Molecular and Biochemical Characterization of a Novel Oxysterol-binding Protein (OSBP2) Highly Expressed in Retina*

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We are interested in understanding the possible function(s) of the oxysterol-binding proteins in mediating oxysterol cytotoxicity in the retina. In this study we describe the cloning, localization, and biological activity of a novel oxysterol-binding protein (OSBP2), and complete the molecular characterization of the previously known OSBP1. Both OSBP genes contain 14 exons and have similar exon sizes and splice sites suggesting they may have arisen from a gene duplication event. OSBP1 is located in chromosome 11q12.1, and OSBP2 is located in 22q12. At the protein level they share 63% overall similarity and although they have unique N termini, both have similar pleckstrin homology domains within the N terminal region. Northern blot analyses indicate that OSBP1 is broadly expressed in human and monkey tissues. OSBP2 is detected mainly in retina, testis, and fetal liver. Western blot analysis using peptide antibodies specific to OSBP1 and OSBP2 detected the proteins in different subcellular fractions in the retinal monkey tissue. OSBP1 is detected mainly in the soluble or cytosolic fraction and nuclei whereas OSBP2 is detected exclusively in the detergent soluble fraction suggesting association with membranes. Immunohistochemical localization of OSBP1 and OSBP2 in the monkey retina placed these two proteins in similar but distinct areas of the inner retina. OSBP2 was found to bind 7-ketocholesterol but to have very little affinity for cholesterol or 25-hydroxycholesterol.

The oxysterol-binding protein (OSBP)1 has been known for many years and has been fairly well characterized biochemically. It was originally detected as a cytosolic protein capable of binding 25-hydroxycholesterol (1). OSBP, as its name implies, is capable of binding a wide variety of oxidized forms of cholesterol with high affinity (2). It was partially purified and characterized in mouse-cultured fibroblast cells (3). The cDNA was first isolated in rabbit (4), then in human (5). OSBP is known to translocate to the Golgi apparatus after binding to oxysterols (6), and this translocation is mediated via a pleckstrin homology domain near the N terminus of the protein (7). OSBP is phosphorylated in the Golgi (8), and this phosphorylation and Golgi transport seem to be dependent on cholesterol trafficking and sphingomyelin hydrolysis (9, 10).

Oxysterols accumulate in tissues and can exert potent pharmacological effects on cellular sterol biosynthesis and uptake. Oxysterols are oxidized byproducts of cholesterol (11) that cause cytotoxic effects on a variety of cells. This oxysterol cytotoxicity is exerted via apoptosis and has been implicated in the pathophysiology of atherosclerosis (12, 13). Oxysterol cytotoxicity has been demonstrated in vascular endothelial cells (14), smooth muscle cells (15), and cultured neuroretinal cells (16). The mechanism by which oxysterols induce cell death is not fully elucidated but some scientific evidence suggests that it may involve the oxysterol-binding proteins (17). Although oxysterols will dramatically reduce the hydroxymethylglutaryl CoA reductase activity and therefore essentially shut down cholesterol synthesis, their induction of apoptosis cannot be rescued by adding excess cholesterol (17). This suggests that OSBP may be playing a central role in the oxysterol-induced apoptosis.

The oxysterol-binding protein is a member of a family of proteins that share structural and possibly functional similarities (18). Our interest in the OSBP is to investigate their role and that of their oxysterol ligands in ocular tissues. More specifically we are interested in determining whether OSBP and oxysterols are involved in the pathogenesis of age-related ocular diseases such as macular degeneration and cataracts. There is existing evidence suggesting that oxysterol cytotoxicity may be playing a role in the formation of cataracts (19).

A recent study found an OSBP-like protein expressed exclusively in metastatic tumor cells (20). The study linked this OSBP-like gene to tumor dissemination. This publication did not provide a complete molecular characterization of this OSBP, but alignments performed in our laboratory indicate that it is likely our novel OSBP. If this is indeed correct, this makes the identification and characterization of this gene even more important. It may also suggest that oxysterols could be playing a role not only in atherosclerosis but in cancer as well.

