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Cloning and nucleotide sequence of cDNA for rhodopsin of the squid *Todarodes pacificus*

Ikuko Hara-Nishimura[a], Maki Kondo[a], Mikio Nishimura[a], Reiko Hara[b] and Tomiyuki Hara[b]

[a]*Department of Cell Biology, National Institute for Basic Biology, Myodaijicho, Okazaki 444, Japan* and [b]*Department of Biology, Kinki University School of Medicine, Osaka-Sayama, Osaka 589, Japan*

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A cDNA for rhodopsin was isolated from a library constructed from poly(A)$^+$RNA of the squid (*Todarodes pacificus*) retina. One positive clone with the longest insert of cDNA (3.1 kb) was selected by employing a PCR-amplified cDNA fragment as a probe. The nucleotide sequence of the cDNA revealed a single open reading frame of 1,344 bp encoding a polypeptide (M_r 49,833), which covered a complete sequence for the squid opsin. This clone had a very long 3′-non-coding region (1.7 kb) including multiple polyadenylation signals, AATAAA, resembling the clones for *Todarodes* retinochrome and retinal-binding protein (RALBP). The analysis of hydropathicity demonstrated the presence of seven transmembrane spanning domains, and a possible retinal-binding site, Lys-305, was found in the 7th domain. *Todarodes* rhodopsin contained characteristic sequences of PPQGY repeated in the C-terminal region, as reported in *Loligo* and octopus rhodopsins. Structural comparison of those cephalopod rhodopsins is also discussed.

Retinal protein; Rhodopsin; Retinochrome; Retinal-binding protein; Visual cell, *Todarodes pacificus*

1. INTRODUCTION

Early investigations on the molecular structure of visual pigments were developed using vertebrate rhodopsins [1,2]. In invertebrates, similar studies have been performed in insects such as *Drosophila* [3] and *Calliphora* [4] and cephalopods such as octopus [5] and squid (*Loligo*) [6]. Comparison of those studied now enables us to predict the relationship between structure and function of the visual pigment molecule.

As ascertained by our previous work, the molluscan visual cell is equipped with the rhodopsin–retinochrome system, which contributes to maintaining the photoreceptive capacity of the visual cell through photopigment regeneration, backed up by the recycling of retinals [7,8]. In this system, 11-*cis*-retinal required for rhodopsin formation is generated by the photoconversion of retinochrome to metaretinochrome in the inner segment of the visual cell, while all-*trans*-retinal chromophore of metarhodopsin is used for retinochrome regeneration. For the intracellular transport of retinal chromophores between the inner and outer segments, a retinal-binding protein (RALBP) serves in such a way that the irradiation of one photopigment assists in the regeneration of the other [9,10]. In order to understand the mechanism for the mutual exchange of retinals between RALBP and meta-pigment, we intended to analyze the molecular structure of a set of the three retinal proteins in the squid, *Todarodes pacificus*. Since cDNAs for RALBP [11] and retinochrome [12] were already cloned and characterized, we here describe the cloning and nucleotide sequence of cDNA for *Todarodes* rhodopsin. The opsin structure will also be compared with those of octopus [5] and *Loligo* [6].

Correspondence address: M. Nishimura, Department of Cell Biology, National Institute for Basic Biology, Myodaijicho, Okazaki 444, Japan.

2. MATERIALS AND METHODS

2.1. *Determination of amino acid sequences*

Todarodes rhodopsin was prepared by our routine method described before [7]. The N-terminal amino acid sequence of the rhodopsin was determined to be Gly-Arg-Asp-Leu-Arg-Asp-Asn-Glu-Thr-Trp-Trp-Tyr-Asn-Pro-Ser-Ile, using an automatic sequence analyzer (Applied Biosystems Model 470A). In order to analyze an internal amino acid sequence, the purified rhodopsin (35 nmol) was dissolved in 70% formic acid and was cleaved by CNBr at room temperature in the dark. After incubation for 24 h, the reaction mixture was diluted with distilled water an lyophilized. The resultant CNBr peptides were subjected to high-pressure liquid chromatography (HPLC, Chemcosorb 5-ODSH column). The elution of peptides was monitored by the absorbance at 215 nm. The amino acid sequence of a peak fraction was determined to be Ile-Ser-Ile-Asp-Arg-Tyr-Asn-Val-Ile-Gly-Arg-Pro.

2.2. *Synthesis of primers for the polymerase chain reaction (PCR)*

For the cloning of rhodopsin cDNA, we constructed 23mer sense oligonucleotides for the above-mentioned internal peptide and 30mer antisense oligonucleotides for the retinyl peptide, the amino acid sequence of which was previously reported by Seidou et al. [13]. The internal and retinyl peptides contained an arginine and a serine, respectively, each of which has six codons. We synthesized two separate pools of oligonucleotides corresponding to each peptide using a Gene Assembler Plus (Pharmacia-LKB), as shown in Fig. 1a. Four-base wobble was replaced by inosine.

