A Novel Rhodopsin Kinase in Octopus Photoreceptor Possesses a Pleckstrin Homology Domain and Is Activated by G Protein βγ-Subunits*

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G protein-coupled receptor kinases (GRKs) play an important role in stimulus-dependent receptor phosphorylation and desensitization of the receptors. Mammalian rhodopsin kinase (RK) and β-adrenergic receptor kinase (βARK) are the most studied members among known GRKs. In this work, we purified RK from octopus photoreceptors for the first time from invertebrate tissues. The molecular mass of the purified enzyme was 80 kDa as estimated by SDS-polyacrylamide gel electrophoresis, and this was 17 kDa larger than that of vertebrate enzymes. Unlike vertebrate RK, octopus RK (ORK) was directly activated by βγ-subunits of a photoreceptor G protein. We examined the effects of various known activators and inhibitors of GRKs on the activity of the purified ORK and found that their effects were different from those on either bovine RK or βARK. To analyze the primary structure of the enzyme, we cloned the cDNA encoding ORK from an octopus retinal cDNA library. The deduced amino acid sequence of the cDNA was highly homologous to βARK over the entire molecule, including a pleckstrin homology domain located in the C-terminal region, and homology to RK was significantly lower. Furthermore, Western blot analysis of various octopus tissues with an antibody against the purified ORK showed that ORK is expressed solely in the retina, which confirmed the identity of the enzyme as rhodopsin kinase. Thus, ORK appears to represent a unique subgroup in the GRK family, which is distinguished from vertebrate RK.

Many G protein-coupled receptors such as rhodopsin and β-adrenergic receptors are known to be phosphorylated in a light- or agonist-dependent manner by a member of the specific protein kinase family called G protein-coupled receptor kinases (GRKs). This stimulus-dependent phosphorylation of the receptors is thought to be involved in the desensitization of these receptors (for reviews, see Refs. 1 and 2). The kinases responsible for phosphorylating the activated forms of rhodopsin and β-adrenergic receptors are rhodopsin kinase (RK or GRK1) (3) and β-adrenergic receptor kinases (βARK1/2 or GRK2/3) (4), respectively. Both of these kinases have been purified to homogeneity, and their specificities and activities have been examined in reconstituted systems (5, 6). In addition to RK (7) and two kinds of βARK (8, 9), at least three other members of the GRK family (GRK4–6) have been cloned in mammals (10–12), and several related genes have also been cloned from other organisms such as Drosophila (13) and Caenorhabditis elegans (14).

It has been demonstrated that βARK is capable of phosphorylating rhodopsin in a totally light-dependent fashion and that RK can phosphorylate the agonist-occupied β-adrenergic receptors (15). Both kinases are insensitive to cyclic nucleotides and Ca2+ and inhibited by 0.1 M NaCl, 1 mM ZnCl2, detergents, and polyanions (16, 17). In addition to these similarities, some differences are present in the characteristics of RK and βARK. Polycations activate RK (16), but not βARK (17). The sequence similarity between the two kinases is not high, and the differences are most evident in the C-terminal regions (7). RK lacks ~120 C-terminal residues present in the corresponding region of βARK. This region in βARK is referred to as the pleckstrin homology (PH) domain and has been identified as the site where the enzyme interacts with G protein βγ-subunits and plasma membranes upon phosphorylation of substrate receptors (18–20). In contrast with the case of βARK, which interacts with and is activated by Gβγ (21, 22), phosphorylation of receptors catalyzed by RK is not affected by Gβγ (23). This is reasonable since RK does not possess a PH domain. Instead of a PH domain, RK has a C terminus sequence unique within the GRK family, the motif termed CAAX boxes (where C is cysteine, A is an aliphatic residue, and X is any amino acid). CAAX boxes are one of the known C-terminal isoprenylation motifs, and bovine RK is farnesylated at its C-terminal cysteine (24). Isoprenylation has been reported to be essential for the expression of full enzymatic activity of RK (25). It is required for light-induced translocation of the enzyme to the disc membranes (26) and therefore seems to play a very important role in the physiological action of RK. Thus, βARK and RK, as far as the present understanding, represent different subgroups in the GRK family in terms of both their structure and regulatory mechanisms.

