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Vertebrate Ancient-Long Opsin Has Molecular Properties Intermediate between Those of Vertebrate and Invertebrate Visual Pigments

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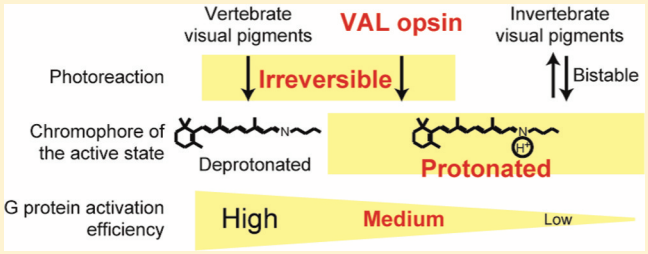
Supporting Information

ABSTRACT: VA/VAL opsin is one of the four kinds of nonvisual opsins that are closely related to vertebrate visual pigments in the phylogenetic tree of opsins. Previous studies indicated that among these opsins, parapinopsin and pinopsin exhibit molecular properties similar to those of invertebrate bistable visual pigments and vertebrate visual pigments, respectively. Here we show that VA/VAL opsin exhibits molecular properties intermediate between those of parapinopsin and pinopsin. VAL opsin from *Xenopus tropicalis* was expressed in cultured cells, and the pigment with an absorption maximum at 501 nm was reconstituted by incubation with 11-*cis*-retinal. Light irradiation of this pigment caused cis-to-trans isomerization of the chromophore to form a state having an absorption maximum in the visible region. This state has the ability to activate Gi and Gt types of G proteins. Therefore, the active state of VAL opsin is a visible light-absorbing intermediate, which probably has a protonated retinylidene Schiff base as its chromophore, like the active state of parapinopsin. However, this state was apparently photoinsensitive and did not show reverse reaction to the original pigment, unlike the active state of parapinopsin, and instead similar to that of pinopsin. Furthermore, the Gi activation efficiency of VAL opsin was between those of pinopsin and parapinopsin. Thus, the molecular properties of VA/VAL opsin give insights into the mechanism of conversion of the molecular properties from invertebrate to vertebrate visual pigments.



Opsins are photoreceptor proteins that play central roles in light sensing in animals. Recent studies have shown that many opsins are expressed not only in photoreceptor cells of the retina but also in secondary neurons of the retina, specific cells in the brain, and other tissues.[1,2] These opsins outside the photoreceptor cells are thought to mediate nonvisual light responses such as entrainment of circadian rhythm, photoperiodism, and light-dependent body color changes. Several lines of evidence have indicated that there are four groups of opsins that are closely related to vertebrate visual pigments in the phylogenetic tree (Figure 1). Among them, the pinopsin and parapinopsin groups have been subjected to detailed investigations of their molecular properties.[3,4] In contrast, investigations of the molecular properties of the other two groups, the parietopsin and VA/VAL opsin groups, have just begun.

Vertebrate ancient (VA) opsin was first isolated from the eyes of the Atlantic salmon,[5] and its splicing variant, vertebrate ancient-long (VAL) opsin, which has a longer C-terminal tail, was then identified in the zebrafish deep brain.[6] VA/VAL opsin is unique in that it has a glutamic acid at position 113, like vertebrate visual pigments, but has a serine at position 181, where most opsins have glutamic acid. Previous studies have indicated that if an opsin has a glutamic acid at position 181 as a counterion, it acts as a bistable pigment that exhibits photoreversible reactions between resting and active states.[7] On the other hand, if an opsin has a glutamic acid at position 113 as a counterion, it produces an active state whose retinylidene Schiff base chromophore is deprotonated, and it spontaneously decomposes into the protein moiety and chromophore. Because VA/VAL opsin has a glutamic acid at position 113 and no glutamic acid at position 181, it was expected that VA/VAL opsin would exhibit a photoreaction similar to those of vertebrate visual pigments.