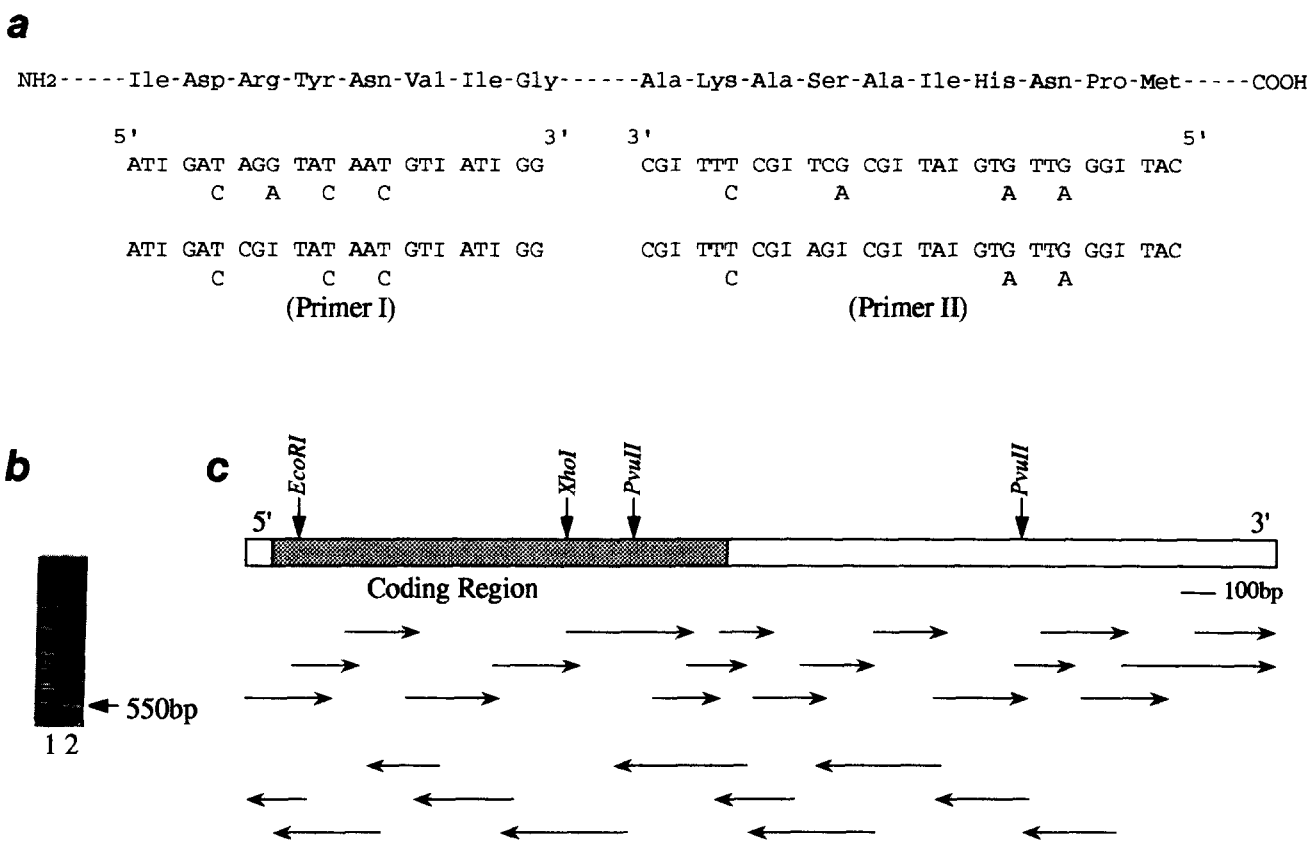

Fig. 1. (a) Synthetic oligonucleotides for the amplification of the rhodopsin cDNA fragment by PCR. Synthetic sense (Primer I) and antisense (Primer II) oligonucleotides correspond in amino acid sequences to the internal CNBr peptides of rhodopsin. (b) Amplified cDNA fragment for rhodopsin. Lane 1, λ phage DNA digested with *Eco*RI and *Hin*dIII. Lane 2, PCR-extended product resolved by agarose gel electrophoresis and visualized after staining with ethidium bromide. The size of amplified DNA was approximately 550 bp. (c) The sequencing strategy for the squid rhodopsin cDNA.

2.3. *Preparation of PCR-amplified DNA*

RNA was prepared from the *Todarodes* retina using the guanidium isothiocyanate method [12]. The first strand DNA was transcribed using RNA and reverse transcriptase. Employing it with the above two primers, a DNA fragment was amplified by PCR. The PCR-extended product precipitated twice by ethanol and 2 M ammonium acetate was labeled with [α-^{32}P]dATP by PCR, and used as a probe for the screening of a cDNA library.

2.4. *Construction of a cDNA library and its screening*

A cDNA library was constructed using vector–primer and poly(A)$^+$RNA exactly according to a previously described method [12]. *E. coli* (DH5α) was transformed with the cDNA. Screening of the library was carried out by colony hybridization using the labeled PCR-extended cDNA fragments as a probe. Approximately 10^5 independent recombinants were screened.