We have previously shown that octopus rhodopsin is phosphorylated in a light-dependent manner and that light-induced phosphorylation of octopus rhodopsin in the microvillar membranes is markedly enhanced by GTPγS (27), which suggests that octopus rhodopsin kinase (ORK), like βARK, could be activated by βγ-subunits of G protein in contrast with bovine RK. Since light depolarizes invertebrate photoreceptor cells,
whereas it hyperpolarizes vertebrate rod and cone photoreceptor cells, the underlying phototransduction machinery, including that for desensitization, in vertebrate photoreceptor cells could be quite distinct from that operating in vertebrate photoreceptors (28). In an effort to explore the molecular and enzymatic properties of ORK, we purified the enzyme to apparent homogeneity for the first time as an invertebrate enzyme and cloned the cDNA encoding it. Here we report the very unique characteristics of ORK, which differ from its vertebrate counterpart and are closer to those of βARK.

**EXPERIMENTAL PROCEDURES**

**Materials**—Mono Q 5/5, concanavalin A-Sepharose, CNBr-activated Sepharose 4B, the Thermo Sequenase fluorescent labeled primer cycle sequencing kit, and the ECL chemiluminescence detection system were purchased from Amersham Pharmacia Biotech. Sulfate-Cellulofine was from Seikagaku Kogyo Inc. (Tokyo). Extractigel-D was from Pierce. [γ-32P]ATP was from NEN Life Science Products. Achromobacter protease I was from Wako Pure Chemical Industries (Osaka, Japan). Taq polymerase and restriction enzymes were from TaKaRa (Otsu, Japan). Protease I was from Wako Pure Chemical Industries. Taq polymerase and restriction enzymes were from TaKaRa (Otsu, Japan). Lambda ZAP II vector was from Stratagene. pT7Blue(R) vector was from Novagen. Other reagents used were the highest grade commercially available.

**Purification of ORK from Octopus Photoreceptor Microvillar Membranes**—Microvillar membranes of octopus photoreceptors were prepared from eyes of Octopus dofleini as described previously (27). Microvillar membranes were isolated by sucrose flotation (repeated twice) from the retinal homogenate. The isolated microvillar membranes were washed three times with 10 mM Tris-HCl (pH 7.4), 10 mM MgCl2, 1 mM dithiothreitol, and 20 μM AMPSF (isotonic buffer). Rhodopsin kinase activity was extracted by freezing and thawing the washed microvillar membranes. The membranes were frozen in liquid nitrogen and then thawed by homogenizing the frozen pellet in 10 mM Tris-HCl (pH 7.4), 0.4 mM KC1, 1 mM dithiothreitol, and 20 μM AMPSF.

The extracted proteins were separated from the membranes by centrifugation at 42,000 × g for 20 min after each freeze-thaw cycle, and five portions of successive extract were pooled together. The extract was diluted to 0.2 mM NaCl and applied to a sulfate-Cellulofine column (2 × 3.5 cm) equilibrated with 20 mM Tris-HCl (pH 7.4), 1 mM dithiothreitol, 20 μM AMPSF, and 0.2 mM NaCl at a flow rate of 0.6 ml/min. After the column was thoroughly washed with the equilibration buffer, the proteins were eluted with a linear gradient of 0.2–1.0 M NaCl in 60 ml (3 ml fractions). The fractions containing ORK were combined; dialyzed against 20 mM Tris-HCl (pH 8.0), 1 mM dithiothreitol, 20 μM AMPSF, and 20 mM NaCl; and applied to a Mono Q 5/5 column equilibrated with the dialysis buffer. The proteins were eluted with a linear gradient of 0.2–1.0 M NaCl in 60 ml and fractions of 1.5 ml. The fractions were pooled, dialyzed against 8 M urea and digested overnight with trypsin. The tryptic peptides were separated by reverse-phase high performance liquid chromatography, and amino acid sequences of the peptides in the peak fractions were analyzed with an Applied Biosystems Model 473A Protein Sequencer.

**Preparation of Octopus Photoreceptor G Protein**—One of the octopus photoreceptor G proteins (32), Gβ, was purified to apparent homogeneity from the detergent extract of the microvillar membranes as described previously (33). Briefly, the 1% sucrose monolaurate extract of the microvillar membranes was applied to a DEAE-cellulose column, and bound proteins including Gβ were eluted stepwise with a buffer containing 0.5 mM NaCl and 1% cholate. A trimeric Gβ preparation was obtained after gel filtration on Sephacryl S-300 HR (Amersham Pharmacia Biotech). To obtain the α-monomer and βγ-dimer, the subunits were separated on Mono Q (Amersham Pharmacia Biotech). Gβ and its subunits thus prepared were homogeneous as revealed by SDS-PAGE.