In this study, we expressed *Xenopus tropicalis* VAL (xtVAL) opsin in cultured cells. We successfully obtained the visible light-absorbing pigment of xtVAL opsin by incubation of the proteins with 11-*cis*-retinal. Irradiation of xtVAL opsin caused isomerization of the 11-*cis*-retinal chromophore into the all-trans form, resulting in formation of a state that activated Gi and Gt efficiently. Unexpectedly, the absorption maximum of the active state was in the visible region, which indicated that deprotonation of the Schiff base was not necessary to activate G proteins in xtVAL opsin. In addition, the active state did not convert back to the original pigment upon light absorption. On

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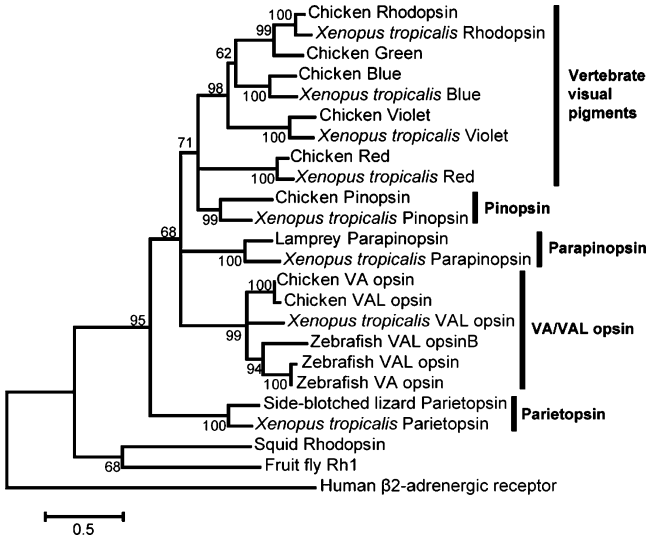

Figure 1. Phylogenetic tree of the vertebrate visual and nonvisual opsins. The human β2-adrenergic receptor (GenBank accession number AAA88015) was used as an outgroup. The amino acid sequences of chicken rhodopsin (GenBank accession number BAA00610), *Xenopus tropicalis* rhodopsin (AAI35235), chicken green (AAA48786), chicken blue (AAA48633), *X. tropicalis* violet (AAI66308), chicken violet (AAA49141), chicken red (CAA40727), *X. tropicalis* red (AAI35756), chicken pinopsin (AAB47565), chicken VA opsin (ABM66817), chicken VAL opsin (ACX32474), zebrafish VA opsin (BAA94289), zebrafish VAL opsin (BAA94288), zebrafish VAL opsinB (AAY56361), lamprey parapinopsin (BAD13381), *X. tropicalis* parapinopsin (BAD17960), side-blotched lizard parietopsin (AAZ79904), *X. tropicalis* parietopsin (ABB88727), squid rhodopsin (CAA49906), fruit fly Rh1 (AAA28733), *X. tropicalis* blue (NCBI Reference Sequence Accession number XP_002937272), *X. tropicalis* pinopsin (XP_002934391), and *X. tropicalis* VAL opsin (XP_002936846) were aligned using MUSCLE,[33] and the phylogenetic tree was constructed using the maximum-likelihood method based on the Whelan and Goldman model.[34] A discrete γ distribution was used to model evolutionary rate differences among sites [five categories (+*G*, parameter = 1.2732)]. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The bootstrap values calculated from 1000 replicates are shown next to the branches, and branches corresponding to partitions reproduced in less than 50% of bootstrap replicates are collapsed. Phylogenetic analysis was conducted using MEGA5.[35]

the basis of these results, we will discuss the unique molecular properties of VA/VAL opsins that are different from those of vertebrate and invertebrate visual pigments.