2.5. *DNA sequence analysis*

The deletion mutants for both sense and antisense strands of the cDNA insert were constructed. DNA sequence analysis was carried out by the dideoxy chain termination method on double-stranded DNA with Sequenase (United States Biochemicals) according to the manufacturer's directions.

3. RESULTS AND DISCUSSION

After 30 rounds of amplification using a combination of the two synthetic oligonucleotides, a PCR-extended product of 550 bp was formed as shown by agarose gel electrophoresis (Fig. 1b). This amplified DNA was employed as a probe in order to screen the squid retina cDNA library. Finally one clone with the longest cDNA insert (3.1 kb) was chosen and sequenced (Fig. 1c). The resulting sequence revealed a single open reading frame of 1,344 bp encoding 448 amino acids (Fig. 2). The deduced protein contained the amino acid sequences of N-terminal region and two CNBr peptides of rhodopsin, indicating that this clone was nothing but that for rhodopsin.

The sequence beginning with the first ATG at nucleotides 87–89 was identical to the N-terminal sequence of rhodopsin. There was no ATG codon upstream from nucleotide 86, but a stop codon TAA at nucleotides 78–80 in frame. This indicated that the ATG for the N terminus was the initiation codon itself, and any processing of the leader sequence did not occur. The initiator methionine residue was cleaved from the protein, so that the net N terminus of squid rhodopsin was glycine. Based on the predicted sequence, the molecular weight of rhodopsin was calculated to be 49,833, slightly smaller than that estimated by SDS-PAGE (51,000) [14]. It was noted that the 3′-noncoding region was very

