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A Novel Rhodopsin Gene from *Octopus vulgaris* for Optobioelectronics Materials

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Abstract

The unique photochromic retinal protein from rhabdomeric octopus membranes – octopus rhodopsin (OctR) has emerged as promising material for biomolecular photonic applications due to its unique properties and advantages. Here we report isolation of the novel full length *octR* gene from retina cDNA of *Octopus vulgaris* eyes and its sequence comparison with rhodopsins of other cephalopods and vertebrates. The isolated gene can be used to develop various expression systems for production of recombinant OctR for structural studies and novel optobioelectronic applications. The alignment of amino acid (a.a.) sequence with different opsins revealed similarity to cephalopoda rhodopsins (Rho) and to human melanopsin from intrinsically photosensitive retinal ganglion cells. The alingment of OctR a.a. sequence with mammalian and cephalopoda Rho with known 3D structures revealed promising substitutions V2C and W292C for developing stable and functionally active recombinant OctR after heterologous expression.

Keywords

Rhodopsin, *Octopus vulgaris*, Photobioelectronics

Introduction

The rapid progress in nanotechnology provides new materials with unique photophysical and surface properties. All-optical switching is necessary to achieve ultrafast and ultrahigh bandwidth information processing. The basic challenge is to design an ultrafast energy-efficient all-optical switch that exhibits high contrast, low-power operations, along with photo and thermal stability and flexibility to reconfigure and tune its characteristics [1]. The prospect of using energy-efficient natural photoreceptors optimized over centuries of evolution to meet these requirements is extremely fascinating. Thus the evolution has led the light, as the source of information, to be caused the appearance, development and improvement of visual system of vertebrates and invertebrates [2].

Rhodopsin is a G protein-coupled receptor (GPCR) that absorbs light quantum thus initiating of enzymatic cascade of phototransduction in photoreceptors [3]. It consists of apoprotein opsin and 11-*cis*-retinal chromophore bound by Shiff-base linkage [4-6]. Several domains are distinguishable in the rhodopsin molecule, namely, the intramembrane hydrophobic domain formed of seven α-helical strands forming a bundle and crossing the photoreceptor membrane and two hydrophilic domains located at both sides of the membrane: cytoplasmic and intradisc (extracellular). These seven α-helices constitutes as much as 60% of its secondary structure and appear oriented mostly perpendicular

to the plane of the disk membrane. The photochemical and spectral characteristics of rhodopsin are determined by one of its most conserved domains, the chromophore center and the chromophore group 11-*cis*-retinal, which interacts with its nearest protein environment.

The unique photochromic retinal protein from rhabdomeric Octopus membranes - rhodopsin - has emerged as an outstanding material for biomolecular photonic applications due to its specific properties and advantages [7, 8]. The unique properties of the OctR is that it is bistable and as a result - photoreversible. This means that in the case of invertebrate pigments, both the rhodopsin and its photoproduct metarhodopsin are chemically stable. Unlike the vertebrate photoproduct, metarhodopsin II, there is no separation of the all-trans retinal chromophore (toxic product) from the opsin. OctR molecules maintain their functional activity at room temperature for several weeks [7]. OctR molecules have an ability to form thin films in gel-matrix [8]. The extremely high ordering of octopus visual membranes, as well as its ability to capture single photons and sensitivity to light polarization [6, 9] suggests the possible use of rhodopsins as a prototype for the photonic qubit detectors.

The 3D struture of cephalopoda Rho from *Todarodes pacificus* has been recently determined (PDB ID: 2Z73) [6]. This data opens new opportunities for generation of recombinant OctR variants with imporved properties (increased stability, higher sensitivity) for various optobioelectronic applications through computer modelling, site-directed mutagenesis, expression and purification.

The purpose of this study was the isolation of full-length *octR* gene as a prerequisite for obtaining recombinant OctR and its sequence comparison with known opsins structures for developing stable and functionally active mutants.