EXPERIMENTAL PROCEDURES

Sample Preparation. The cDNA of xtVAL opsin (NCBI Reference Sequence accession number XM_002936800) was tagged with the epitope sequence of the anti-bovine rhodopsin monoclonal antibody Rho1D4 (ETSQVAPA) at the C-terminus and was inserted into mammalian expression vector pCAGGS. The plasmid DNA was transfected into the HEK293T cell line using the calcium phosphate method. After 6−12 h, the transfection medium was replaced with fresh medium containing 2 μM 11-*cis*-retinal, and the cells were kept in the dark thereafter. Forty-eight hours after the transfection, the HEK293T cells were collected by centrifugation. The following procedures were conducted on ice under dim red light unless otherwise noted. To estimate the expression level of VA/VAL opsins, the proteins were extracted with 1% CHAPS in buffer A [50 mM HEPES (pH 7.0), 140 mM NaCl, and 3 mM $MgCl_2$]. To obtain purified xtVAL opsin, the CHAPS extract was applied to a column conjugated with the Rho1D4 antibody. The purified xtVAL opsin was eluted with buffer A containing 0.75% CHAPS, 1 mg/mL PC, and 0.45 mg/mL synthetic peptide of the Rho1D4 epitope sequence. To determine whether VAL opsin is bistable, xtVAL opsin-containing cell membranes were prepared by sucrose flotation as previously described.[8] For the measurement of G protein activation efficiencies, xtVAL opsin was solubilized from the collected HEK293T cells with 0.2% dodecyl β-D-maltoside (DM) in buffer A.

Gt and Gi were prepared as previously described.[9] Briefly, Gt was purified from bovine rod outer segments according to a previous report.[10] The rat Giα subunit was expressed in *Escherichia coli* strain BL21 by using the pQE6 vector containing Giα1 cDNA and was purified as described previously.[11] The purified Giα1 was mixed with an equal amount of purified Gt$\beta\gamma$.

Spectrophotometry. Absorption spectra were recorded using a Shimadzu UV2450 spectrophotometer and an optical cell (width, 2 mm; light path, 1 cm). To control the sample temperature, an optical cell holder was connected to a Neslab RTE-7 temperature controller. The sample was irradiated with light from a 1 kW tungsten halogen lamp (Rigaku Seiki) that had been passed through a glass cutoff filter or a band-pass filter.

Retinal Extraction and High-Performance Liquid Chromatography (HPLC) Analysis. The retinal isomer composition was analyzed by HPLC (Shimadzu LC-10AT VP) with a silica column (150 mm × 6.0 mm, A-012-3; YMC) according to previous studies.[12] In short, the retinals of light-irradiated and nonirradiated purified samples were extracted in the form of retinaloximes and analyzed by HPLC. The retinal composition was calculated from the area of the peaks based on the absorption coefficients previously reported.[13]

G Protein Activation Assay. The G protein activation efficiencies of the pigment-containing membranes were assayed by the GTPγS binding method as previously described.[14] The measurement was taken at 10 °C before or after a 30 s irradiation with yellow light (>500 nm). The activation efficiency of bovine rhodopsin was also measured as a standard, and two G protein subtypes, Gi and Gt, were tested. The assay mixture contained 0.01% DM, 1 μM [^{35}S]GTPγS, 140 mM NaCl, 8 mM $MgCl_2$, 1 mM DTT, 50 mM HEPES (pH 7.0), 10 nM pigment, and 0.6 μM G protein. Additionally, 4 μM GDP was added for the measurement of Gi activation.

RESULTS

Spectral Properties of xtVAL Opsin. We tried to express chicken VA and VAL (cVA and cVAL, respectively) opsins and *X. tropicalis* VAL (xtVAL) opsin in cultured cells. The expression of cVA opsin could not be detected, and the level of expression of cVAL opsin was much lower than that of xtVAL opsin (data not shown). Therefore, we used xtVAL opsin as a model. We expressed xtVAL opsin in HEK293T cells cultured in the presence of 11-*cis*-retinal and purified it in the form of CHAPS/PC mixed micelles after it was solubilized with 1% CHAPS in buffer A (Figure 2A). The absorption maximum of purified xtVAL opsin was 501 nm. This maximum differed by 4 nm from that of zebrafish VAL opsin (505 nm).[6] Next, we determined the molar extinction coefficient of xtVAL opsin by the acid denaturing method, as previously described (Figure