One of the genes presented in our study is the original OSBP and will be referred to as OSBP1. The novel homolog will be referred to as OSBP2. We have cloned and characterized the OSBP2 gene as well as completed the molecular characterization of the OSBP1 gene. A detailed comparative analysis of OSBP1 and OSBP2 at the molecular and biological level is presented.

EXPERIMENTAL PROCEDURES

Monkey Retinal Tissue—Fresh eye tissue was obtained from rhesus monkeys (Macaca mulatta, 2–3 years old) through the courtesy of the
Center for Biologic Research and Testing, U.S. Food and Drug Administration (Bethesda, MD). Animal studies were conducted in accordance with the NIH guidelines on the care and use of animals in research. The monkeys were anesthetized then exsanguinated, and the enucleation was performed within 3 to 4 min after death. The eyes were immediately kept in liquid nitrogen and stored at −75 °C for further processing. Each retinal sample was from a pool of ten eyes.

**DNA Sequence**—The DNA was sequenced using a PE-Applied Biosystems Model 377 automated fluorescence sequencer (Beckman Instruments). The sequencing reactions were performed according to the manufacturer’s specifications.

**Screening of the Human Retina cDNA Library and Rapid Amplification of cDNA Ends (RACE)—**A human retina cDNA library in lambda ZAP (Stratagene, La Jolla, CA) was screened using standard methodology. OSBP1- and OSBP2-specific PCR probes were generated from EST sequences. The 5'-RACE was performed using human retina cDNA synthesized on magnetic Dynabeads® (Dyna Inc., Oslo, Norway) as previously described (21). The same solid-phase cDNA was used to perform 3'-RACE using a degenerate oligo(dt)-primer (NNTTTTTTTTTTTTTTTTTTTTTT). The amplified products were either sequenced directly or cloned using the TOPO-TA cloning kit (Invitrogen, Carlsbad, CA).

**Subcellular Fractionation of Whole Monkey Retina—**Subcellular fractionation of the retina was performed as previously described (22). Fresh rhesus monkey (Macaca mulatta) retinas (10 retinas) were homogenized in 50 ml of 10 mM HEPES buffer, pH 7.2, containing 5 mM MgCl₂, 4% (w/v) sucrose and Complete Inhibitor (1 tablet/50 ml, Roche Molecular Biochemicals). The homogenate was subjected to a low speed (300 × g) centrifugation to separate the nuclei (P1) and the remaining supernatant was centrifuged at high speed (27,000 × g) to pellet the subcellular organelles (P2). The P2 pellet was subsequently subjected to a 0.5 M NaCl wash (P2-salt) and a 2% Triton X-100 wash (P2-detergent). The remaining pellet (P2 residue) was not further processed. The protein amounts for each of the fractions are: S2 (50 ml, 2 mg/ml) 100 mg, P2-salt (15 ml, 0.3 mg/ml) 4.5 mg, and P2-detergent (15 ml, 0.5 mg/ml) 7.5 mg.

**Northern Blot Analysis and Quantification—**Human RNAs were either purchased from CLONTECH (Palo Alto, CA) or purified directly from human and monkey tissues using the RNeasy Kit from Qiagen (Valencia, CA). The Northern blots were probed with 32P-labeled PCR from human and monkey tissues using the RNeasy Kit from Qiagen or purchased from CLONTECH (Palo Alto, CA) or purified directly from human and monkey tissues using the RNeasy Kit from Qiagen. The blots were washed in 0.1 M NaCl wash (P2-salt) and a 2% Triton X-100 wash (P2-detergent). The amplified products were either sequenced directly or cloned using the TOPO-TA cloning kit (Invitrogen, Carlsbad, CA).

**Preparation of Antibodies to Human OSBP1 and OSBP2**—An alignment of the known OSBP peptides was performed using MegAlign software (DNASTAR Inc., Madison, WI) to identify peptide regions specific to each OSBP. Two unique peptides were synthesized for OSBP1 (EQYKHQLEETK and PLGTHICIFHGHHTY), and one peptide was synthesized for OSBP2 (TVTTEAKEDSKRAEGS). The OSBP1 antibodies detect a truncated protein in the retina but the correct size peptide is detected in the P1 nuclear fraction and the P2 residue. The anti-OSBP2 antibodies react only with a protein at the correct molecular weight present in the detergent soluble fraction of the retina. The anti-OSBP2 antibodies react vigorously and specifically with recombinant OSBP2, but the anti-OSBP1 antibodies do not cross-react with recombinant OSBP2.