Materials and Methods

Isolation of total RNA from octopus eyes

Freshly caught octopuses (*O. vulgaris*) were bought directly from fishermen in Camogli (Genova, Italy), eyes were isolated with scalpel, shipped in dry ice and kept at low temperature freezer (-70°C) until needed. Frozen octopus eyes were hemisected, and the retinas were collected and washed with buffer (4% NaCl, 50 mM Na-phosphate buffer pH 6.8), as described previously [8]. The isolated retinas were homogenized with the help of ExtractRNA kit (Evrogen, Russia). Extraction and purification of total RNA were performed according to the Evrogen protocol.

cDNA preparation

RNA samples were diluted and subsequently treated with DNase I (Promega, Madison, WI, USA). Full length rhodopsin from the octopus retina was then constructed with SMARTer™ RACE cDNA Amplification Kit (Clontech - Takara BIO company, USA). SMARTScribe Reverse Transcriptase was used in 5'-RACE cDNA synthesis with 5'-RACE CDS Primer A and SMARTer II A oligonucleotide and in 3'-RACE cDNA with 3'-RACE CDS Primer A according to the protocol (Clontech - Takara BIO Company, USA).

Amplification and analysis of Rho gene from *O. vulgaris*

OctR gene was amplified with Advantage® 2 PCR Kit (Clontech - Takara BIO Company, USA). The initial primers for 5'-RACE PCR (OctV_01 5`GGGTTGTGGATAGCTGAAGCT) and 3'-RACE PCR OctV_02 5`ATGGCGGCATCCAAGAAAAT) were designed using the Vector NTI *v. 11.0* software [10] (Table 1). The design of oligonucleotides, sequences analysis and alignment, the estimation of isoelectric points, 3D structures analysis were performed in Vector NTI software. The data of opsins sequences were taken from GenBank data base (http://www.ncbi.nlm.nih.gov/genbank/), the data of Rho 3D structures were taken from RCSB Protein Data Bank (http://www.rcsb.org/pdb/home/home.do).

Table 1: Sequences of oligonucleotides, used in this work.

Oligonucleotide	Oligonucleotide (5' - 3')
OctV_01	ATGGCGGCATCCAAGAAAAT
OctV_02	GGGTTGTGGATAGCTGAAGCT
OctD_01	ATGGTGGAATCAACAACGTTA GTAAACC
OctD_02	AGCTTGATAGGCCTGGTTGTCG
OctD_03	TGGTGGTATAATCCAACCGTAG ACATC
OctD_04	TCCATCCTCATTGGGCCAAGTT CGATCCCATC
OctD_05	GCAAACCATAAAGTTGACAAGCA
OctD_06	CTACCCACCACAAGGCTACCCACC

Results and Discussion

Octopus is component of the benthic fauna. By now more than 200 octopus species are known, but the only one full length sequence of rhodopsin gene has been published (GenBank: X07797.1). This sequence was developed from giant Pacific octopus (*Enteroctopus dofleini*) after cDNA sequencing in 1988 [11] (Table 2). All other data were received after genomic sequencing and provide information about central exon, with covers 45-65 percent of *rho* gene and code information about 3-6 (of 7) transmembrane domains (TMD) [12-17] (Table 2). The full length sequence of synthetic rhodopsin gene from the Japanese patent [18] (Table 2) has 79,6% identity with rhodopsin gene from *E. dofleini*, but is codes the same protein. Nucleotide substitutions were designed to optimize heterologous expression. Since *rho* gene is rather conservative and has systematic value [12], the majority of this sequences were published after sequencing of characteristic parts of octopuses genomes in phylogenetic studies [12-17]. In particular 679 bp of *O. vulgaris rho* gene (code 2-5 TMD) was sequenced in the study "The ink sac clouds octopod evolutionary", where fragments of four mitochondrial genes (12S rRNA, 16S rRNA, cytochrome oxidase I, cytochrome oxidase III), and three nuclear genes (rhodopsin, octopine dehydrogenase and pax-6) were analyzed for evolutionary purposes [15].