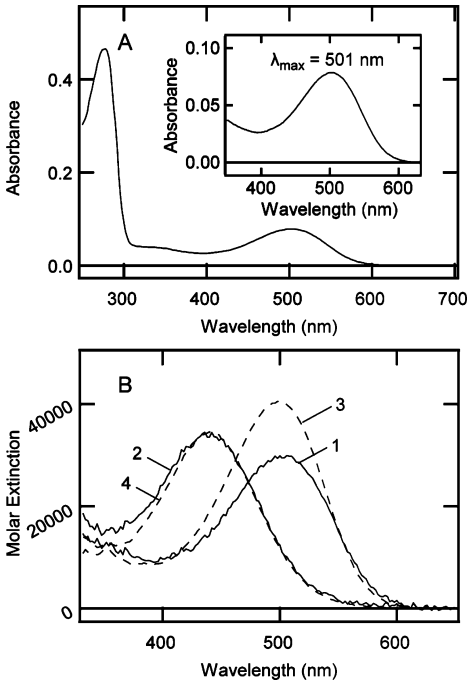

Figure 2. Spectral properties of xtVAL opsin. (A) Absorption spectrum of purified xtVAL opsin. The absorption maximum of the α-band was at 501 nm, and the A_{280}/A_{501} ratio was 5.9. The inset is an enlarged view of the absorption spectrum between 330 and 650 nm. (B) Absorption spectra of xtVAL opsin and bovine rhodopsin before and after acid denaturation. All the spectra were recorded at 0 °C. Solid curves 1 and 2 are the absorption spectra of xtVAL opsin before and after acid denaturation, respectively. Dashed curves 3 and 4 are the absorption spectra of bovine rhodopsin before and after acid denaturation, respectively.

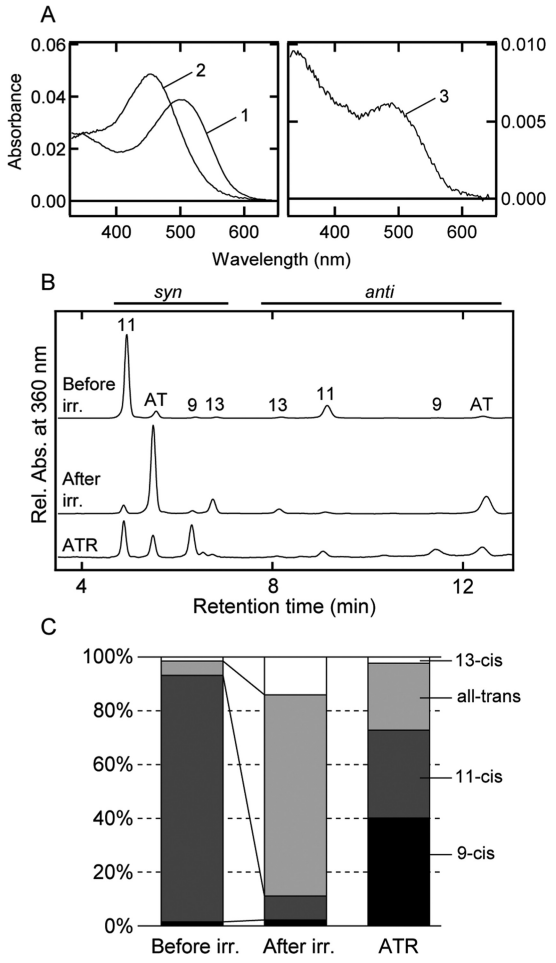

Figure 3. Isomeric composition of retinal in xtVAL opsin. (A) Absorption spectra of the purified xtVAL opsin sample that was incubated with 11-*cis*-retinal during the expression in HEK293T cells (curve 1) and the sample after >500 nm light irradiation (curve 2) and of xtVAL opsin sample that was incubated with all-*trans*-retinal during the expression in HEK293T cells (curve 3). All the spectra were recorded at 0 °C. (B) HPLC pattern of the purified xtVAL opsin sample that was incubated with 11-*cis*-retinal during the expression in HEK293T cells (Before irr.), the sample after >500 nm light irradiation (After irr.), and the xtVAL opsin sample that was incubated with all-*trans*-retinal during the expression in HEK293T cells (ATR). The retinal chromophore was extracted from the samples in the form of retinal oximes and analyzed by HPLC. (C) Calculated compositions of retinal isomers in the samples based on each peak area in the chromatogram and the extinction coefficients previously reported. Compositions of the retinal isomers of the sample before irradiation (Before irr.) were 1.82, 91.4, 1.45, and 5.32% for the 9-cis, 11-cis, 13-cis, and all-trans forms, respectively. Those of the sample after >500 nm light irradiation (After irr.) were 2.49, 8.93, 14.0, and 74.5% for the 9-cis, 11-cis, 13-cis, and all-trans forms, respectively. Those of the sample prepared by incubation with all-*trans*-retinal during the expression in HEK293T cells (ATR) were 40.3, 24.9, 2.28, and 32.6% for the 9-cis, 11-cis, 13-cis, and all-trans forms, respectively.