**Cloning and Sequencing of the orb Gene**—Poly(A) + RNA was prepared from an octopus retina with Oligotex-dT30 Super (TaKaRa) and used to construct a cDNA library in Lambda ZAP II vector. A pair of PCR primers were synthesized on the basis of the partial amino acid sequences determined from peptide fragments generated by Achromobacter protease I digestion of the purified ORK; orb-fl, 5'-CCCGAG(A/G)ACA/GICA/AGCA/TCAAG+3'; and orb-r1, 5'-CA(A/G)AAAAG(TT/TC)TC/TAIAGTCT/GTG-3' (where I is deoxynosine). DNA fragments were amplified by PCR using this pair of primers and the octopus retinal cDNA library as a template. The PCR products that matched the predicted size between the primer regions (~360 base pairs) were cloned into the plasmid vector pT7Blue(R), and the inserts were analyzed for their DNA sequences. DNA sequence was determined according to the chain termination procedure using the Thermo Sequenase fluorescent labeled primer cycle sequencing kit and an SQ-3000 DNA sequencer (Hitachi, Tokyo). All nucleotide sequences were determined for both strands from several independent clones. All PCR clones from 3 different clones were sequenced. Two clones showed an identical insert, and they contained the sequence corresponding to the peptide fragment obtained from the purified protein (clone P-1). To determine the amino- and carboxyl-terminal sequences, 5'and 3'-PCRs were carried out with the same cDNA library as a template. For the amino-terminal sequence, the first 5'-PCR was carried out with a gene-specific primer, orb 5'-1 (5'-ATTCCTCAGTCA/GTGGAAATC-3'), and a vector-specific primer, P6 (5'-TGGTACGCTGGTCAGTAAGAG-3'). The purified ORK was adjusted to 1 nmol) was dialyzed overnight with 10 mM Tris-HCl (pH 7.4), 50 mM KCl, 10 mM MgCl2, and 0.5 mM [γ-32P]ATP (1 μCi/μl) at 15 °C in the dark or light. The reaction was terminated by addition of an equal volume of electrophoresis buffer. Incorporation of 32P was visualized by autoradiography or measured with a Fuji Bioimage BAS2000 analyzer after SDS-PAGE on 11% gels. Amino Acid Sequencing—The purified ORK (~1 nmol) was dialyzed against 8 mM urea and digested overnight with Achromobacter protease I (EC 3.4.21.50) in 50 mM Tris-HCl (pH 9.5) and 4 μl urea. The digested peptides were separated by reverse-phase high performance liquid chromatography, and amino acid sequences of the peptides in the peak fractions were analyzed with an Applied Biosystems Model 473A Protein Sequencer.

**Phosphorylation of Rhodopsin**—Phosphorylation of octopus rhodopsin was carried out according to the method described previously (27) with some modifications. Briefly, the rhodopsin-containing vesicles (~3 μM rhodopsin) was incubated with the sample containing ORK in a buffer containing 20 mM Tris-HCl (pH 7.4), 50 mM KCl, 10 mM MgCl2, and 0.5 mM [γ-32P]ATP (1 μCi/μl) at 15 °C in the dark or light. The reaction was terminated by addition of an equal volume of electrophoresis buffer. Incorporation of 32P was visualized by autoradiography or measured with a Fuji BioImage BAS2000 analyzer after SDS-PAGE on 11% gels.

**Electrophoresis and Immunoblot Analysis**—SDS-polyacrylamide gel electrophoresis was performed as described by Laemmli (34). Protein

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blotting onto polyvinylidene difluoride membrane was performed following the method of Towbin et al. (35) using a transfer buffer containing 0.1% (w/v) SDS and 15% (v/v) methanol. For immunological detection, horseradish peroxidase-conjugated anti-IgG antibodies and the ECL chemiluminescence detection system were used according to the manufacturer’s instructions.

