Cone visual pigments are present in gecko rod cells

(DNA cloning/rod and cone/transmutation theory)

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Communicated by C. Ladd Prosser, March 23, 1992

ABSTRACT

The Tokay gecko (Gekko gekko), a nocturnal lizard, has two kinds of visual pigments, P467 and P521. In spite of the pure rod morphology of the photoreceptor cells, the biochemical properties of P521 and P467 resemble those of iodopsin (the chicken red-sensitive cone visual pigment) and rhodopsin, respectively. We have found that the amino acid sequence of P521 deduced from the cDNA was very similar to that of iodopsin. In addition, P467 has the highest homology with the chicken green-sensitive cone visual pigment, although it also has a relatively high homology with rhodopsins. These results give additional strength to the transmutation theory of Walls [Walls, G. L. (1934) Am. J. Ophthalmol. 17, 892–915], who proposed that the rod-shaped photoreceptor cells of lizards have been derived from ancestral cone-like photoreceptors. Apparently amino acid sequences of visual pigments are less changeable than the morphology of the photoreceptor cells in the course of evolution.

One of the central tenets of vertebrate vision is that there are two fundamentally different types of photoreceptors, rods and cones: cones are associated with daylight (photopic) vision, whereas rods are associated with twilight (scotopic) vision. Over 50 yr ago, however, Walls (1) suggested that some reptiles, especially members of the gecko family, were anomalous because, although they possessed what were clearly rods from a morphological point of view, these rods had several cone-like properties. Walls proposed that in geckos the rods had transmuted from cones (transmutation theory). Others provided morphological (2) and biochemical (3) evidence in favor of this hypothesis. Goldsmith (4) has provided a critique of a more general version of the transmutation hypothesis.

An often unappreciated question in vision is what actually is the difference between rods and cones. In many vertebrate eyes a difference in shape is rather clear, but in others it is not. A better criterion than shape is probably the plasmamembrane topology. In rods, the vast majority of the visual pigments reside in the disks, which are separated from the extracellular medium by the plasma membrane, whereas in cones all visual pigment-containing membranes are in contact with the extracellular medium. Nocturnal geckos contain only rods (5–7). Another useful distinction is the electrophysiological properties of the photoreceptors. Rods are ~100 times more sensitive than cones, and a rod response is about two to five times slower than that of a cone (8). Gecko rods respond as typical rods (9).

The Tokay gecko (Gekko gekko) has two kinds of visual pigments. According to spectrophotometry on the extracted pigments (3) and microspectrophotometry on the visual cells (10, 11), the dominant pigment absorbs light maximally at 521 nm (P521), whereas the minor one absorbs at 467 nm (P467). P521 and P467 have biochemical similarities to iodopsin (chicken red-sensitive cone visual pigment) and rhodopsin, respectively (3, 12); the absorption maxima of both P521 and iodopsin are shifted to shorter wavelengths by removing chloride ions from the medium (chloride effect). The chromophores of P521 and iodopsin are easily attacked by chemicals, such as hydroxylamine, even in the dark. In contrast, neither P467 nor rhodopsin shows the chloride effect, and neither is bleached by hydroxylamine in the dark.

The present study clearly shows that both P521 and P467 are cone-type pigments in terms of amino acid sequences, even though all the gecko photoreceptor cells are morphologically rods. These observations not only support the transmutation theory but also indicate that the morphology of the photoreceptor cells changes between rod and cone shape independently of the visual pigments in the cells.

MATERIALS AND METHODS

Library and Probes. Poly(A)+ RNA (2.0 µg) was prepared from retinas of Gekko gekko according to standard procedures (13). cDNA was prepared with the cDNA Synthesis System Plus kit (Amersham). To prepare the library the cDNA was ligated to AZAPII arms and packaged with Gigapack II Gold (Stratagene).