**SDS-Polyacrylamide Gel Electrophoresis and Western Blot Analysis—**Protein samples were run on 7.5% polyacrylamide gels and blotted onto nitrocellulose filters. The blots were probed with 32P-labeled PCR from human and monkey tissues using the RNeasy Kit from Qiagen (Valencia, CA). The blots were washed in 0.1 M NaCl wash (P2-salt) and a 2% Triton X-100 wash (P2-detergent). The amplified products were either sequenced directly or cloned using the TOPO-TA cloning kit (Invitrogen, Carlsbad, CA).

**Immunocytochemistry—**Paraflin-embedded monkey retina sections were de-paraffinized twice each with xylene and chloroform followed by 100% ethanol phosphate-buffered saline/Tween 20. The sections were blocked using 1:100 diluted goat serum for 15 min. The tissue sections were then rinsed and incubated with anti-OSBP1 (1:200) or anti-OSBP2 (1:100) antibody for 2 h followed by additional washes with TBS/Tween 20. The secondary antibody (mouse anti-rabbit alkaline phosphatase conjugate, same as for Western blots above) was applied at 1:500 dilutions for 1 h. After the PBS/Tween 20 washes, the sections were developed using Fast Red (Fast Red tablets, Roche Molecular Biochemicals) for 15 min according to the manufacturer’s instructions.

**Identification and Cloning of cDNAs for OSBP1 and OSBP2—**We initially cloned the extended 3'-end portion of the OSBP1 cDNA while mapping human ESTs to 11q13. Using a PCR probe derived from these EST sequences, we screened a human fetal retina cDNA library and isolated a clone that extended the previously cloned OSBP1 cDNA (5) −2 kb. We were not aware of the identity of our cDNA until its 5'-end sequence matched the 3'-end portion of the previously reported OSBP1 cDNA (5), GenBank™ accession number NM_002556. We then constructed the complete cDNA from the sequence of our overlapping clones and the ESTs in GenBank™. The entire cDNA sequence was also confirmed by sequencing the genomic clone (see below). The full-length cDNA for OSBP1 is 5083 bp in length and can be obtained from GenBank™, accession number AF185696.

The OSBP2 gene was first identified through a BLASTN (24) search of GenBank™ using the OSBP1 cDNA sequence. A large portion of the OSBP1 peptide was represented in matching regions of chromosome 22 (accession number AC004542).
These regions were sequential but not contiguous, thus defining exon and intron structures. The genomic sequences and several testes-derived ESTs were used to construct a partial cDNA sequence for OSBP2, which served as a basis for designing primers for generating a specific probe. Solid-phase 5'- and 3'-RACE was performed to complete the cDNA sequence using human retina cDNA synthesized on magnetic Dynabeads® (21). Two types of cDNA clones were isolated by 3'-RACE differing in the length of the 3'-untranslated sequence. The most abundant OSBP2 cDNA in the retina is 2791 bp in length. A longer message of 4238 bp is found primarily in testis but can also be detected in small amounts in retina. The open reading frame of both messages encodes a peptide of 878 amino acids. Polyadenylation signals are found at positions 2763–2768 and 4206–4211. The different sizes for OSBP2 seem to be consistent with the use of these two different polyadenylation signals. The cDNA sequence for human OSBP2 is available from GenBank™ (accession number AF288741). The cDNA sequence and comparison with OSBP1 is shown in Fig. 1. The similarity between OSBP1 and OSBP2 mRNA is 38.7% overall and 52% in the coding region. At the protein level, OSBP1 and OSBP2 share 63% identity (Fig. 2).