Table 2: Sequences of octopus *rho* genes.

	Molecular type	***bp***		***GenBank: accession no.***
Octopus kaurna	genomic DNA	711	TMD, part	AY545169.1, [13]
Octopus bimaculoides	genomic DNA	884	TMD, part	AY545172.1, [13]
Octopus rubescens	genomic DNA	873	TMD, part	AY545170.1, [13]
Octopus berrima	genomic DNA	828	TMD, part	AY545168.1, [13]
Octopus californicus	genomic DNA	683	TMD, part	HM572214.1, [14]
Octopus ornatus	genomic DNA	861	TMD, part	AY616926.1, [16]
Sasakiopus salebrosus	genomic DNA	618	TMD, part	GQ226025.1, [12]
Bathypolypus sponsalis	genomic DNA	549	TMD, part	GQ226024.1, [12]
Benthoctopus sp.	genomic DNA	612	TMD, part	GQ226023.1, [17]
Vulcanoctopus hydrothermalis	genomic DNA	464	TMD, part	GQ226020.1, [12]
Octopus vulgaris	genomic DNA	679	TMD, part	HM104297.1, [15]
Octopus rhodopsin	synthetic construct	1371	TMD, part	E02902.1, [16]
Enteroctopus dofleini	cDNA, library	1675	5`UTR, full length RHO, 3`UTR	X07797.1, [11]
Octopus vulgaris	**cDNA, RACE**	**1368**	**full length RHO**	KR902901, ***current article***