The AZAPII cDNA library was probed with a mixture of cDNAs encoding chicken visual pigments (ANECO [iodopsin], ref. 14; AF7G [green], AF1 [blue], λARc2 [violet], and λARh [rhodopsin], refs. 15 and 22), after labeling with [α-32P]dCTP by the use of the Random Primer labeling kit (Takara Shuzo, Kyoto).

Screening, Cloning, and Sequencing. The library was plated on agarose and incubated until the plaques were evident in a bacterial lawn. Plaques were transferred to nylon membranes (Hybond-N+, Amersham) and probed with the mixed cDNA probe discussed above under the following conditions: (i) prehybridized for 1 hr at 37°C in 5% sterilized skim milk (Difco); (ii) hybridization overnight at 42°C in hybridization buffer [5× standard saline citrate (SSC) (1× SSC = 0.3 M sodium chloride/0.03 M sodium citrate, pH 7.0)/40% forma- mide/10 mM sodium phosphate/0.25% sterilized skim milk (Difco)/10% dextran sulfate/denatured salmon sperm DNA at 100 µg/ml]; (iii) after hybridization, nylon membranes were washed for 10 min three times in 2× SSC/0.1% SDS at 50°C. The probed nylon membranes were then autoradiographed overnight for screening. Those plaques containing cDNA inserts that hybridized to the probe were identified by

Abbreviations: λmax, absorption maximum; P521, dominant visual pigment of gecko, which absorbs light maximally at 521 nm; P467, minor visual pigment of gecko, which absorbs light maximally at 467 nm.

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The sequences reported in this paper have been deposited in the GenBank data base (accession nos. M92035 and M92036).
FIG. 1. Sequences of cDNAs encoding gecko visual pigments. GZ1 encodes P467 (A), and GZ8 encodes P251 (B). The long open reading frame of each clone started from the first ATG codon. The polyadenylation signal AATAAA in GZ1 is underlined but is absent in GZ8. Putative transmembrane domains (I–VII) are shown by lines under the deduced amino acid sequences.

A

B
a positive signal on the film. Phage stocks were then made by isolating positively identified plaques.

The cDNA inserts from the isolated phage stocks were subcloned into the bacterial plasmid pBluescript SK(-) prepared from the isolated phage clones according to the in vivo excision protocol of AZAPII (Stratagene). These plasmids or unidirectionally deleted ones (13) were sequenced by using [α-32P]dCTP and the Sequenase V2.0 system in the presence of single-stranded DNA-binding protein (United States Biochemical).

**Identities of Amino Acid Sequences.** After the amino acid sequences of vertebrate visual pigments (14–22) were aligned visually to optimize similarity, the values of amino acid identities (percent identities) between each pair of the sequences were calculated at every position, excluding positions where gaps exist in either of the two sequences.

**RNA Blot Analysis.** Blots of glyoxylated poly(A)+ RNA (13) (1.5 µg per lane) from gecko (Gekko gekko) retinas were hybridized with random-primed 32P-labeled insert DNAs (3.0 × 10^6 dpm per lane) in the hybridization buffer as described above. After hybridization, blots were washed with the 4× SSC/0.1% SDS at 50°C and exposed for 16 hr at room temperature to x-ray film.

**RESULTS AND DISCUSSION**

**Isolation of cDNA.** A mixture of five kinds of chicken visual pigment cDNA (chicken iodopsin, green, blue, violet, and rhodopsin) was used as a probe to screen an oligo(dT)-primed cDNA library (1.1 × 10^6 clones; constructed in a phage vector, AZAPII) from the retinas of Gekko gekko. Sixty-two positive signals were obtained, among which 15 clones were isolated. These clones were classified into two groups; one group strongly hybridized with the cDNA of chicken rhodopsin and chicken green, the chicken green-sensitive cone pigment (class I; six clones), and the other clones hybridized with the chicken iodopsin cDNA (class II; nine clones). Two clones, designated as GZ1 (class I) and GZ8 (class II), were further characterized. The nucleotide sequences of clones GZ1 (Fig. 1A) and GZ8 (Fig. 1B) consisted of 1667 and 2118 nucleotides, respectively, in each of which a long open reading frame encoding a visual pigment was found. As expected, the deduced amino acid sequence of GZ8 (365 residues) was strikingly similar to that of iodopsin (14) (Table 1), suggesting that GZ8 encodes P521. On the other hand, the high homology in amino acid sequence between GZ1 (355 residues) and rhodopsin (17–21) suggested that GZ1 encodes P467. Interestingly, GZ1 showed the highest homology with chicken green-sensitive cone pigment (22) (Table 1) among all visual pigments for which primary structures are known.

