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Isolation and Nucleotide Sequence of a Partial cDNA Clone for Bovine Opsin

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Bovine cDNAs were cloned by using a mixture of 18-base-long synthetic deoxyribonucleotides as a hybridization probe. The longest cDNA clone (pBO-1) contained an 811-bp insert that included the 434 bp of the coding region corresponding to the C-terminal 144 amino acid residues of opsin peptide and the 377 bp of the 3'-untranslated region. The size of opsin mRNA was determined as 23 S by Northern blot hybridization. Bovine liver DNA gave rise to a single band of 2.8 kb, 1.1 kb and 7.9 kb each with Eco RI, Hind III and Bam HI, respectively, by Southern blot hybridization with pBO-1 as probe. Therefore, bovine opsin gene may occur once per haploid genome.

Visual pigments are photoreceptor proteins of visual cells present in animal retinas. On absorption of light, visual pigments bleach through several intermediates (1-3). Coupling with the bleaching an amplified excitatory response is caused in the visual cells (4). All of the visual pigments that have been reported have 11-cis retinal as their chromophore. Bacteriorhodopsin, which is present in the purple membrane of a number of extremely halophilic bacteria, also has retinal as the chromophore. It has been well characterized and its gene has been isolated (5,6).

Although bovine retina possesses only one kind of visual pigment, called rhodopsin, some animals including human, chicken, frog and goldfish have other kinds of visual pigments for color vision in addition to rhodopsin which are detected by spectroscopic methods (7-9). Little is known about the biochemical properties of these color visual pigments. Only recently, the primary

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structure of bovine opsin (apoprotein of visual pigment) has been determined (10,11). We are interested in the properties of these visual pigments, the structure and regulation of expression of their genes and the mechanism how animals aquire color visual pigments in the process of evolution. In order to solve these problems, direct analysis of visual pigment gene is indispensable. As the first step of the experiments, we have isolated the bovine opsin cDNA clone and determined the partial nucleotide sequence.

MATERIALS AND METHODS

Bovine retinas were homogenized in 0.25M sucrose, 50mM Tris-HCl pH 7.5, 25mM KCl, 5mM $MgCl_2$, 0.1 % diethylpyrocarbonate and centrifuged at 7,000 x g for ten minutes. Post mitochondrial supernatant was centrifuged at 186,000 x g for two hours at 4°C. Pellet was homogenized in 0.1M NaCl, 0.1M Tris-HCl pH9.0, 1mM EDTA, 1 % SDS. RNA was extracted by phenol-chroloform-isoamyl alcohol (50:50:2). Poly$(A)^+$ RNA was prepared by two-cycles of oligo(dT)-cellulose column chromatography (12).

Single-stranded cDNA and subsequently double-stranded cDNA were synthesized at 42°C from 30 µg of bovine retina mRNA by using avian myeloblastosis virus reverse transcriptase as described previously (13). Ds-cDNA was treated with S1 nuclease, inserted into Pst I site of pBR322 employing oligo(dC)-oligo(dG) tailing method and then used to transform E. coli strain χ1776.

The mixed oligonucleotides shown in Table 1 were synthesized by phosphotriester method on polystyrene (14) by using programmed synthesizer (Solid Phase Synthesizer Model 25A, Genetic Design Co.,) (15).

Bacterial colonies which contained the opsin cDNAs were screened by colony hybridization with oligonucleotide probe by the method of Wallace et al (16) with some modifications. Seven colonies that were hybridized with the mixture of synthetic probes were isolated from 6,400 transformants. One plasmid, designated pBO-1, which contained the longest cDNA sequence was chosen for further characterization.

Plasmid DNA was prepared essentially as described by Currier and Nester (17).

DNA sequencing was done by the chemical degradation method of Maxam and Gilbert (18).

The size of opsin mRNA was determined by Northern blot hybridization as described by Thomas (19).

Bovine liver DNA was analyzed by Southern blot hybridization with pBO-1 DNA (see below) as probe. Bovine liver DNA was digested with Eco RI, Hind III or Bam HI, resolved by electrophoresis on 0.8 % agarose gel, transferred to nitrocellulose by the method of Southern (20). pBO-1 probe was hybridized and then washed under several different conditions.