Gene Structure and Localization of OSBP1 and OSBP2—The OSBP1 gene was isolated by PCR screening using Incyte...
Genomics (St. Louis, MO). The 3′-end PAC clone, p17641, was isolated using primers 6436 (ATGTAGCTTATCCACTGTA) and 6437 (GGGCTTTGGATAGAGTGAG). This clone contained exons 8 to 14 but lacked the 5′-end region of the gene. Two other PACs (p19144 and p19145) were isolated using primers 3F (GCAGCGCCTGGAGGAAAAACA) and 4R (CTACTTCTCAG). These PACs contained the 5′-end but lacked the middle region containing exons 4–7. Additional attempts to clone the middle region in the PAC libraries failed. A BAC library was then screened using primers 3F (TCACCGTGTCAT), which amplified a 494-bp product across exons 8 and 9. Three BAC clones (p20714, p20715, and p20716) were isolated, which contained the 5′-end and middle regions of the gene. The gene was found to consist of a total of 14 exons. The complete size of the introns was determined using sequence available from GenBank™ accession number AP00442 recently submitted by The Eugene McDermott Center for Growth and Development.

The relative levels of expression of the OSBPs were quantified by normalizing the SYB Green II (Molecular Probes) stained 28S ribosomal RNA band to the OSBP1 and OSBP2 isotope hybridization signal using a Storm 860 PhosphorImager as previously described (29). The macula is a retinal substructure that among mammals is unique to primates. This structure is responsible for the exquisite visual acuity and color vision enjoyed by our species. We have no evidence that this alternatively spliced message is translated. The relative levels of expression of the OSBPs were quantified by normalizing the SYB Green II (Molecular Probes) stained 28S ribosomal RNA band to the OSBP1 and OSBP2 isotope hybridization signal using a Storm 860 PhosphorImager as previously described (29).
| INTRON | DONOR SIZE (bp) | ACCEPTOR SIZE (bp) | EXON 5' | EXON 3' |
|--------|----------------|--------------------|---------|---------|
| **OSBP1 gene structure at 11q12.2** | | | | |
| 5' flanking | ... | ... | ... | ... |
| gtaactgct | -4722 | ttattacag | 1 | ... |
| gttcaggac | -1648 | ttcctacag | 2 | ... |
| gttgatgca | -6647 | tccctacag | 3 | ... |
| gttagagac | 254 | ttccttcag | 4 | ... |
| gttacttct | 191 | ttgctcctacag | 5 | ... |
| gttacctagtct | >35 kb | ttcctacag | 6 | ... |
| gttgaggaas | 85 | tttctcctacag | 7 | ... |
| gttgatgtta | >12 kb | gttctcctacag | 8 | ... |
| gttgagggaaa | ~1185 | attttcctacag | 9 | ... |
| gttagggaaa | -1845 | cccctccctacag | 10 | ... |
| gttgaggtac | -1123 | ttcttcctacag | 11 | ... |
| gttatgacac | 85 | ttcccttcctacag | 12 | ... |
| gttgatcttgg | ... | 3' flanking | ... | ... |

| **OSBP2 gene structure at 22q12** | | | | |
| 5' flanking | ... | ... | ... | ... |
| gttatggag | 45,607 | accttcctacag | 1 | ... |
| gttgtagggc | 129,059 | ctcctcctacag | 2 | ... |
| gttgaggtc | 1,6743 | ccccttcctacag | 3 | ... |
| gttgagggg | 504 | tgtctcctacag | 4 | ... |
| gtttagggag | 836 | ttctctcctacag | 5 | ... |
| gttgaggggg | 254 | atgtgagggggg | 6 | ... |
| gttgagggtggc | 1,091 | caccctcctacag | 7 | ... |
| gttgagggggtgc | 2,148 | tgggtgctcctacag | 8 | ... |
| gttgagcggcag | 220 | tgccttcctacag | 9 | ... |
| gttcaggccgc | 71 | cccctcctacag | 10 | ... |
| gttgagggggc | 83 | tgtccctcctacag | 11 | ... |
| gttgaggtggc | 11,835 | cccctccctcctacag | 12 | ... |
| gttgagggggt | 127 | cccctccctcctacag | 13 | ... |
| cccctgccttc | ... | 3' flanking | ... | ... |

The intron-exon junctions for OSBP1 and OSBP2.
were separated into the neural retina and the corresponding retinal pigment epithelium (RPE) choroid. Therefore, four different pieces of tissue were examined, the neural macula (NM), the macular pigment epithelium choroid, the peripheral neural retina, and the peripheral pigment epithelium choroid. It should be noted that the RPE is a single cell layer between the neural retina and the choroid. In young monkeys, the separation of the choroid from the neural retina may lead to some contamination of the neural retina with RPE. However, in our experience most of the RPE cell bodies remain with the choroid. If done carefully, there is no observable contamination of the peripheral pigment epithelium choroid with neural tissue.

