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Rat pineal S-antigen: sequence analysis reveals presence of α-transducin homologous sequence

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S-antigen (S-Ag) is a soluble, highly antigenic protein, the administration of which induces autoimmune uveitis. This protein is found in the retina and pineal. Retinal S-Ag from three species has been sequenced. In this study rat pineal S-Ag was sequenced. Clones were isolated from a rat pineal λgt11 cDNA library by probing with a 300 bp fragment of mouse retinal S-Ag cDNA containing the 5'-coding region. The largest clone isolated (RPS-118; 1364 bp) contained the entire coding sequence. Comparison of the rat pineal and mouse retinal S-Ag nucleotide sequences indicated a high homology (95%). The deduced amino acid sequence was found to contain 403 residues (≃44 992 Da). Comparison of the rat pineal and mouse retinal S-Ag amino acid sequences also revealed high homology (97%). The similarity of both the nucleotide and amino acid sequences of rat pineal and mouse retinal S-Ag indicates that expression of the S-Ag gene in both tissues is similar. Further analysis of the rat pineal S-Ag sequence indicated that it contained essentially the same major uveitopathogenic region of S-Ag present in bovine retina; minor uveitopathogenic sites were somewhat different. As is true of retinal S-Ag, rat pineal S-Ag contains the same consensus phosphoryl-binding site present in many GTP/GDP-binding proteins and a homologous sequence found in the C-terminus of α-transducin. These sequences may play a role in the action of pineal S-Ag in transmembrane signal transduction.

Antigen, S-; Pineal; cDNA; Autoimmune; Uveitis; Pinealitis; Signal transduction

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Abbreviations: S-Ag, S-antigen; bp, base pairs; EAU, experimental autoimmune uveitis; EAP, experimental autoimmune pinealitis; Gs, GTP-binding protein which stimulates adenylate cyclase; kbp, kilo base pairs; PDE, phosphodiesterase; P*, photoexcited rhodopsin. R*-P, phosphorylated photoexcited rhodopsin; β-Ad-P, phosphorylated β-adrenergic receptor

1. INTRODUCTION

S-antigen is a soluble, highly antigenic protein which is present in only two tissues, the retina and pineal gland [1,2]. This protein first attracted attention when it was shown to cause experimental autoimmune uveitis in susceptible animal strains, most notably the Lewis rat [1]. EAU is an inflammatory condition characterized by destruction of retinal photoreceptor cells (see [3,4]). The histological features of the S-Ag-induced EAU are similar to those of some forms of uveitis in humans, a major cause of blindness [4]. This is the basis of the speculation that S-Ag might play some role in certain forms of uveitis.

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Sequence analysis of mouse [5], bovine [6,7] and human [8] retinal S-Ag indicates that it has 403, 404, and 405 amino acid residues, respectively (≃ 45 kDa). Sequence analysis has also enhanced our understanding of how this protein functions. First, the antigenic regions of S-Ag have been identified using a series of synthetic peptides which covered the entire sequence [9–13]. Second, one region of S-Ag has been found to be highly homologous to a region of α-transducin [3,5–8], the GTP-binding regulatory protein which mediates the transmembrane stimulation of phosphodiesterase (PDE) by phosphorylated photoexcited rhodopsin (R*-P). The presence of homologous sequences in retinal S-Ag and α-transducin has provided reason to suspect that these two proteins might bind to the same target site or sites, and that binding to an α-transducin-binding site might be the basis for the biological action of S-Ag (see [3]). Two possible modes of action of S-Ag have been proposed. One is that binding to R*-P inactivates the receptor [14,15]; the second is binding to PDE inhibits activity [16].

In contrast to our knowledge of retinal S-Ag, little is known about pineal S-Ag. The presence of S-Ag in the pineal was first indicated by the demonstration that administration of retinal S-Ag causes EAP in the Lewis rat [1,2]. The presence of S-Ag in the mammalian pineal seems to be an evolutionary holdover reflecting the photoreceptive function of the pineal in lower vertebrates. For example, the frog pineal organ is clearly photosensitive, has well developed photoreceptors, and also contains rhodopsin, α-transducin, and S-Ag [17]. In contrast, the mammalian pineal is not photosensitive, does not have photoreceptors, and does not seem to contain rhodopsin or α-transducin [18,19].