2B). We compared the absorption spectra of xtVAL opsin and bovine rhodopsin under neutral and acidic conditions. On the basis of the molar extinction coefficient of rhodopsin at 498 nm (40600 M^{-1} cm^{-1}),[15] the molar extinction coefficient of xtVAL opsin at λ_{max} was estimated to be 29800 M^{-1} cm^{-1}.

Photoreactions of xtVAL Opsin at 0 °C and Chromophore Configurations. Curves 1 and 2 in Figure 3A show absorption spectra of the original xtVAL opsin and its photoproduct produced by irradiation with yellow light at 0 °C. Irradiation of the pigment caused a blue shift of the absorption maximum from 501 to 455 nm. The photoproduct was unstable at 0 °C in the CHAPS/PC purified sample, and it then decayed into a species absorbing at 372 nm, which would correspond to a mixture of a free unprotonated retinylidene Schiff base and free retinal, with a time constant of ~10 min (see Figure 1 of the Supporting Information). To investigate the chromophore configurations of the original and irradiated states, we extracted the chromophores and analyzed them by HPLC. The results showed that predominantly the 11-cis form was extracted from the original state, and irradiation caused an increase of the amount of the all-trans form (Figure 3B,C). Thus, the photoreaction of xtVAL opsin is accompanied by cis−trans isomerization of the retinal chromophore. We also investigated the direct binding of all-*trans*-retinal to xtVAL opsin by incubation of xtVAL opsin with all-*trans*-retinal during the expression of xtVAL opsin in the cultured cells. We obtained a small but detectable amount of the pigments in addition to the relatively large amount of unreacted retinal even after performing the purification procedure (curve 3 in Figure 3A). HPLC analysis showed that the sample contained large amounts of 9-cis and 11-cis forms, with a relatively small amount of the all-trans form [9-cis, 40.3%; 11-cis, 24.9%; 13-cis, 2.28%; all-trans, 32.6% (Figure 3B,C)]. It should be noted that we added only all-*trans*-retinal (>98.0%) to the medium. Therefore, it is reasonable to speculate that the pigments formed contained 9-*cis*- or 11-*cis*-retinal, which formed by thermal isomerization of all-*trans*-retinal during the expression

of xtVAL opsin. The presence of large amounts of 9-*cis*- and 11-*cis*-retinal in the sample strongly suggested that the opsin trapped 9-*cis*- or 11-*cis*-retinal under the condition that included the presence of a large amount of all-*trans*-retinal. Therefore, we concluded that xtVAL opsin has little, if any, ability to directly bind to all-*trans*-retinal.

Next we investigated the photochemical behavior of the photoproduct produced from the original xtVAL opsin. To exclude the effect of detergent used for the extraction of the pigment, we prepared the HEK293T cell membrane fractions with xtVAL opsin. We also prepared the membrane fractions of bovine rhodopsin and lamprey parapinopsin to compare the photochemical behavior of these typical pigments. As reported previously, yellow light irradiation of bovine rhodopsin produced a mixture of meta I (λ_{max} = 480 nm) and meta II (λ_{max} = 380 nm) intermediates (Figure 4B, curve 1),[16] and then