RESULTS

Purification of ORK—About half of the rhodopsin kinase activity in the microvillar membranes was extracted by freezethaw methods. The extracted proteins were loaded onto a heparin-like polyanion affinity matrix sulfate-Cellulofine column. As shown in Fig. 1, all of the rhodopsin kinase activity was bound to the column (no rhodopsin kinase activity was detected in the flow-through fractions) and eluted as a single activity peak on a linear gradient of 0.2–1.0 M NaCl. ORK was eluted at ~0.4 M NaCl, and fractions 11–17 contained an 80-kDa protein as a major component (data not shown). These fractions were pooled, dialyzed to reduce salt concentration, and further purified by Mono Q chromatography. Fig. 2 shows the elution profile of ORK on a Mono Q 5/5 column. The proteins were eluted by a linear gradient of 20–200 mM NaCl. ORK was eluted at ~100 mM NaCl as a single peak. The peak fractions eluting from the Mono Q column consisted of only one detectable protein with an apparent molecular mass of 80 kDa as determined by SDS-PAGE followed by Coomassie Blue staining (Fig. 3). Purification to apparent homogeneity was thus achieved at this step. The summary of the purification is documented in Table I and Fig. 3.

The purified ORK catalyzed phosphorylation of the purified rhodopsin, which had been reconstituted into phospholipid vesicles, in a light-dependent manner (Fig. 4). Phosphorylation of rhodopsin did not occur in the dark, whereas intensive phosphorylation was observed when the reaction was carried out in the light.

Activation of ORK by G Protein Subunits—Since phosphorylation of octopus rhodopsin in the membrane preparation markedly increased with addition of GTPγS (27), a photoreceptor G protein could be involved in regulation of rhodopsin phosphorylation. Thus, we investigated the effects of octopus photoreceptor G, on rhodopsin phosphorylation in a well defined reconstituted system using highly purified proteins. Phosphorylation of the purified octopus rhodopsin in phospholipid vesicles by the purified ORK was examined in the presence or absence of the subunits of G, isolated from the microvillar membranes. As demonstrated in Fig. 5, addition of the purified βγ-subunits of G, increased rhodopsin phosphorylation 2.5-fold. On the other hand, the purified α-subunit of G, showed no

**TABLE I**

| Purification step | Total protein | Total activity | Specific activity | Recovery | Purification -fold |
|------------------|---------------|----------------|------------------|----------|-------------------|
| Freeze-thawed extract | 159 | 426.1 | 2.68 | 100 | 1 |
| Sulfate-Cellulofine | 10 | 182.0 | 18.2 | 42.7 | 6.8 |
| Mono Q | 1.7 | 78.2 | 46.0 | 18.4 | 17.2 |

FIG. 1. Elution profile of ORK on a sulfate-Cellulofine column. The diluted membrane extract was applied to a sulfate-Cellulofine column (2 × 3.5 cm). The absorbance at 280 nm of eluted proteins was monitored. Inset, an autoradiogram of phosphorylation of the purified rhodopsin with the eluted fractions. E, membrane extract; FT, flow-through fractions. Pooled fractions for the purified protein are indicated (hatched area).

FIG. 2. Elution profile of ORK on a Mono Q 5/5 column. The pooled fractions from the sulfate-Cellulofine column were applied to a Mono Q 5/5 column (0.5 × 5 cm). A, the absorbance at 280 nm of eluted proteins was monitored (——). Rhodopsin kinase activity in the eluted fractions (●●●●) was assayed as described under “Experimental Procedures.” B, the fractions eluted from the column were subjected to SDS-PAGE on a 12% gel. Protein bands were stained with Coomassie Brilliant Blue R-250. arb, arbitrary units.

FIG. 3. SDS-PAGE of samples obtained at each step of ORK purification. Aliquots of each purification step (10 μg of protein in lanes 1–3 and 5 μg in lane 4) were analyzed by SDS-PAGE on an 11% gel and stained with Coomassie Brilliant Blue R-250. Lane 1, microvillar membranes; lane 2, freeze-thawed extract; lane 3, sulfate-Cellulofine pool; lane 4, final Mono Q pool.
effect regardless of the presence of GDP or GTPγS. The heat-denatured βγ-subunits were completely inactive, showing that the functionally intact subunits are required for activation.

These results agree with those obtained with βARK, which possesses a PH domain, but are quite different from the results obtained with RK.