The mammalian pineal functions primarily to produce and release melatonin, a function it is known to share with all vertebrate pineal cells and to a limited degree with the retina. Melatonin production is stimulated by norepinephrine acting through α_1- and β_1-adrenergic receptors. β_1-Adrenergic receptors and rhodopsin belong to the same family of receptors which rely upon GTP-binding regulatory proteins to mediate transmembrane signal processing. The GTP-binding protein which mediates β_1-adrenergic stimulation of adenylate cyclase is Gs. Based on studies with the β_2-adrenergic receptor, it has been proposed that S-Ag might bind to a Gs-binding region of this receptor and thereby inhibit function [20]. It seems reasonable to extend this line of thought to the β_1-adrenergic receptor because it contains those sequences thought to be required for the action of S-Ag on the phosphorylated β_2-adrenergic receptor R*-P [21].

In an effort to better understand the function of S-Ag in the pineal gland, we decided to determine the sequence of the polypeptide present in the rat pineal gland; we were especially interested if the pineal polypeptide contained the homologous sequence found in α-transducin. The rat gland was chosen because this is the best studied pineal model. The results of this effort are reported here.

2. MATERIALS AND METHODS

A group of 100 pineal glands was obtained during day time from male rats (200 g, Sprague Dawley). The tissue was homogenized in ≃ 10 vols of a 10:1 mixture of buffer (10 mM Tris, pH 8.4, containing 0.14 M NaCl and 1.5 mM MgCl2) and 5% Nonidet P 40. The homogenate was centrifuged (1 min, 6500 × *g*, room temperature); the supernatant was removed and mixed with 500 μl of phenol. Cytoplasmic RNA was extracted and ethanol precipitated using routine procedures [22]. PolyA RNA was isolated by oligo(dT)-cellulose column purification [22]. A λgt11 library (817i) was constructed using a commercially available kit (Pharmacia, Piscataway, NJ). Inserts were packaged using Protoclone λgt11 arms (Promega Biotech, Madison, WI) and the Pacagene system.

Five petri dishes (23 cm), each containing approximately 2×10^5 recombinant phages and 1×10^8 *Escherichia coli* Y1090, were incubated at 37°C for 7–8 h. DNA was transferred to nitrocellulose filters for hybridization. The filters were incubated as described elsewhere [22]. The hybridization probe was prepared with a synthetic oligonucleotide (from nt 300 to 321) designated for primer extension in the 5′-direction; a 1.6 kbp mouse S-Ag cDNA was used as the template [5]. The hybridization was performed overnight at 42°C in a solution containing 50% (v/v) formamide, 5 × SSC, 1 × SSC and 0.1 × SSC at 20°C and subjected to autoradiography. The plaque purification procedure was repeated at least three times until all plaques of phage showed a positive signal.

The cDNA insert was excised with *Eco*RI restriction enzyme (International Biotechnologies, Inc., New Haven, CT), purified on a 5% polyacrylamide gel and subcloned into the *Eco*RI site of the Bluescribe M13 plasmid (Stratagene, La Jolla, CA) for DNA sequence determination. The sequencing primers were synthesized using a Biosearch DNA synthesizer (model 7800, Burlington, MA). A portion of the DNA sequence was determined by BioTechnica International, DNA Sequence Determination Service Division (Cambridge, MA).

Hydrophobicity/hydrophilicity analysis of the deduced amino acid sequence was performed using the procedure of Garnier et al. [23]. The secondary structure of the deduced polypep-

tide was examined using the algorithm of Needlman and Ununsch [24].

3. RESULTS AND DISCUSSION

Screening of 2×10^6 phages identified 20 positive plaques. The clones were plaque-purified; the inserts were excised (*Eco*RI) and sized on 5% polyacrylamide gels. Inserts ranged from 1.2 to 1.4 kbp in length and all contained the same *Hae*III restriction fragment as revealed by polyacrylamide gel analysis. The clone containing the largest insert (1.4 kbp) was designated RPS 118. This insert was subcloned into Bluescribe M13 plasmid DNA sequence vector for sequencing. DNA sequence determination was performed at least twice in one direction for both strands of RPS-118 without discrepancies.

The DNA sequence is shown in fig. 1. A single large open reading frame was found with an ATG initiation codon at position 28 and a TGA termination codon at position 1237. The potential polyadenylation signal (AATAAA) is located at position 1351 in the 3′-noncoding region. The insert studied here seems to be shorter than the mRNA because Northern blot analysis has indicated that rat pineal and bovine retinal S-Ag mRNAs are the same size [5,25]. The published length of retinal S-Ag mRNA from several species is about 1600 bp. Hence, it would appear that the insert we have studied in this report lacks a 250 bp sequence in the 5′-noncoding region. Comparison of the rat pineal and the mouse retinal sequences reveals that the overall homology is approx. 95% (fig. 1), and that this high homology was apparent both in the 3′-noncoding and in the coding sequences. Analysis of the differences in nucleotides in the coding sequence indicated that 81% were found in the third position of triplet codons.