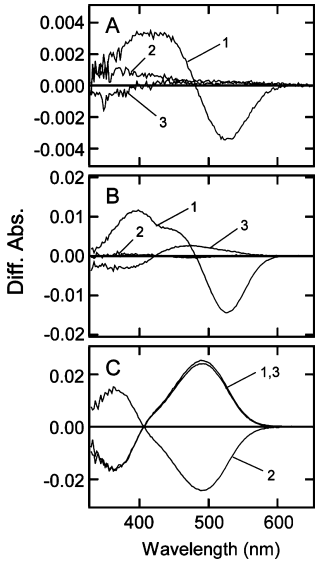

Figure 4. Verification of the bistable nature of xtVAL opsin. All of the spectra were recorded at 0 °C. (A) Spectral change of xtVAL opsin caused by >500 nm light irradiation (curve 1), subsequent 436 nm light irradiation (curve 2), and an additional <400 nm light irradiation (curve 3) for 1 min. (B) Spectral change of bovine rhodopsin caused by >500 nm light irradiation (curve 1), subsequent 436 nm light irradiation (curve 2), and an additional <400 nm light irradiation (curve 3) for 1 min. (C) Spectral change of lamprey parapinopsin caused by <400 nm light irradiation (curve 1), subsequent >500 nm light irradiation (curve 2), and an additional <400 nm light irradiation (curve 3) for 1 min.

UV light irradiation of the meta II intermediate of bovine rhodopsin produced the meta III intermediate, which has a protonated Schiff base;[17] however, the photoproduct did not convert back to the original state (Figure 4B, curve 3). In contrast, repeated UV and visible light irradiation of lamprey parapinopsin caused reversible conversion (bistable nature) between the original state and the photoproduct (Figure 4C).[4] Irradiation of xtVAL opsin in the membranes caused a photoreaction quite similar to that observed with the purified sample; namely, it produced a photoproduct having an absorption maximum in the visible region. However, subsequent irradiation of the photoproduct did not cause a significant absorbance change (Figure 4A, curves 2 and 3). This means that the photoproduct of xtVAL opsin is apparently photoinsensitive; that is, xtVAL opsin is not a bistable pigment.

G Protein Activation by xtVAL Opsin. We then examined whether the photoproduct produced from xtVAL opsin has the ability to activate G proteins. As VA/VAL opsin is closely related to the vertebrate visual pigments, we examined whether VA/VAL opsin activates Gi-type G proteins in comparison with bovine rhodopsin (Figure 5). We used

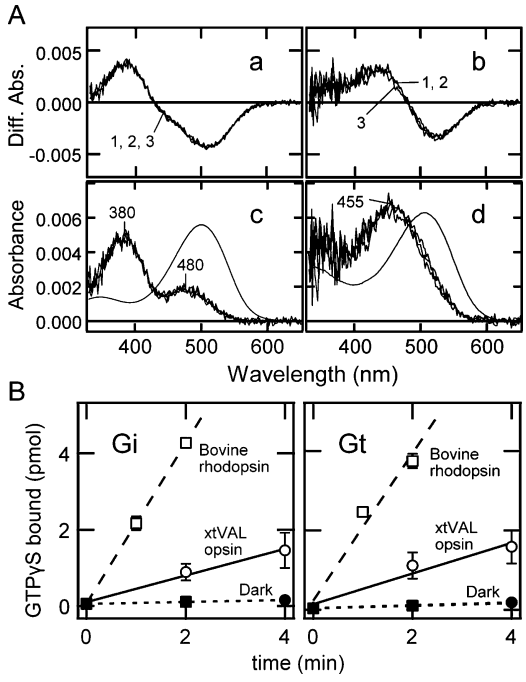

Figure 5. G protein-activating state of xtVAL opsin. (A) Spectral change of bovine rhodopsin (a) and xtVAL opsin (b) in 0.1% DM extract. Absorption spectra were recorded immediately and 2 and 4 min after >500 nm light irradiation for 30 s at 10 °C (curves 1−3). Panels c and d show the calculated absolute spectra of the photoproducts of bovine rhodopsin and xtVAL opsin. The absorption maxima are shown. (B) Light-dependent Gi and Gt activation by xtVAL opsin and its efficiency. Bovine rhodopsin and xtVAL opsin solubilized in buffer A supplemented with 0.01% DM were subjected to the Gi (left) and Gt (right) activation assays. The samples were irradiated for 30 s with yellow light (>500 nm) or were kept in dark before the assays. All the assays were conducted at 10 °C. Data were plotted vs incubation time and fitted by least-squares lines for the dark state of xtVAL opsin (●, dotted lines), light-activated xtVAL opsin (○, solid lines), the dark state of bovine rhodopsin (■, dotted lines), and light-activated bovine rhodopsin (□, dashed lines). Symbols and error bars represent the mean and standard deviation estimated on the basis of three independent experiments. The light-dependent G protein activation efficiencies of xtVAL opsin and bovine rhodopsin were estimated to be 0.35 and 2.1 pmol/min for Gi and 0.39 and 1.9 pmol/min for Gt, respectively.