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      CAGGTC CACTGGTTTA CTGTAGTGGT TCCCATAAGG CAGGCACCAG TACACAGAGA CCAAATTTTG AAAAAGAATT GTAATTCGCC      86
ATG GGT AGA GAT CTC AGA GAC AAT GAG ACT TGG TGG TAT AAC CCT TCC ATC GTA GTC CAT CCT CAT TGG AGA GAA     161
 M   G   R   D   L   R   D   N   E   T   W   W   Y   N   P   S   I   V   V   H   P   H   W   R   E      25
TTC GAT CAA GTG CCT GAT GCA GTT TAC TAC TCT CTC GGT ATT TTC ATC GGT ATC TGT GGT ATT ATT GGT TGC GGT     236
 F   D   Q   V   P   D   A   V   Y   Y   S   L   G   I   F   I   G   I   C   G   I   I   G   C   G      50
GGC AAT GGC ATT GTC ATA TAC CTC TTC ACC AAG ACG AAA TCC CTG CAG ACA CCA GCC AAC ATG TTC ATC ATT AAC     311
 G   N   G   I   V   I   Y   L   F   T   K   T   K   S   L   Q   T   P   A   N   M   F   I   I   N      75
TTG GCT TTC TCC GAT TTC ACT TTC TCA TTG GTG AAC GGT TTC CCG CTC ATG ACA ATT TCT TGT TTC TTA AAG AAA     386
 L   A   F   S   D   F   T   F   S   L   V   N   G   F   P   L   M   T   I   S   C   F   L   K   K     100
TGG ATC TTT GGA TTT GCT GCC TGC AAA GTC TAT GGC TTC ATC GGT GGT ATT TTC GGT TTT ATG TCC ATC ATG ACT     461
 W   I   F   G   F   A   A   C   K   V   Y   G   F   I   G   G   I   F   G   F   M   S   I   M   T     125
ATG GCT ATG ATC TCC ATT GAT CGA TAC AAT GTC ATT GGA AGA CCA ATG GCG GCG TCA AAG AAA ATG AGC CAC AGG     536
 M   A   M   I   S   I   D   R   Y   N   V   I   G   R   P   M   A   A   S   K   K   M   S   H   R     150
AGG GCC TTC ATT ATG ATC ATC TTC GTA TGG CTC TGG TCA GTC TTG TGG GCT ATC GGA CCC ATT TTC GGA TGG GGC     611
 R   A   F   I   M   I   I   F   V   W   L   W   S   V   L   W   A   I   G   P   I   F   G   W   G     175
GCA TAC ACC TTA GAG GGA GTC CTC TGC AAT TGC TCT TTT GAT TAC ATC AGC CGC GAT TCA ACA ACC AGG TCC AAC     686
 A   Y   T   L   E   G   V   L   C   N   C   S   F   D   Y   I   S   R   D   S   T   T   R   S   N     200
ATC TTG TGC ATG TTT ATT TTG GGT TTC TTC GGT CCT ATT CTC ATC ATT TTC TTC TGC TAC TTC AAT ATT GTA ATG     761
 I   L   C   M   F   I   L   G   F   F   G   P   I   L   I   I   F   F   C   Y   F   N   I   V   M     225
TCC GTG TCT AAC CAC GAA AAA GAG ATG GCA GCT ATG GCG AAG AGG CTG AAT GCC AAG GAA TTG CGA AAA GCC CAG     836
 S   V   S   N   H   E   K   E   M   A   A   M   A   K   R   L   N   A   K   E   L   R   K   A   Q     250
GCC GGA GCA AAC GCT GAA ATG AGA TTG GCA AAG ATC TCA ATT GTC ATC GTC TCC CAG TTC TTG CTC TCC TGG AGT     911
 A   G   A   N   A   E   M   R   L   A   K   I   S   I   V   I   V   S   Q   F   L   L   S   W   S     275
CCA TAT GCT GTT GTG GCT CTT CTC GCC CAG TTC GGT CCT CTC GAG TGG GTT ACA CCT TAT GCC GCT CAG TTG CCT     986
 P   Y   A   V   V   A   L   L   A   Q   F   G   P   L   E   W   V   T   P   Y   A   A   Q   L   P     300
GTC ATG TTC GCC AAG GCC TCT GCT ATC CAC AAC CCC ATG ATC TAC TCC GTA TCT CAT CCT AAG TTC CGT GAG GCT    1061
 V   M   F   A   K   A   S   A   I   H   N   P   M   I   Y   S   V   S   H   P   K   F   R   E   A     325
ATC AGT CAA ACC TTC CCA TGG GTC CTC ACA TGT TGT CAA TTC GAC GAT AAG GAA ACT GAA GAT GAC AAG GAT GCA    1136
 I   S   Q   T   F   P   W   V   L   T   C   C   Q   F   D   D   K   E   T   E   D   D   K   D   A     350
GAA ACT GAA ATT CCA GCT GGT GAA TCA TCT GAT GCT GCA CCC AGT GCG GAT GCT GCC CAA ATG AAA GAA ATG ATG    1211
 E   T   E   I   P   A   G   E   S   S   D   A   A   P   S   A   D   A   A   Q   M   K   E   M   M     375
GCT ATG ATG CAG AAG ATG CAA CAA CAA CAG GCC GCC TAC CCA CCA CAG GGA TAT GCA CCA CCA CCA CAG GGA TAC    1286
 A   M   M   Q   K   M   Q   Q   Q   Q   A   A   Y   P   P   Q   G   Y   A   P   P   P   Q   G   Y     400
CCA CCA CAG GGA TAC CCA CCA CAG GGA TAC CCA CCA CAA GGA TAC CCA CCA CAA GGT TAC CCA CCA CCA CCC CAA    1361
 P   P   Q   G   Y   P   P   Q   G   Y   P   P   Q   G   Y   P   P   Q   G   Y   P   P   P   P   Q     425
GGA GCA CCA CCC CAG GGA GCA CCC CCA GCA GCC CCA CCC CAG GGA GTT GAC AAC CAG GCT TAC CAG GCT TGA        1433
 G   A   P   P   Q   G   A   P   P   A   A   P   P   Q   G   V   D   N   Q   A   Y   Q   A   *         448
GCAACCAACC TAAGAACTCT GTAGACTTAT TTTGTAGATG CTGCAAGAAA GAATTATTCT GAGAAAAAAT ATAGTTCGAT GGAACCTGTC CGTATCTTCT 1533
AAAGAGAGGC GTCCGAGTGA AGAAGACAAA AAGAAGATTG AACTTATCAT TAAAACGAAG TTGCACGCAT GAAAATCGTG CCAAAAGAAA GAAGATGATG 1633
AAGCGGTGAC TAACATATCT GAATTATATA CAGATGAGCT TTCACAGAGA AGCCGTGTAT TAGGAACTTT TCCACCTTTC CTATTTCAAT CAAATTTAAT 1733
TAACTAATAT TCAGGACATT TTCGGACATA GACAATTTTC TTGAAAAGTA GATAAATACA TTTTAGAAAA AATTGTGTAT ATATATATAT ATATATATAT 1833
ATATATATAA TCTTGCACGT ATTTTGAATA GTATAAGTCT GGCGCAAAAT CGACTCAATT ACGACTTTGA GCGCTGCCTT AAGTAAATGC GTTGTCTACA 1933
GGTAACATCA ACAAAAAATA AACAAAAGAC TATATGCACG AGCAACATAC ACGCACATAA ATGTATACTT ATTTACATAC GTATCTATTT AGCAATCATT 2033
ATATACATAT ATATCTTCGA CTCTTATATG TACGTGCATA TATGCATATG TATGGATGTT GGTGGGTATG AGCGTGAGTG TTTGCGTGTG TCAGTGCATA 2133
TGATGTATCA GTGTGCGTAT GTGCTTTTTT TACTGTACGA TCAGAGGAGT TGGTCTGGCT AGCGTCTCTT CGGAATTGTG ACATCGCCAC CTGGTGTGGG 2233
TTTCTCGCCT GAAAGGACGT GGAGTGATTT TTTTCTAATG GAAGATAGAT TTTTAGAACG TTTGTTTCAT TCTTCAGCTG TTTCGAGTGA CCTCTCTCTC 2333
ATTTTTTATC AGTCATTCCG AATCTATCAT CACTGGTTGA TCAATTGTTT TGTTTCTTCT TTTTTTGATA TGTGTTAGAA AGGAAAAGAG ATACAACAAT 2433
AATAACAATG ACAACCAGCA ATAGATCAAT ACTCGATTGA TGAAGATTTT CATCGCGCGA ATCTATAATA GAGACTGGTA CAAACAAACG AGAATTGATT 2533
ACAACCCCTC GGAAATTGAT TCTAAAATCG GTTTTGTATC GTATGCACAA TGCTCGATAA AAGCAAAGAC CGTGATTGTA TCTATCGGCA GGTTTCCTTC 2633
CGTTTCTAAA TTTTGATCAT CTACTCTAGG ATTGAAAAAA TAAAATTAAG AAAATATTGA CCAAAAAATA ATATTATAAC CCCCTCAAAA ATATATTTAA 2733
AAAATTAAAT AAAAATAACA TTTTTGGTAG CTAAATTATT TTTTTCTTTT TAAATTGCTT AATAATGTGG TTATCTTTCT TGCAATTTTT TTTCTCTTTT 2833
TAATTTTTTT AGTCAGTCAG TTTTTATTTC GTTTTTATTT TTTCTCTCCT GTAAAGTTTT TTTTGGGGTT TTGACTTTTT TGGTCTTTTT GTTTGCTTAC 2933
TTGTTATTGA TTTTTTTTAA AATTTTTTCT CGTATTTCTT TTCCAAAAGA TGAAATAAAC TATTTTACGG TAGTTTTTAT TCTTGAAAAA TGTCTTCCCC 3033
AAGCCGAGTT GCAGCATTGG AAGCACTCAA TAAAAATCGA ATTAACAAAT poly(A)                                                   3083
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Fig. 2. cDNA nucleotide sequence and deduced amino acid sequence of *Todarodes* rhodopsin. The nucleotide sequence is numbered on the right of each line, and the predicted amino acid sequence is similarly numbered on the right. Underlined amino acid sequences agree with those of the N-terminal region and CNBr peptides of rhodopsin. Underlined nucleotides at the 3′-noncoding region represent possible signals for polyadenylation. (...) represents a stop codon in a frame at 5′-noncoding region.