Table 1 Oligonucleotide probes for the isolation of opsin cDNA

		308	309	310	311	312	313	
Amino acid sequence		Met	Met	Asn	Lys	Gln	Phe	
Possible codons	5'	AUG	AUG	AA^U_C	AA^A_C	CA^A_G	UU^U_C	3'
Probes	3'	TAC	TAC	TT^A_G	TT^T_C	GT^T_C	TT^A_G	5'

RESULTS AND DISCUSSION

Restriction map and nucleotide sequence of pBO-1

Restriction map, sequence strategy and the nucleotide sequence of pBO-1 were shown in Fig. 1. The sequence contained 434 bp of the coding region

A

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                                    210                                 220
              Ile Tyr Met Phe Val Val His Phe Val Ile Pro Leu Ile Val Ile Phe
TC ATC TAC ATG TTC GTG GTA CAC TTC GTC ATC CCC CTG ATT GTC ATA TTC

                                    230                                 240
Phe Cys Tyr Gly Gln Leu Val Phe Thr Val Lys Glu Ala Ala Ala Gln Gln Gln Glu Ser
TTC TGC TAC GGG CAG CTG GTG TTC ACC GTC AAG GAG GCG GCT GCC CAG CAG CAG GAG TCG

                                    250                                 260
Ala Thr Thr Gln Lys Ala Glu Lys Glu Val Thr Arg Met Val Ile Ile Met Val Ile Ala
GCC ACC ACT CAG AAG GCG GAG AAG GAG GTC ACC CGC ATG GTG ATC ATC ATG GTC ATC GCT

                                    270                                 280
Phe Leu Ile Cys Trp Leu Pro Tyr Ala Gly Val Ala Phe Tyr Ile Phe Thr His Gln Gly
TTC CTA ATC TGC TGG CTG CCC TAC GCT GGG GTG GCG TTC TAC ATC TTC ACC CAT CAG GGC

                                    290                                 300
Ser Asp Phe Gly Pro Ile Phe Met Thr Ile Pro Ala Phe Phe Ala Lys Thr Ser Ala Val
TCT GAC TTT GGC CCC ATC TTC ATG ACC ATC CCG GCT TTC TTT GCC AAG AGT TCA GCC GTC

                                    310                                 320
Tyr Asn Pro Val Ile Tyr Ile Met Met Asn Lys Gln Phe Arg Asn Cys Met Val Thr Thr
TAC AAC CCC GTC ATC TAC ATC ATG ATG AAC AAG CAG TTC CGG AAC TGC ATG GTC ACC ACT

                                    330                                 340
Leu Cys Cys Gly Lys Asn Pro Leu Gly Asp Asp Glu Ala Ser Thr Thr Val Ser Lys Thr
CTC TGC TGT GGC AAG AAC CCG CTG GGT GAC GAC GAG GCC TCC ACC ACC GTC TCC AAG ACA

                                348
Glu Thr Ser Gln Val Ala Pro Ala
GAG ACC AGC CAG GTG GCG CCT GCC TAAGCCCCTCCAGGGACTCCGTGGCCAGCTGCAGGAGTCCCTCAGCC

CCCACCCCACCCCAGCCTCAGCAGCTCCATCAGGAGCCGCGCCTGTCGGAACCAGCTCTCACAGGCTCCCTGAGTGGAA

CACAAAGACCAACCAACCAAATGCAAAAGAATCAACGAGAGAAACAGGAGGCGCCTCACGTGGCAGGGGCGGCCCGATC

TGGAGTCCTGATTTCCCGGGGGCCCGCTGCAGATCCACTCCCCCCAGCTCATCTCTCAGCTACACAAGAGCTCTTGCTC

TGGAAAAGTGTCCCAGCTTAGGGATAAGTGAGTAGCACATGACGGGGCATGCCTGAGGTGCTTATTAATAAATGCTAGG

TGGAGGAAAGAAGG
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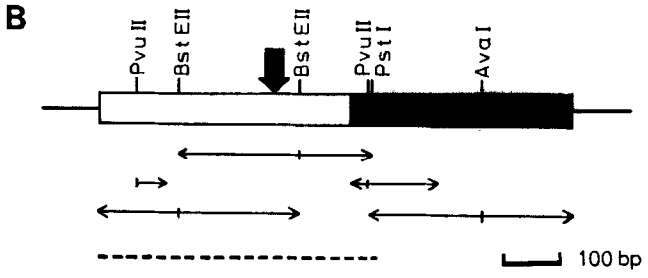