extracts solubilized with 0.2% DM for these experiments, because CHAPS could affect the integrity of the G protein[18] and the coupling between the receptor and the G protein.[19] We determined the spectral properties of DM-solubilized xtVAL opsin and bovine rhodopsin before the G protein activation assay. DM-solubilized bovine rhodopsin exhibited preferential formation of the G protein-activating meta II, which has an absorption maximum in the UV region.[20] The UV absorption reflects the deprotonation of the retinylidene Schiff base chromophore.[21] In contrast, irradiation of xtVAL opsin predominantly produced a photoproduct having a protonated retinal Schiff base (Figure 5A). These observations were similar

to those obtained using the CHAPS/PC mixed micelles and the membranes. The photoproduct was stable for at least 4 min at 10 °C, which was long enough for the G protein activation assay. The DM-solubilized xtVAL opsin and bovine rhodopsin were diluted to reduce the DM concentration to 0.01% in the assay. The assays were performed at 10 °C before or after yellow light irradiation. The result showed that light activation of xtVAL opsin caused the GTPγS binding ability of Gi and Gt to be strengthened, although the efficiencies of activation of Gi and Gt by xtVAL opsin were 5−6-fold lower than those by bovine rhodopsin (Figure 5B). It should be noted that the cell membrane fractions containing xtVAL opsin exhibited significant activation of Gi and Gt. This suggests a difference in the conformational changes undergone by the protein to reach the G protein activating state between xtVAL opsin and bovine rhodopsin. Because of the extremely low expression yields of xtVAL opsin, we were unable to prepare an xtVAL opsin sample that met the experimental conditions required to measure the Gq activation.[14,22] On the basis of all of these observations, we concluded that xtVAL opsin can really function as GPCR coupling with Gi-type G proteins and that the G protein activating state of xtVAL opsin is the photoproduct that has a visible absorption spectrum. This indicates that the retinylidene Schiff base chromophore remains protonated in the G protein-activating state of xtVAL opsin.

DISCUSSION

It has generally been considered that the UV and visible absorptions of retinal proteins reflect the unprotonated and protonated retinylidene Schiff base chromophores, respectively.[16,21] This study thus showed that the active state of VAL opsin is not "meta II", which has a deprotonated Schiff base, but rather an intermediate state having a protonated Schiff base, like the active state of invertebrate-type rhodopsins, including parapinopsin. However, it did not show a bistable nature between the dark state and the active state, which is observed in invertebrate-type rhodopsins. In other words, the active state of xtVAL opsin is similar to invertebrate-type rhodopsins with regard to the protonation state of the Schiff base but is similar to vertebrate visual pigments in its irreversible photoreaction. It is well-known that pinopsin exhibits photochemical reactions similar to those of bovine rhodopsin and produces a meta II-like intermediate,[3] while parapinopsin exhibits photochemical behavior similar to that of the invertebrate bistable pigment.[4,7] In this context, it should be noted that the efficiency of the G protein activation by VA/VAL opsin is between those of pinopsin and parapinopsin, which were reported to be similar and ∼20 times weaker than that of bovine rhodopsin.[3,7] Therefore, it appears that VA/VAL opsin represents an intermediate state between those of parapinopsin and pinopsin, and the unique molecular properties of VAL opsin are very informative for tracing the process of evolution of the vertebrate opsins. Additionally, it has been shown that the amplitude of conformational change correlates with the efficiency of G protein activation.[23] Thus, VA/VAL opsin would show a medium-amplitude conformational change between those of vertebrate rhodopsin and parapinopsin, which could be related to the unique properties of the active state of VA/VAL opsin. Alternatively, the difference in the sequences of the cytoplasmic loops may contribute to the difference in the efficiency of G protein activation.[14] On the other hand, it should be noted that the mutant bovine rhodopsin E113A/A117E can form the active state with the protonated retinylidene Schiff base, and the activation efficiency is almost equivalent to that of metarhodopsin II of the wild type.[24,25] On the basis of this observation, the neutralization of Glu113 caused by the transfer of the proton from the Schiff base would be more important for the high efficiency of G protein activation than for the Schiff base deprotonation itself. Thus, in the process of evolution from the ancestor of vertebrate visual and nonvisual opsins, strong G protein activation ability in vertebrate visual opsins would have been achieved by two steps: acquisition of Glu113 as the counterion and formation of the active state accompanied by the transfer of a proton to Glu113.