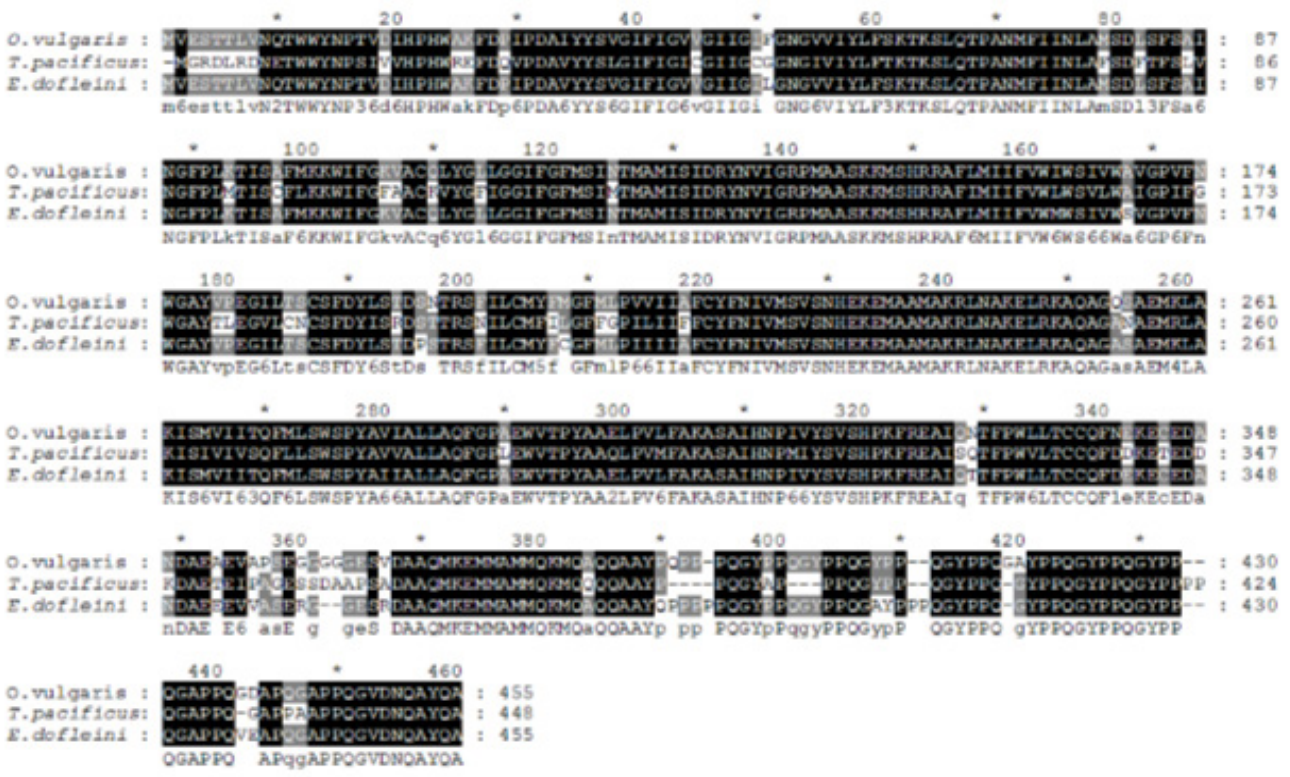

Figure 1: Comparison of the amino acid sequences of cephalopoda Rho. Dashes are alignment gaps. Sequence data used in the figure were taken from GenBank data base: *T. pacificus* (X70498.1), *E. dofleini* (X07797.1). Sequence data for *O. vulgaris* are results of the present study are results of the present study (accession number: KR902901).

For developing optobioelectronics materials full length recombinant Rho is required. All present attempts of heterologous expression of *rho* gene were based on *E. dofleini* sequence [11]. In previous studies we achieved photoreversibility and photostability in thin films of native rhodopsin from *O. vulgaris* [8]. Although vision rhodopsin of cephalopoda is conservative protein, some mismatches may slightly change its biophysical properties, probably, as result of adaptation to different habitat and sea water transparency. More over even single mutation can greatly change opsin properties [19,20]. Thus we amplified *rho* gene from *O. vulgaris* retina. The alignment with *E. dofleini rho* gene revealed more than 90% of identity and some characteristic mismatches after translation in protein structure (M162I, S168A, C208M, D341N, E353A, R360G, R365V) (Figure 1). To estimate the possible role of

these alterations in OctR molecule we compared our sequence with the sequence of squid Rho from *T. pacificus* for which the 3D structure was established) [6] (Figure 1). Several variable positions deserve special discussion. For instance, the analysis of *T. pacificus* Rho 3D structure shows that 208L lies in central part of TMD. This s indicates that orthologous C208 in *E. dofleini* Rho lies in lipid bilayer and does not participate in S-S bridge formation. Therefore substitution C208M in *O. vulgaris* is unlikely to change the stability of Rho molecule. The importance of the cysteine a.a. reside location in Rho molecule was demonstrated in resent study with bovine Rho [21]. Introduction of additional cysteins into water soluble parts of Rho may lead the formation of additional S-S bridges that greatly change protein proportions, such as thermal stability and stability in detergent solutions. At the same time cysteines. resides from TMDs loaded into lipid bilayer, there substitutions probably not greatly change Rho properties [20].

The replacement of 2 negative and two positive charged amino acids to neutral amino acids in TMDs at positions D341N, E353A, R360G, R365V retains for both proteins the same total charge (-0.77 at pH=7.0) and isoelectric point (6.67). Such changes may influence Rho properties by forming/reducing additional internal salt bridges required for its function. The C-terminal part of *rho* molecules is more variable and more indels can be found in this region (Figure 1).

Comparison of invertebrate and vertebrate rhodopsins

To localize structurally and functionally important evolutionary conserved regions of OctR we have performed sequence comparison between cephalopoda Rho, evolutionary distant vertebrate Rho, namely human and bovine, and human melanopsin (Figure 2).