long, where 5 hexanucleotides AATAAA (polyadenylation signal) occurred at nucleotides 1950–1955, 2672–2677, 2741–2746, 2987–2992 and 3062–3067. The distance from the 5th AATAAA to a poly(A) stretch was as short as 16 nucleotides.

As seen in Fig. 3, the analysis of hydropathicity for

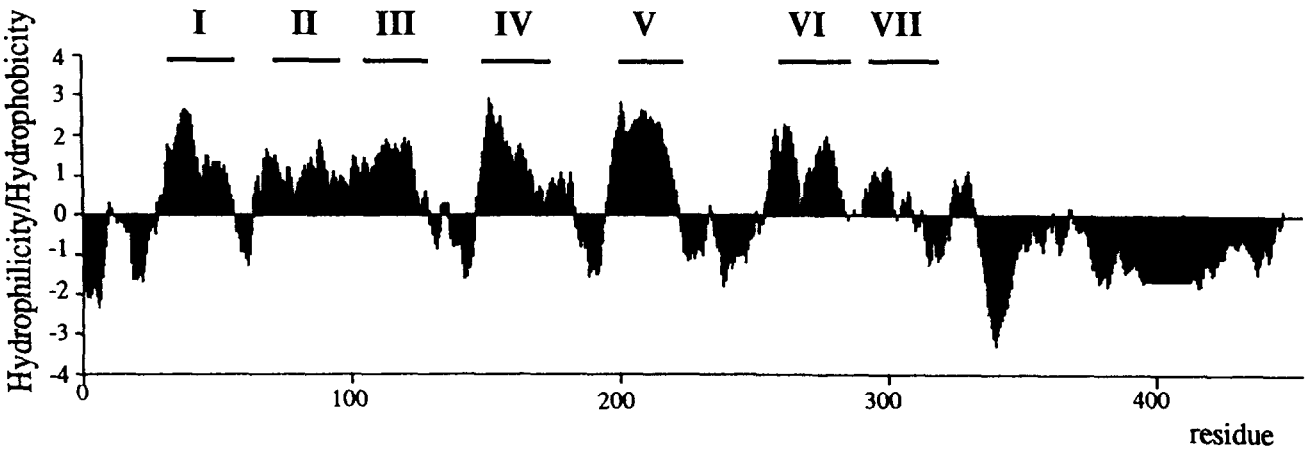


Fig. 3. Hydropathicity profile of the deduced amino acid sequence of *Todarodes* rhodopsin. Hydropathic indexes were calculated according to Kyte and Doolittle [15] for a window size of 10 amino acid residues. Numbers on the abscissa denote those assigned for the deduced amino acid sequence in Fig. 2.

the predicted rhodopsin according to Kyte and Doolittle [15] showed that 7 clusters of hydrophobic sequences with a high proportion of aromatic amino acids occurred in the rhodopsin molecule. This profile strongly suggested that *Todarodes* rhodopsin also possesses 7 transmembrane helices, as ascertained for bovine rhodopsin [1,2,16] and bacteriorhodopsin [17].