The open reading frame was found to code for a polypeptide of 403 amino acid residues with a calculated molecular mass of 44 992 Da. Comparison of the deduced amino acid sequences of the rat pineal and mouse retinal polypeptides indicated that there was 97% homology (fig. 2). Only ten of the 403 residues were different. Four were conserved changes; the rest were: Met→Ile (76); Ala-Pro→Val-Leu(100–101); Leu→Glu (106); Arg→Leu (108); Ala→Pro (160); Cys→Ser (190) and His→Asn (205). Similar differences in the deduced

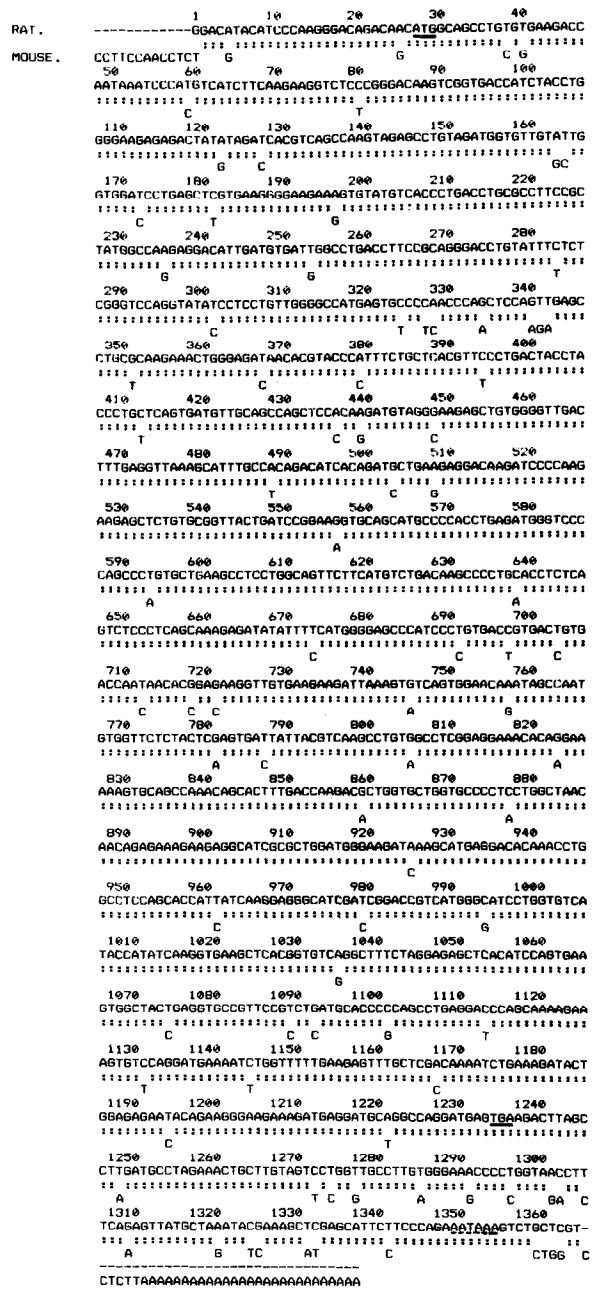

Fig.1. Comparison of the nucleotide sequences of rat pineal (RPS-118, upper sequence) and mouse retinal (lower sequence) S-Ag cDNA. The nucleotide sequence for mouse retinal S-Ag was taken from Tsuda et al. [5]. An identical nucleotide is indicated by a colon; a dash represents an undetermined nucleotide. The solid underlining indicates the beginning and the end of the protein coding region. The broken underlining indicates the polyadenylation signal. The numbers identify specific nucleotides in the sequence.