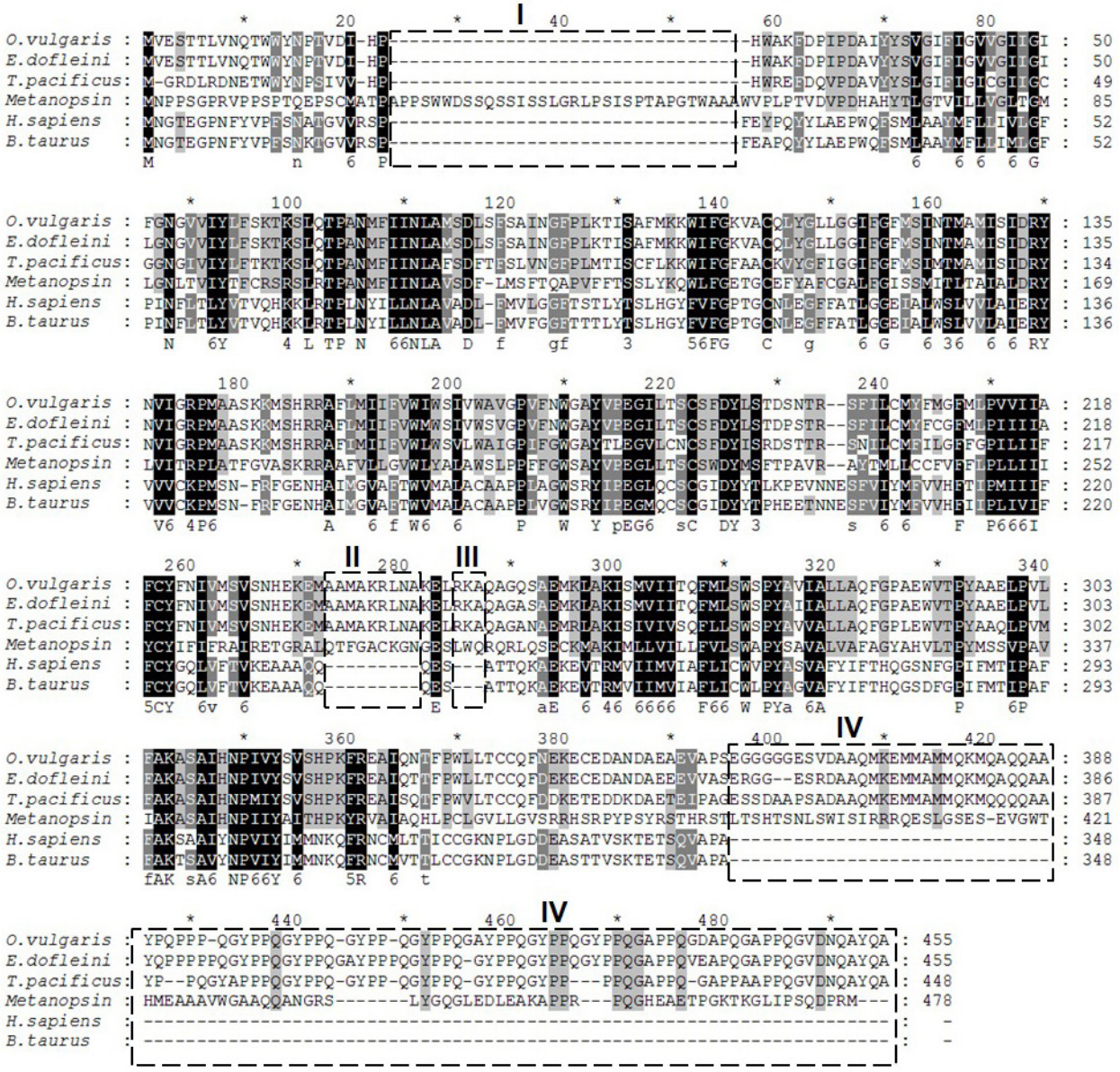

Figure 2: Comparison of the amino acid sequences of cephalopoda and mammal photosensitive opsin-like proteins. Dashes are alignment gaps. Boxes: I – specific region for – melanopsin protein; II - IV – mutual regions for cephalopoda rhodopsins and melanopsin. Sequence data used in the figure were taken from GenBank data base: *E. dofleini* – Rho (X07797.1), *T. pacificus* – Rho (X70498.1), melanopsin – melanopsin protein from *H. sapiens* (NM_033282.3), *H. sapiens* – Rho (NM_000539.3). *B. taurus* – bovine Rho (P02699). Sequence data for *O. vulgaris* Rho are results of the present study (accession number: KR902901).