The amino acid sequence homology between squid and bovine rhodopsins is shown in Fig. 4, where the seven transmembrane domains of bovine opsin are indicated as dotted areas [16]. Squid rhodopsin contained 34.8% identical amino acids in the region of 37 to 309 residues of bovine opsin. Low homology was observed in two transmembrane domains, helices 1 (24% identity) and 3 (17% identity). The other helices displayed an amino acid identity as high as 40% (38–43%). Most of the hydrophilic loops showed approximately 30% amino acid identity, with highest (50%) identity in the cytoplasmic loop between helices 1 and 2 (abbreviated as loop 1–2). In squid rhodopsin, helix 1 contained much more glycine, and the size of the loop between helices 5 and 6 (loop 5–6) was much larger than in bovine opsin. A characteristic feature of squid rhodopsin was a long C-terminal tail containing large quantities of proline and glutamine which form many blocks of repetitive sequences Pro-Pro-Gln-Gly-Tyr (PPQGY).

In Fig. 5 a structural model of *Todarodes* rhodopsin is proposed, based on the data in Fig. 4. At the extracellular side, two possible sites for *N*-linked glycosylation [18] were actually observed at Asn-8 and Asn-185. During Edman degradation of the rhodopsin, PTH-asparagine was not detected at the 7th cycle of the degradation, indicating that Asn-8 was glycosylated. The two cysteine residues, Cys-108 and Cys-186, may form an important disulfide bond to complete the correct protein structure [19]. The essential sequence Asp-Arg-Tyr for G-protein binding [20] was also conserved near the cytoplasmic end of helix 3 of squid opsin. The site of covalent binding of the 11-*cis*-retinal chromophore was determined to be Lys-305 located in helix 7, around which the sequence was the same as that of the retinyl peptide reported previously [14]. Intramembranous proline residues which may serve to accommodate the polyene chain of retinal into the transmembrane segments [21] were observed at positions 90, 170, 212, 276, 300 and 312 in helices 2, 4, 5, 6, 7 and 7, respectively. Unlike bovine rhodopsin, squid rhodopsin has a proline residue in helix 2 but not in helix 1. In the C-terminal region, there is no distinct serine- and threonine-rich part which has been indicated for light-dependent phosphorylation in vertebrate [2,22] and insect rhodopsins [3]. Close to helix 7, two cysteines, Cys-336 and Cys-337, may be palmitoylated by analogy with bovine rhodopsin [23].