A Northern blot was performed using RNA extracted from the four different tissues and probed with the OSBP1- and OSBP2-specific probes. OSBP1 showed similar levels of expression in the NM, macular pigment epithelium choroid, and peripheral neural retina, but little or no expression in the peripheral pigment epithelium choroid. It should be noted that the RPE is a single cell layer between the neural retina and the choroid. In young monkeys, the separation of the choroid from the neural retina may lead to some contamination of the neural retina with RPE. However, in our experience most of the RPE cell bodies remain with the choroid. If done carefully, there is no observable contamination of the peripheral pigment epithelium choroid with neural tissue.

Expression of OSBP1 and OSBP2 Proteins in Monkey Retina—To determine whether OSBP1 and OSBP2 proteins were expressed in retina, antibodies to peptides unique to human OSBP1 and OSBP2 were raised in rabbits. Tissue punches from different areas of the monkey retina (macula versus peripheral retina) were used to prepare protein fractions for SDS-polyacrylamide gel electrophoresis. OSBP1 was clearly detectable by Western blot analysis in the monkey neural macula and peripheral retina but not in the RPE/choroid punches, whereas OSBP2 was undetectable in the same samples (data not shown). A subcellular fractionation was performed in whole monkey retina (including RPE and choroid) to see whether the proteins differentially fractionated with the various organelle fractions. Western blots of the subcellular fractions (Fig. 5) detected OSBP1 in all fractions (Fig. 5B), whereas OSBP2 was detected only in the P2-detergent fraction (Fig. 5C). In most of the fractions OSBP1 was detected as a truncated or processed 50-kDa form, but in the nuclear fraction the full-length peptide (~80 kDa) was detected. OSBP2 was detected as a 90-kDa protein, and its extraction from the P2 pellet with detergent suggests that it may be associated with membranes.
FIG. 5. Western blot of subfractionated whole monkey retina. The whole monkey retina (including the pigment epithelium and choroid) was subfractionated as previously described under “Experimental Procedures.” Each lane was loaded with ~20 μg of protein. Panel A is a Coomassie Blue-stained gel representative of the two blotted gels. Panels B and C are Western blots probed with OSBP1 and OSBP2 antibodies, respectively. An additional lane was included in panel C containing recombinant thioredoxin OSBP2 as a positive control. The immunoreactivity in both blots can be completely eliminated by adding the immunizing peptides to the primary antibodies.

FIG. 6. Immunocytochemical localization of OSBP1 and OSBP2 in monkey retina. The monkey retina tissue preparation and the immunohistochemical process were performed as described under “Experimental Procedures.” The macula/fovea region was photographed at half the magnification of that shown for the peripheral retina and control. The size of the black bar is 100 μm and was determined using a micrometer. The controls shown are peripheral retina, which was identically treated except that the primary anti-OSBP1 and anti-OSBP2 antibodies were pre-treated with the immunizing peptide.
Immunocytochemical Localization of OSBP1 and OSBP2 in Monkey Retina—OSBP1 and OSBP2 were localized in the monkey retina using the peptide antibodies described above. Representative photographs of the macula/fovea region and peripheral retina are shown in Fig. 6. The controls were treated identically except that the primary antibodies were pre-incubated with the immunizing peptides. The black bar represents 100 μm in length. In the macula/fovea region, OSBP1 localizes to the ganglion cell layer, inner plexiform layer, inner nuclear layer (INL), outer plexiform layer, and the RPE. Little or no labeling was observed in the outer nuclear layer or the rod outer segments. The OSBP1 labeling in the ganglion cell layer seems to involve Müller cells, and in the INL, it seems to occur in a subset of bipolar cells. OSBP2 localizes to the ganglion cell layer but in a different pattern from OSBP1, suggesting preference for a subset of ganglion cells. Like OSBP1, OSBP2 localizes to the inner plexiform layer and INL. In the INL, the OSBP2 was localized to a subset of cells that are adjacent to the inner plexiform layer, possibly amacrine cells. Diffuse OSBP2 labeling is observed in other cells of the INL, suggesting some localization to bipolar, Müller, and/or horizontal cells. OSBP2, unlike OSBP1, does not seem to be present in the outer plexiform layer, but like OSBP1, is also absent from the outer nuclear layer and rod outer segments. The RPE also seems to contain OSBP2. Similar results were observed for both OSBPs in the peripheral retina. The peripheral retina contains significantly fewer inner retinal cells than the macula/fovea region, thus the heavily labeled ganglion and amacrine cells are more sparse in this area of the retina.