First of all it should be noted that cephalopoda Rho are more similar to melanopsin that to vertebrate Rho (Figure 2). Cephalopoda rhodopsins are larger than their vertebrate counterparts and other invertebrate rhodopsins, mainly due to a unique C-terminal extension composed of 9–10 repeats of the consensus sequence Pro-Pro-Gln-Gly-Tyr interspersed with proline-rich sequences [22-24].

The nonvisual photoreceptor melanopsin, found in the neurons of vertebrate inner retina, absorbs blue light and triggers the "biological clock" of mammals by activating the suprachiasmatic nuclei (a small region of the brain that regulates the circadian rhythms of neuronal and hormonal activities over 24 h cycles) [19, 25–29]. Melanopsin has unique I region (Figure 2) in the N-terminus and three regions conserved with cephalopoda Rho in TMD (II and III regions) and C-terminus (IV region). Melanopsins are unique among vertebrate photoreceptors because their amino acid sequence shares greater similarity to invertebrate than vertebrate rhodopsin, they are also bistable [30]. and use an invertebrate-like signal transduction cascade [31]. Human melanopsin has 35.4% homology with human Rho, and 44.7% with *O. vulgaris* Rho (Figure 2). The homology model of melanopsin (maximal absorbance λmax = 447 nm) based on the crystal structure of squid rhodopsin (λmax = 490 nm) shows that 43 nm spectral shift is due to increased bond-length alternation of the protonated Schiff base of 11-cis-retinal chromophore, induced by N87Q mutation and water-mediated H-bonding interactions with the Schiff base linkage [19]. This phenomenon is analogous to spectral changes observed in the G89Q bovine rhodopsin mutants [32]. The alignment with novel OctR structure reveals N88 position for mutagenesis for developing "blue light" bistable recombinant Rho.

The heterologous expression of functionally active Rhp molecules is a rather difficult task. Structure-based design of more stable Rho variants opens new opportunities to overcome existing problems with successful Rho expression in mammalian cells [21, 33]. For instance, mutations N2C/D282C in bovine Rho were designed to introduce additional S-S bond between the N-terminus and loop E3 increasing the Rho thermal stability 10°C and enabling robust handling of recombinant opsin in detergent solution and subsequent crystallization of obtained recombinant protein [21]. Orthologous substitutions V2C and W292C may be designed in OctR based on sequence alignments with bovine and squid Rho to design more stable recombinant OctR for optobioelectronic applications.

Conclusion

Novel *octR* gene was isolated from *O. vulgaris* retina. The encoded protein is highly homologous to other known cephalopoda Rho and may contain evolutionary significant amino acids changes relevant to its spectral properties.

The *octR* gene can be used to develop expression systems for production of recombinant Rho and its mutant analogs. Protein engineering may be particularly useful to overcome known problems in production of functionally active recombinant Rho. We plan to pursue this route of computer modelling/mutation prediction/mutagenesis/protein expression and purification to obtain recombinant OR suitable for structural studies and optobioelectronic applications. The introduction of additional S-S bond may improve handling of opsin in detergent solution and increase rhodopsin thermal stability to obtain high-quality recombinant rhodopsin for subsequent crystallisation [21]. Single mutations may shift photosensitivity (to blue light) and keep main characteristics (stability, bistable) endurable for optobioelectronics [19]. Recombinant Rho with altered properties may contribute to vast amount of optobioelectronic methods such as the developing optical logic element based on photoconversion of retinal-containing protein with different light pulses [34].

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