Fig.2. Comparison of the amino acid sequences of rat pineal S-Ag (deduced from RPS-118, above) and mouse retinal S-Ag (below). The amino acid sequence for mouse retinal S-Ag is from Tsuda et al. [5]. Identical residues are identified by a colon; the single letter code is used to designate amino acids.

sequence of retinal S-Ag polypeptides at the same positions exist between the human and mouse polypeptides and the mouse and bovine polypeptides [3,5–8]. Analysis of the predicted secondary structure of rat pineal S-Ag indicated that it is nearly identical to that of mouse retinal S-Ag (data not shown). The marked similarities between rat pineal S-Ag and mouse retinal S-Ag mRNA [21] and the deduced amino acid sequence when taken together with the evidence that only a single gene codes for this protein in the mouse and human (unpublished results), indicate that expression of the S-Ag gene is similar in the pineal and retina.

Consistent with the generally high degree of

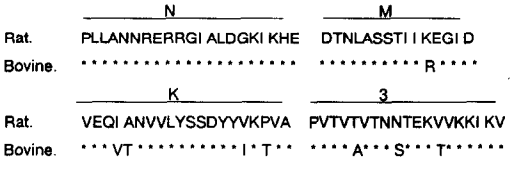

Fig.3. Amino acid sequence similarities of pathogenic sites in rat pineal and bovine retinal S-Ag [6,7]. Identical residues are indicated by an asterisk. The range of rat presumptive pathogenic peptides N (283–303), M (303–318), K (242–261) and 3 (222–240) and bovine pathogenic peptides N (282–302), M (303–317), K (241–260) and 3 (221–239) are indicated by lines. Peptides M and N in bovine S-Ag are considered to be major uveitopathogenic sites and peptides K and 3 are considered to be minor uveitopathogenic sites. The single letter code is used to designate amino acid residues.

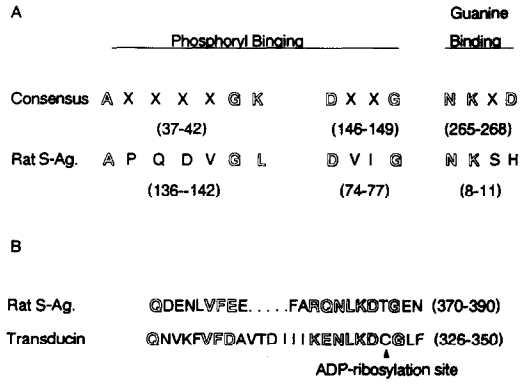

Fig.4. Amino acid sequence similarities of rat pineal S-Ag and α-transducin. (A) The GTP/GDP-binding consensus sequences of most GTP/GDP-binding proteins are presented. The numbers in parentheses represent amino acid residues. Shadowed letters identify similar residues. X represents any residue. The first two elements are known to bind the phosphoryl portion of guanine nucleotides and the third element is the guanine-binding site. (B) Similarity at the carboxyl-terminus. Shadowed letters indicate the same residues including conserved changes (S=T, I=V=L, R=K and D=E). The arrowhead identifies the ADP-ribosylation site (Cys) of α-transducin. The numbers in parentheses identify the residues.

homology, it was found that regions of rat pineal S-Ag of special interest were also generally similar to those of retinal S-Ag. For example, two regions of the retinal polypeptide which correspond to the two highly uveitopathogenic synthetic peptides M and N [9–13] are virtually identical in the rat pineal sequence (fig. 3). Two other regions, which correspond to the less uveitopathogenic peptides K and 3 [9–13], are somewhat different. It is possible that such differences might explain differences in the uveitopathogenicity of S-Ag peptides among different species.

Rat pineal S-Ag also contains the same sites as those in retinal S-Ag which have been proposed to be involved in the interaction of the S-Ag and R*-P [20,21,26,27]. These include the consensus phosphoryl-binding sequence which is found on many GTP/GDP-binding proteins (fig. 4A) and the sequence shared with α-transducin (fig. 4B). It has been proposed that these sites might play an important role in the function of S-Ag [3]. Thus, it seems reasonable to suspect that S-Ag might function in the pineal to regulate the β-adrenergic receptor. Certainly this issue deserves further investigation. In addition, other possible roles of S-Ag also

require further study, including the proposal that it regulates PDE [16].

REFERENCES

[1] Wacker, W.B., Donoso, L.A., Kalsow, C.M., Yankeelov, J.A. jr and Organisciak, D.T. (1977) J. Immunol. 119, 1949–1958.

[2] Kalsow, C.M. and Wacker, W.B. (1978) Invest. Ophthalmol. Vis. Sci. 17, 774–783.

[3] Shinohara, T., Donoso, L.A., Tsuda, M., Yamaki, K. and Singh, V.K. (1988) in: Progress in Retina Research (Osborne, N. and Chader, J. eds) vol. 8, pp. 51–66, Pergamon Press, London.