Oxysterol Binding Activity of OSBP1 and OSBP2—Oxysterol-binding activity was detected in the monkey retina subfraction by incubating with tritiated oxysterols and separating the proteins by HPLC. The S2 (soluble) and P2-detergent fractions were incubated with [3H]cholesterol (CH), [3H]25-hydroxycholesterol (25HC) and [3H]7-ketocholesterol (7KC). The proteins were fractionated by size using a two-column system (TSK-4000, TSK-3000). The proteins containing bound sterols were detected using an in-line radiochromatography detector. The free highly hydrophobic oxysterols and cholesterol bind to the pre-column and are not eluted under these conditions. Binding activity was found in both the soluble protein fraction S2 and the P2-detergent fraction (Fig. 8, A and B). In the S2 soluble fraction (Fig. 7A), CH binding activity was located mainly in the void volume of the column, suggesting a possible nonspecific association with macromolecules. Another peak was detected around 28 min, which bound 25HC preferentially, but also 7KC and CH. The P2-detergent fraction (Fig. 7B) also showed two CH binding peaks at 11 and 14 min, but the main peak occurred at 30 min, preferentially binding 7KC. The 30-min peak showed no CH binding activity and very little 25HC binding activity. Because OSBP2 was detected only in the P2-detergent fraction and no 7KC binding activity was detected in the S2 fraction, we suspected that this activity is because of OSBP2 binding. Western blot analysis was performed across the 30-min peak (Fig. 7C) to delineate the elution profile of OSBP2. The results indicate that the 30-min 7KC binding activity co-elutes with the OSBP2 immunoreactivity. The results also suggest that OSBP2 may have preferential binding for 7KC.

Several additional controls were performed to demonstrate that the 7KC binding activity found in the P2-detergent fraction are because of the presence of OSBP2. 1) Treatment of the P2-detergent fraction with Proteinase K at room temperature in neutral buffer (conditions that do not effect the 7KC binding) completely obliterated all binding activity in a few hours. 2) Pre-extraction of the S2 and P2-detergent fraction with petroleum ether to remove free and/or bound sterols did not significantly change the results shown in Fig. 7. 3) Addition of anti-OSBP2 antibody to the P2-detergent fraction moved the 7KC binding activity to a larger size peak around 20 min and further addition of anti-OSBP2 removed all 7KC binding activity. These controls indicate that the 7KC activity found in the P2-detergent fraction is because of OSBP2 specifically.

**DISCUSSION**

We are reporting the molecular and biological characterization of a novel oxysterol-binding protein, OSBP2, and extend the molecular characterization of the previously reported OSBP1. We became interested in OSBP2 because of its high and almost unique expression in the retina as well as its striking similarity to OSBP1. Both genes contain 14 similarly sized exons and nearly identical intron-exon junctions (Fig. 1, Table I). The genes...
share a high degree of similarity both at the nucleotide (Fig. 1) and protein levels (Fig. 2) and may have derived from a gene duplication event. At the peptide level, OSBP1 and OSBP2 share the oxysterol-binding domain and the pleckstrin homology domain (7) and are almost identical toward the C terminus. Recent evidence from other investigators (18) and from unpublished data within our group indicates that oxysterol-binding proteins are a large and complex family of genes.