One of the marked differences between vertebrate visual opsins and invertebrate ones is the position of the counterion of the retinylidene Schiff base. The position of the counterion in invertebrate-type opsins is the glutamic acid at position 181 (based on bovine rhodopsin numbering). In the vertebrate visual opsins, the position of the counterion is displaced to Glu113, even though the glutamic acid at position 181 is well-conserved among all the members except for long-wavelength-sensitive cone pigments.[7,26−28] In the members of the VA/VAL opsin group, positions 113 and 181 are completely conserved as glutamic acid and serine, respectively. This strongly suggests that the glutamic acid at position 113 serves as the counterion of the protonated retinylidene Schiff base in VA/VAL opsin. The conserved serine at position 181 in VA/VAL opsin is also expected to contribute to the unique photochemical properties of xtVAL opsin. To assess the role of serine at position 181, we tried to construct the single mutant S181E of xtVAL opsin but did not obtain a detectable photoabsorbing pigment (data not shown). This suggests that the serine plays an important role in maintaining the 11-*cis*-retinal in the chromophore binding pocket. In bovine rhodopsin, it has been shown that the substitution of the glutamic acid at position 181 moderately affects the G protein activation efficiency and the decay rate of meta II and considerably increases the hydroxylamine reactivity of the Schiff base.[29] However, the spectral properties of the dark and photoactivated states of the mutant rhodopsins, including the E181S mutant, are almost the same as those of the wild type, indicating that the mutation at this position caused little, if any, effect on the absorption and G protein activation characteristics of bovine rhodopsin. The failure of expression of the S181E mutant of xtVAL opsin suggested that the retinal binding pockets of rhodopsin and VAL opsin have different architectures, including the role of the residue at position 181.

The expression pattern of VA/VAL opsin has been studied in detail in some animal species. Chicken VA/VAL opsin is expressed in the hypothalamus,[30] zebrafish VAL opsin in the hypothalamus and retinal horizontal cells,[6] and salmon VA opsin in the pineal gland, subhabenula, and retinal horizontal and amacrine cells.[31,32] The distribution of xtVAL opsin in *X. tropicalis* remains unknown, but it is also considered likely to be expressed in the retinal interneurons and in cells in the deep brain. xtVAL opsin can couple with the Gi-type G protein group, and the sequences of the putative G protein-interacting domains, the second and third cytoplasmic loops, are highly conserved among VA/VAL opsin members (see Figure 2 of the Supporting Information). Therefore, it is likely that VAL opsin functionally drives a common Gi-type G protein group-mediated signal transduction cascade within the retina and the brain.

In summary, we successfully characterized the molecular properties of vertebrate ancient-long opsin found in *X. tropicalis*. We confirmed that light irradiation resulted in the isomerization of 11-*cis*-retinal to the all-trans form and subsequently in G protein activation, as observed for vertebrate visual opsins. Interestingly, the spectroscopic properties of the active state are in part similar to those of vertebrate visual opsins and in part similar to those of invertebrate opsins. Therefore, further investigations comparing VA/VAL opsin and various closely related opsins should provide valuable information about the determinants of the diversified natures of visual and nonvisual opsins.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Decay kinetics of the photoproduct of xtVAL opsin under various conditions (Figure 1) and amino acid sequence alignment of bovine rhodopsin and VA/VAL opsins (Figure 2). This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS

xtVAL, *X. tropicalis* vertebrate ancient long; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]propanesulfonate; DM, dodecyl β-D-maltoside.

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