**Comparison of Effects of Various Activators and Inhibitors of Mammalian GRKs on ORK Activity**—To characterize ORK biochemically, we studied the effects of various compounds known to affect mammalian GRKs on ORK activity. Polycations such as polyamines and polylysine act as activators of bovine RK (16), but they were potent inhibitors of ORK (Fig. 6A). Spermine weakly (up to 20% activation) activated ORK at low concentrations, but showed strong inhibition at higher concentrations. Its IC_{50} was ~5 μM. Spermidine (Fig. 6A) and polylysine (data not shown) did not show any activation, and their IC_{50} values were 10 μM and 50 μg/ml, respectively. Polyanions such as heparin, dextran sulfate, and polyglutamic acid inhibit both bovine RK (16) and βARK (17), and they also exhibited similar inhibition of ORK; heparin and dextran sulfate were strong inhibitors, and polyglutamic acid was a weak inhibitor (Fig. 6B). The IC_{50} values for heparin and dextran sulfate were ~1 and 0.3 μg/ml, respectively. The receptor-mimetic peptide mastoparan, which acts as an activator of both RK (36) and βARK (37), was, on the contrary, a potent inhibitor of ORK (Fig. 6C), and its IC_{50} was ~0.1 mM.

**Cloning and Sequencing of the cDNA Encoding ORK**—To isolate the octopus cDNA clone encoding ORK for determination of its primary structure, PCR was conducted with an octopus retinal cDNA library as a template. Degenerative primers for screening of the library were synthesized on the basis of the partial amino acid sequences determined from peptide fragments generated by Achromobacter protease I treatment of the purified ORK. Sequences from several peptide fragments showed homology to βARK, and two regions were selected to synthesize the degenerative primers (data not shown).

The full-length ork gene contains a single 2070-base open reading frame encoding a 80-kDa polypeptide, and the deduced amino acid sequence matched all the protein sequences obtained from an Achromobacter protease I digest of the purified enzyme (Fig. 7). Thus, we concluded that ork cDNA encodes the ORK that we had purified. The amino acid sequence deduced from the nucleotide sequence displayed 64% identity to bovine βARK1. The amino acid identity to bovine RK was 34%, which is significantly lower than that to βARK. It is also notable that PH domain-like sequence, which is found in mammalian βARK
but not in RK, was found in the C-terminal region of ORK, although the sequence similarity to bARK was lower in this region than in other regions.

**DISCUSSION**

In this paper, we report the first rhodopsin kinase that has been purified from invertebrate photoreceptors to apparent homogeneity using octopus photoreceptors. Rhodopsin kinase activity is present in both the soluble and membrane fractions of octopus retinal homogenate. As we have previously reported, rhodopsin kinase activity is present even in thoroughly washed microvillar membranes (27). This indicates that a considerable amount of ORK remains as a membrane-bound form in the washed microvillar membrane preparation; thus, we intended to isolate the enzyme from the membrane extract. We found that roughly more than half of the rhodopsin kinase activity is detached from the washed microvillar membranes by freeze-thaw extract contains relatively small number of proteins and is free of detergents that interfere with phosphorylation of rhodopsin, it is suitable as a starting material for purification. We used sulfate-Cellulofine, a sulfated cellulose resin, for the affinity chromatography since it gave better resolution of proteins than the widely used immobilized heparin under our experimental conditions. By two steps of successive chromatography.

**FIG. 7.** Sequence alignment of ORK with other members of the GRK family. Shown is an alignment of the deduced amino acid sequence of ORK with bovine bARK1 (8), Drosophila GPKR-1 (15), and bovine RK (7). The alignment was generated using University of Wisconsin Genetics Computer Group programs and optimized for the largest number of matches with the minimum number of gaps (shown by dashes). Identical and similar amino acids between ORK (top rows) and at least two other sequences are indicated by asterisks and dots, respectively. The regions where protein sequence was obtained from an Achromobacter protease I digest of the purified ORK are underlined. The PH domain of bovine bARK1 is shown in italics.

**FIG. 8.** Tissue distribution of ORK. Homogenates prepared from various octopus tissues (17 μg of protein for microvillar membranes and 3 mg for other tissues) were subjected to Western blot analysis with the antibody raised against the purified ORK. Lane 1, isolated photoreceptor microvillar membranes; lane 2, retina; lane 3, brain; lane 4, optic lobe; lane 5, testis; lane 6, liver; lane 7, muscle; lane 8, salivary gland; lane 9, skin; lane 10, a blot of retina incubated with the antibody that had been preincubated with the purified ORK.
Purification and Characterization of Octopus Rhodopsin Kinase

ory on sulfate-Cellulofine and Mono Q columns, ORK was purified to apparent homogeneity. The apparent molecular mass of the purified ORK was estimated as 80 kDa by SDS-PAGE, which differs from that of RK (67 kDa) (5), but is similar to that of βARK (80 kDa) (6). It is also consistent with the molecular mass predicted from the sequence of the ork gene (80 kDa).