Although OSBP1 and OSBP2 are structurally very similar, they undergo different transcriptional and posttranslational regulation. Although OSBP1 mRNA is readily detectable by Northern blot in most human tissues, OSBP2 mRNA expression is relatively specific to retina, pineal, testis, and fetal liver (Fig. 3). The expression in fetal liver may suggest that there is developmental regulation of OSBP2 and may correlate with earlier observations that implicated oxysterols in apoptosis control during development (11). The OSBP1 mRNA is more highly expressed in the macula than in the peripheral retina (Fig. 4A) but the protein seems to be equally distributed. This may be because of the much higher metabolic activity of the macula, which may induce a greater OSBP1 turnover. In contrast, OSBP2 mRNA seems to be readily detectable in both the macula and peripheral neural retina but the protein is only detectable by Western blot in the P2-detergent retinal subfraction (Fig. 5C). These Western blots (Fig. 5) suggest that there is more OSBP1 than OSBP2 protein in the retina. It should be noted that there is 13 times more total protein in the S2 fraction than in the P2-detergent fraction. Thus, unless there is a very large difference in the antibody affinities (>10-fold), there is probably more OSBP1 than OSBP2 in the retina.

Immunocytochemical localization of OSBP1 and OSBP2 in similar but not identical regions of the retina (Fig. 6). The localization of OSBP1 and OSBP2 to the inner retina is complex involving several retinal cell types and possibly subtypes, and it is difficult to speculate as to its meaning without further examination by electron and/or confocal microscopy. However, the localization of these OSBPs to the RPE is of particular interest for several reasons. The RPE expresses the LDL receptor (25) and its proximity to the choriocapillaris makes it a likely target for the uptake of blood LDL. The RPE is also probably serving as a cholesterol source for other retinal cells such as the photoreceptors that constantly synthesize rod outer segment membranes. LDL is one of the major sources of oxidized cholesterol and considering the cytotoxicity demonstrated by these molecules, it is likely that the RPE has some form of mechanism to bind these oxysterols as they are released from the LDL complex.

The oxysterol binding activity of OSBP2 was determined by incubating the detergent-extracted P2 pellet fraction (P2-detergent) with tritiated oxysterols (25HC and 7KC) and fractionating the proteins by HPLC (Fig. 7B). The S2 soluble fraction, which lacks OSBP2, was also treated identically to serve as a control (Fig. 7A). These results suggest that the majority of the 7KC binding activity is present in the P2-detergent fraction and that this activity is associated with immunoreactivity to OSBP2 (Fig. 7C). Although the experiments in Fig. 7 were not designed to determine binding constants, the data suggest that OSBP2 preferentially binds 7KC and has little or no affinity for 25HC or CH. The binding observed in the void volume (11 to 12 min) is probably because of nonspecific association of these sterols with macromolecules such as hyaluronid acid, which is present in all of our retinal fractions. There may also be other OSBPs in these fractions that associate with these macromolecules. Unpublished results from our laboratory indicate that there are at least 12 different oxysterol-binding protein-like genes in the human genome and many of these are present in the retina. Thus, until these OSBPs are better understood, oxysterol-binding activity in any tissue should be considered to originate from a mixture of binding proteins. The relatively low oxysterol binding activity detected in the S2 fraction may be because of the low concentration of oxysterols used (0.4 nM) or perhaps the narrow choices of oxysterols used (7KC and 25HC). There are over 80 different forms of oxidized cholesterol (26), and as can be seen by the relatively high affinity of OSBP2 for 7KC, different OSBPs may have different affinities for different oxysterols. The identification of the oxysterol binding activity of OSBP2 was facilitated by several factors: OSBP2 fractionated relatively cleanly to the P2-detergent soluble fraction, the availability of a specific OSBP2 antibody, and the high affinity of OSBP2 for 7KC, which allowed it to bind 13% of the total counts at a concentration of 0.4 nM. This allowed the detection of OSBP2 with very small amounts of tritiated oxysterols. Although OSBP1 is also present in the P2-detergent fraction, no OSBP1 immunoreactivity was present in this fraction.

OSBs and their oxysterol ligands may be playing an important role in the pathogenesis of age-related ocular diseases. A recent study has shown that there is a gradual accumulation of LDL cholesterol in the choriocapillaris and Bruch’s membrane (27) with aging. This coupled with the high metabolic rate and cholesterol needs of the retina, especially the macula, may make this area of the eye especially susceptible to oxysterol accumulation. The functions of the OSBs and particularly OSBP2 with its high and almost exclusive expression in the retina could play an important role in macular degenerations.

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