-antigen peptides M and N to k and 3. Peptides M, N, K and 3 of bovine S-antigen are uveitogenic sites for experimental animals [24,44]. Solid lines between sequences indicate the identical amino acid residues and dotted lines indicate conserved amino acid residues. (I = V = L, K = R).

tionally, in the same protein, a Lys or Tyr replacement of the Asp in Asn-Lys-X-Asp abolishes GTP-binding activity [34]. We did not observe binding of the ATP to S-antigen (not shown), which is consistent with other results [12–14]. Thus, it is reasonable to speculate that R*P binds to putative phosphoryl pocket of S-antigen.

The second interesting regional similarity of S-antigen is with a site near the carboxyl terminus of $T\alpha$ where pertussis toxin catalyzes ADP-ribosylation [35] as shown in fig.3. Modification of α -transducin by pertussis toxin results in marked reduction of the light dependent GTPase activity as well as the light stimulation of PDE [36]. This suggests that the carboxyl terminus of $T\alpha$ plays a critical role in the interaction with R*. We speculate that S-antigen and $T\alpha$ bind to R* at the same site.

Thus, the structure of S-antigen is consistent with the observation of Kuhn et al. [12–14] that it interacts with R* at the same site as $T\alpha$ in a competitive manner [12]. Initial S-antigen and R* binding may be very weak, as reported previously [12–14]. However, when rhodopsin is phosphorylated with rhodopsin kinase, secondary binding may take place: S-antigen phosphoryl pockets may bind to the phosphate groups of R*P leading to tighter binding than R*/ $T\alpha$ thereby quenching the capacity of $T\alpha$ to activate PDE. The $T\alpha$ binding site on rhodopsin is estimated to be at the carboxyl terminus, residues 317–339³⁷ or loop V³⁸, which is close to the multiple phosphorylation sites [11]. Furthermore, carboxyl terminus amino acid residues (approximately 10) were cleaved from the phosphorylated rhodopsin by proteinase. This rhodopsin increases PDE activity higher than the original level [39]. This result suggests that the carboxyl terminus does not interact with $T\alpha$. The phosphorylation of rhodopsin is an important pro-

cess for inactivation of the phototransduction cascade.

S-antigen is also present in the pineal gland where there is no detectable rhodopsin [40]. We speculate that S-antigen binds to phosphorylated β -adrenergic receptors since the β -adrenergic receptor has some sequence similarity to rhodopsin and is also known to be inactivated by phosphorylation [41]. A recent report indicates that S-antigen indeed inhibits the β -adrenergic signal transduction cascade [42].

Recently, the immunopathogenic sites of bovine S-antigen, peptides M, N, K and 3 for the induction of EAU in the Lewis rat have been characterized [24]. Human S-antigen is also capable of inducing EAU in experimental animals including Lewis rats, guinea pigs and monkeys (in preparation) with similar pathology [43]. Interestingly, human S-antigen has an identical sequence to bovine S-antigen M and N peptides (306–317), furthermore it has sequence similarity between peptides M and N and peptides K and 3 (fig.4). The multiple repeated structures may play an important role in the induction of EAU. These findings would indicate that EAU induced by peptides M and N could have clinical relevance to some types of human uveitis. As mentioned previously, however, human S-antigen seems to be polymorphic, perhaps indicating that the susceptibility to disease is in part due to the heterogeneity of the S-antigen structure in each individual.

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