ORK also resembles mammalian βARK in terms of regulation of activity. ORK is activated by βγ-subunits of a photoreceptor G protein, Gβγ. This is consistent with our previous observation that phosphorylation of rhodopsin in the microvillar membrane preparation is enhanced in the presence of GTP (27), which suggests regulatory activity of a G protein. The result that ORK is activated by Gβγ implies that the C-terminal region of ORK can serve as an interface domain between Gβγ, as expected from its sequence similarity to the PH domain present in βARK. RK does not possess a PH domain (7), and it is not activated by Gβγ (23) either. Isoprenylation, which occurs at the C terminus of RK but not of the octopus enzyme, plays an important role in regulation of the enzymatic activity (25, 26). Thus, the mode of regulation of rhodopsin kinase seems to be quite different between vertebrate and invertebrate photoreceptors.

Mammalian rhodopsin couples with the photoreceptor-specific transducin, whereas octopus rhodopsin couples with multiple photoreceptor G proteins (38), including the more widely expressed Gαq (33). ORK also has many properties common to βARK, which is expressed in a wide range of tissues. Together with the fact that the depolarizing photoreponses of invertebrate photoreceptors are the same as those of the typical mammalian neurons, these findings show that invertebrate photoreceptors have adopted signaling machinery common to typical mammalian neurons in their evolutionary process.

In terms of sensitivity to activators and inhibitors, ORK differs from both βARK and RK. Mastoparan, a wasp venom that activates βARK (37) and bovine RK (36), does not activate, but instead inhibits ORK. Since mastoparan is thought to mimic the third intracellular loop of the receptor that acts as an interface on activation of G protein by the receptor (39), one may assume that its effects on βARK are also alike (37). Mastoparan does not affect GTP binding to octopus photoreceptor Gαq; thus, it probably does not mimic octopus rhodopsin against both Gαq and ORK. As one may presume from their sequence differences, ORK is not activated by activators of bovine RK such as polyamines (16). On the contrary, polyamines are very potent inhibitors of ORK. Polyamines also inhibit βARK (17), although less potently than they inhibit the octopus enzyme. Polyamines such as spermin, dextran sulfate, and polyglutamic acid inhibit all three enzymes (16, 17), but their potencies vary with regard to each kinase. Finally, ORK is affected by all the drugs tested in a different way compared with both mammalian βARK and RK.

Sequence analysis of the ork gene shows striking structural similarity of ORK to βARK, but only moderate similarity to RK. The amino acid sequence identity of ORK is much higher to βARK (64% identity) than to RK (34% identity). In addition, ORK seems to possess a PH domain in the C-terminal region, which is thought as an interaction domain with G protein βγ-subunits present in βARK, but absent in RK (1, 2, 40). On the other hand, the CAAX motif for C-terminal isoprenylation, which is present in RK (7), is not found in ORK. In addition, a phylogenetic analysis of the ORK family reveals that ORK pairs with the βARK group, not with the RK group (data not shown). From these results, we conclude that ORK is evolutionarily closely related to an ancestor of mammalian βARK and belongs to a family distinct from RK. Drosophila GPRK-1 (13) has also been reported to be highly homologous to βARK, although no adrenergic signaling system has been identified in Drosophila. Taken together, it is interesting to hypothesize that a βARK-like enzyme may represent a prototype of all GRKs including RK and that ORK (possibly as well as other invertebrate rhodopsin kinases) may remain in a less differentiated structure than highly differentiated RK.

Mammalian βARK is expressed in a wide range of tissues, and Drosophila GPRK-1 also does not show retina-specific expression (13). Since ORK is structurally similar to these enzymes, its expression also may not be limited to the retina, but may range over a variety of tissues. When several octopus tissues were subjected to Western blot analysis with an antibody generated against the purified ORK, immunoreactivity to the antibody was detected only in the retina among the tissues tested, and the expression level of ORK in the retina was very high. This abundant and specific expression pattern of ORK in the retina, which is quite similar to that of RK, affirms that this enzyme is indeed “rhodopsin kinase” and is not merely one representative of invertebrate GRKs targeting multiple receptors.

In conclusion, ORK is structurally closely related to βARK, but has enzymatic properties that are unique among the known GRKs. Thus, we propose that it represents a novel subgroup, possibly that of invertebrate rhodopsin kinases, in the GRK family. Validity of this hypothesis will be examined through characterization of the corresponding enzymes in photoreceptors of other invertebrates.

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