[4] Gery, I., Mochizuki, M. and Nussenblatt, R.B. (1986) in: Progress in Retina Research (Osborne, N. and Chader, J. eds) vol. 5, pp. 75–109, Pergamon Press, London.

[5] Tsuda, M., Syed, M., Bugra, K., Whelan, J.P., McGinnis, J.F. and Shinohara, T. (1988) Gene 73, 11–20.

[6] Yamaki, K., Takahashi, Y., Sakuragi, S. and Matsubara, K. (1987) Biochem. Biophys. Res. Commun. 142, 904–910.

[7] Shinohara, T., Dietzschold, B., Craft, C.M., Wistow, G., Early, J.J., Donoso, L.A., Horwitz, J. and Tao, R. (1987) Proc. Natl. Acad. Sci. USA 84, 6975–6979.

[8] Yamaki, K., Tsuda, M. and Shinohara, T. (1988) FEBS Lett. 234, 39–43.

[9] Donoso, L.A., Merryman, C.F., Shinohara, T., Sery, T.W. and Smith, A. (1987) Arch. Ophthalmol. 105, 838–840.

[10] Donoso, L.A., Merryman, C.F., Sery, T.W., Shinohara, T., Dietzschold, B., Smith, A. and Kalsow, C.M. (1987) Curr. Eye Res. 6, 1151–1159.

[11] Singh, V.K., Yamaki, K., Donoso, L.A. and Shinohara, T. (1988) Curr. Eye Res. 7, 78–92.

[12] Singh, V.K., Nussenblatt, R.B., Donoso, L.A., Yamaki, K., Chen, C. and Shinohara, T. (1988) Cell Immunol. 115, 413–419.

[13] Singh, V.K., Donoso, L.A., Yamaki, K. and Shinohara, T. (1989) Autoimmunity, in press.

[14] Kuhn, H. and Wilden, U. (1984) FEBS Lett. 176, 473–478.

[15] Wilden, U., Hall, S.W. and Juhn, H. (1986) Proc. Natl. Acad. Sci. USA 83, 1174–1178.

[16] Zuckerman, R.Z. and Cheasty, J.E. (1986) FEBS Lett. 207, 35–41.

[17] Vigh-Teichman, I. and Vigh, B. (1986) in: Pineal and Retinal Relationships (O'Brien, P.J. and Klein, D.C. eds) pp. 401–413, Academic Press, New York.

[18] Van Veen, T., Ostholm, T., Gierschik, P., Spiegel, A., Somers, R., Korf, H.W. and Klein, D.C. (1986) Proc. Natl. Acad. Sci. USA 83, 912–916.

[19] Vigh, B. and Vigh-Teichman, I. (1981) Cell Tissue Res. 221, 451–463.

[20] Benovic, J.L., Juhn, H., Weyand, I., Codina, J. Caron, M.G. and Lefkowitz, R.J. (1987) Proc. Natl. Acad. Sci. USA 84, 8879–8882.

[21] Yarden, Y., Rodriguez, J., Wong, S.K.-F., Brandt, D.R., May, D.C., Burnier, J., Harkins, R.N., Chen, E.Y. and Ramachandran, J. (1986) Proc. Natl. Acad. Sci. USA 83, 6795–6799.

[22] Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

[23] Garner, J., Osguthorpe, D.J. and Robson, B. (1978) J. Mol. Biol. 120, 97–120.

[24] Needleman, S.B. and Wunsch, C.D. (1970) J. Mol. Biol. 48, 443–453.

[25] Shinohara, T., Craft, C.M., Stein, P., Zigler, J.S., jr, Wistow, G., Katail, A., Gery, I. and Klein, D.C. (1986) in: Pineal and Retinal Relationships (Klein, D.C. and O'Brien, P. eds) pp. 331–355 , Academic Press, New York.

[26] Dixon, R.A., Kobilka, B.K., Strader, D.J., Benovic, J.L., Dohlman, H.G., Frielle, T., Bolanowski, M.A., Bennett, C.D., Rands, E., Diehl, R.E., Mumford, R.A., Slater, E.E., Sigal, I.S., Caron, M.G., Lefkowitz, R.J. and Strader, C.D. (1986) Nature 321, 75–79.

[27] Benovic, J.L., Mayor, F., jr, Somers, R.L., Caron, M.G. and Lefkowitz, R.J. (1986) Nature 321, 869–872.