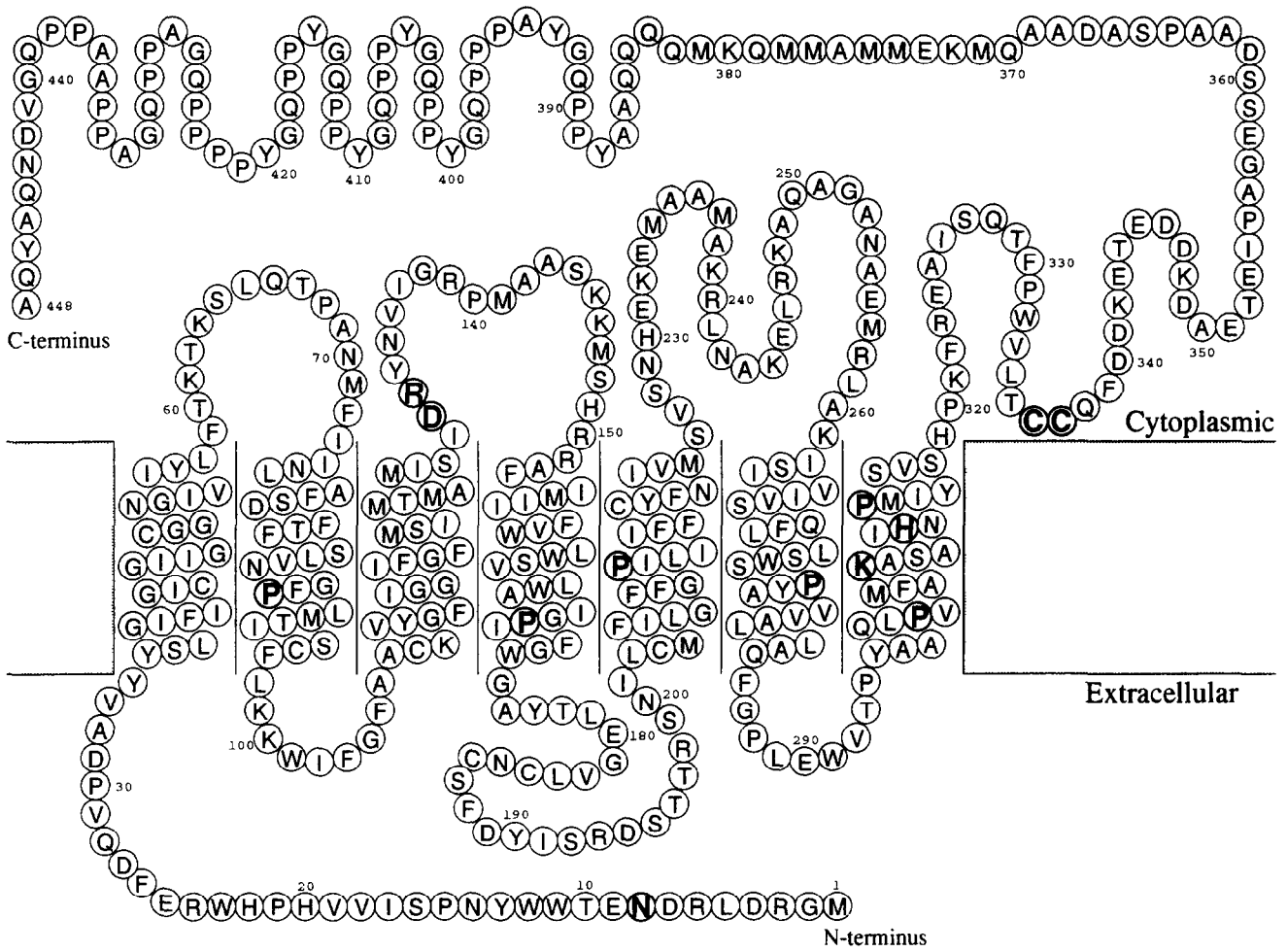
Fig. 6 is presented to compare the opsin structures of *Todarodes*, *Loligo* [6] and octopus [5] rhodopsins. They are composed of 448, 452 and 455 amino acid residues respectively, and similar to each other in molecular weight. *Todarodes* rhodopsin displays a high identity (90.5% between helices 1 and 7) with *Loligo* rhodopsin but a lower identity (78.9%) with octopus rhodopsin. Unlike octopus rhodopsin, *Todarodes* and *Loligo* rhodopsins have the same amino acid sequence in helices 2 and 7, accompanied by only one different residue in helix 6. In these three rhodopsins, the sequences of the cytoplasmic loops 1–2, 3–4 and 5–6 are well conserved without marked difference of amino acid residues. They are also entirely the same on various properties described in the preceding paragraph, i.e. the glycosylation site close to the N-terminus, the couple of cysteines in the extracellular space, the conserved sequence of Asp-Arg-Tyr outside helix 3, the presence of some intramembranous prolines and the acylation with fatty acids in the C-terminal region. As has been suggested [6,13], the histidine residue located at the 5th position following the retinal Schiff's base attachment site (Lys-

Fig. 4. Comparison of amino acid sequences between squid and bovine rhodopsins. The homology was analyzed by DNASIS (Hitachi). (:), identical amino acid. (.), homologous amino acid. (*), possible site for *N*-linked glycosylation. Dotted areas indicate transmembrane domains in bovine rhodopsin.

305) seems to be characteristic of cephalopod rhodopsins, since it is replaced by tyrosine in vertebrate visual pigments. The tyrosine in helix 3 (Tyr-111) may play a role equivalent to the retinal Schiff's base counterion [6], well conserved in all cephalopod rhodopsins.

In the C-terminal region of cephalopod rhodopsins, serine and threonine are rather scattered two to three residues in the accessory loop following helix 7, but five in *Todarodes* and only two each in *Loligo* and octopus in the subsequent stretch. It was also noticed that, even in the C-terminal region except the PPQGY repetitive tail, cephalopod rhodopsins are far more abundant not only in alanine (8–11 residues) but also in aspartic and glutamic acid (14 residues) than vertebrate visual pigments. The region from Asp-347 to Glu-358 appears to form a binding domain for calcium [24]. We consider that this domain may play an important role for rhodopsin regeneration, probably associated with the retinal exchange reaction between RALBP and meta-pigment in the rhodopsin–retinochrome system.