Fig. 1. Nucleotide sequence (A) and restriction endonuclease map with strategy for sequencing (B) of pBO-1. White and black boxes indicate the coding region and 3'-untranslated region, respectively. The horizontal arrows indicate the fragments that were sequenced. The thick arrow indicates the position of synthetic oligonucleotide probe. Dotted line shows the region used as a probe for Southern and Northern blot hybridization.

(amino acid residues 204 to 348) and 377 bp of the 3'-untranslated region. The amino acid sequence deduced from the nucleotide sequence was identical to that determined by amino acid sequencing techniques except for one amino acid difference. At position 213, we identified valine instead of isoleucine which had been reported by Ovchinnikov (10) and Hargrave et al (11). This may reflect either polymorphism of bovine opsin gene or the misreading by the reverse transcriptase. This will be determined by sequencing another cDNA clone. The putative polyadenylation sequence, AATAAA was present 21 bases away from the 3'-terminal end of pBO-1 clone. However, the poly(A) tract was not present within this region. The experiments for the isolation of longer cDNA clones which include the 5'-terminal region and 3' poly(A) sequence were now in progress.

Northern blot hybridization

The result of Northern blot hybridization shown in Fig. 2A suggests that the size of opsin mRNA was 23 S which corresponds to 3,000 bases long. Bovine opsin consists of 348 amino acid residues and has no signal peptide at the NH_2-terminus (21), which means that the number of nucleotides needed to encode

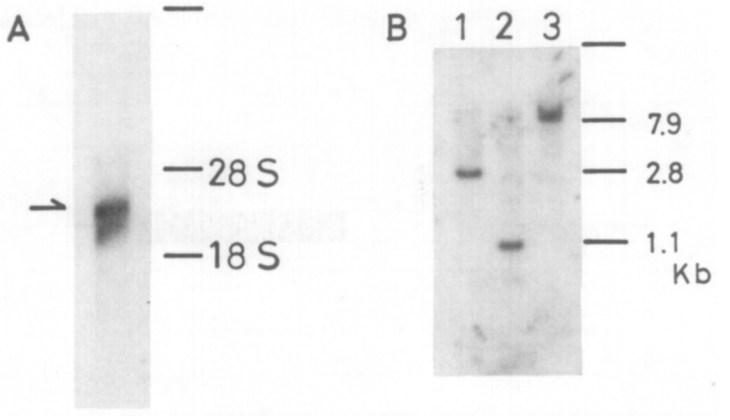

Fig. 2A. Autoradiogram of Northern blot hybridization. Chicken liver rRNA was used as the marker. The arrow indicates the opsin mRNA whose S value is approximately 23.
B. Southern blot analysis of genomic DNA. Bovine liver DNA was digested with Eco RI (1), Hind III (2) and Bam HI (3). Filter was hybridized to the probe and washed at 65°C with 0.1 x SSC, 0.1 % Sodium N-lauroyl Sarcosinate. A single restriction fragment hybridized to the probe in each case.

opsin is 1,044. Therefore, the result suggests that the opsin mRNA has very long untranslated regions.

Southern blot analysis of genomic DNA

Fig. 2B shows the Southern blot analysis of the total genomic DNA of bovine liver. With three different restriction enzymes, only a single restriction fragment hybridized to pBO-1 probe in each digest. It means that bovine opsin is encoded most probably by a single-copy sequence. To check the presence of pseudogenes, filters were washed under less stringent conditions, however, no other bands were observed.

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