Cephalopod rhodopsins possess a long tail of repetitive sequences containing proline and glutamine, as dis-



tinguished from vertebrate visual pigments. In either of the cephalopod rhodopsins, a pentapeptide repeat occurs about ten times, showing at least six complete sets of a PPQGY sequence. Although the role of this repetitive structure is not yet clear, it was discussed [6] that it may be involved in the maintenance of the highly organized structure of rhabdomeric microvilli through rhodopsin–rhodopsin interactions. Before and after the above-mentioned repetitive structure, there are two regions composed of 16 (367–382) and 13 (436–448) amino acid residues, respectively. The amino acid sequences in each region are fully identical in all cephalopod rhodopsins. Interestingly, the former conserved region is enchased with many methionines, and the sequence between positions 370 and 382 is virtually in a symmetrical arrangement centering around Ala-376. In any case, the three cephalopod rhodopsins showed a fairly high similarity in opsin structure. However, the 3′-noncoding region of cDNA was extraordinarily longer (1653 bp) in *Todarodes* than in *Loligo* (402 bp [6]), octupus (236 bp [5]) and *Calliphora* (348 bp [4]). Since such a long 3′-noncoding region was also observed in the cases of retinochrome (1053 bp [12]) and RALBP (1623 bp [11]) cDNAs in *Todarodes*, it may be due to its phylogenetic characteristics in vision.

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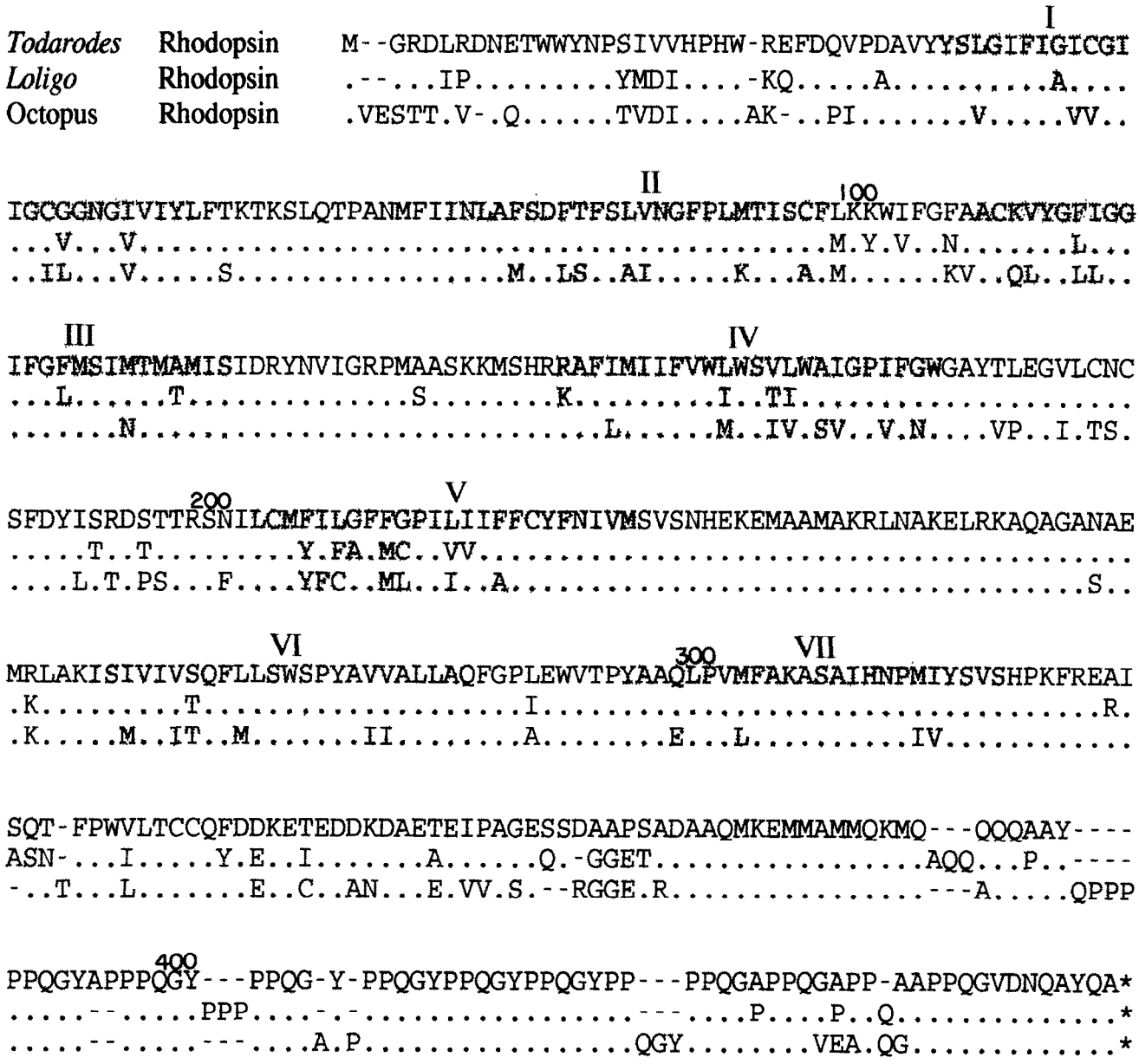

Fig. 6. Comparison of cephalopod rhodopsins. Amino acid sequences of rhodopsins in squid (*Todarodes*), squid (*Loligo*, [6]) and octopus [5]. (.) represents the amino acid identical with that in the *Todarodes* rhodopsin. Dottes areas indicate transmembrane domains according to Fig. 4.

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