In an RNA blot analysis (Fig. 2), GZ1 and GZ8 hybridized with the 1.9-kb and 2.6-kb bands, respectively, in gecko retinal mRNA. The ratio in density between these two bands was estimated at 1/17. This ratio agrees well with the ratio of the pigments P467 and P521 found in digitonin extracts of the retina (1/10; ref. 23), supporting the hypothesis that GZ1 and GZ8 encode P467 and P521, respectively.

**Classification of the Gecko Visual Pigments.** The isoelectric points (pI) of P467 (9.7) and P521 (9.8) were calculated from their deduced amino acid sequences (22). They indicate that both pigments are basic, which is a common feature of the cone visual pigments studied so far (14, 16, 22), in contrast with acidic pl for vertebrate rhodopsins (17–21). Furthermore, the amino acid sequences of P521 and P467 are closest to those of iodopsin and the chicken green-sensitive cone pigment, respectively (Table 1).

**Color Regulation of P521 and P467.** The vertebrate visual pigments can be divided into at least four groups on their amino acid sequences. The groupings are well correlated with one based on the absorption maximum of the visual pigments (22). We designate the groups as long-wavelength sensitive, short-wavelength sensitive, and middle-wavelength sensitive, with two subgroups M1 and M2. M1 includes the chicken blue-sensitive cone pigment, whereas M2 includes P467, the chicken green-sensitive cone pigment and all of the rod pigments. Of the pigments in group M2, the chicken green-sensitive cone pigment has the greatest sequence similarity to gecko P467 (Table 1). Accordingly, the sequence alignment among the visual pigments that fall into the same group enabled us to select the most likely amino acid residues involved in the color regulation of the visual pigments. Our criteria for selecting such residues are as follows: (i) They are situated in the putative transmembrane segments. (ii) The results of site-directed mutagenesis are fully taken into consideration (24–28). (iii) Polar and polarizable amino acid residues are boxed, and the highest value in each column is in boldface type.

![Fig. 2. Northern (RNA) blot analysis of gecko retinal mRNA with cDNA clones GZ1 and GZ8. Numbers at right mark sizes. GZ1 and GZ8 yielded 1.9-kilobase (kb) and 2.6-kb bands, respectively. In addition, GZ8 yielded minor bands (6.2 kb and 4.2 kb), suggesting different splicing or multiple transcription.](image-url)
Table 2. Divergent amino acid residues affecting spectral properties of visual pigments in the long-wavelength group

<table>
<thead>
<tr>
<th>Visual pigment</th>
<th>( \lambda_{\text{max}} ) nm</th>
<th>Amino acid position</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gecko P521</td>
<td>521* V F V A W F A S</td>
<td></td>
</tr>
<tr>
<td>Human green</td>
<td>542† T W T A A F A C</td>
<td></td>
</tr>
<tr>
<td>Human red</td>
<td>558† T W T S A Y T C</td>
<td></td>
</tr>
<tr>
<td>Chicken iodopsin</td>
<td>571† T W T S C Y T C</td>
<td></td>
</tr>
</tbody>
</table>

Numbers of amino acid positions refer to those of bovine rhodopsin (17, 18). Single-letter codes for amino acid residues are used. In each column, replacement of an amino acid residue with another (boldface type) could shift \( \lambda_{\text{max}} \) to longer wavelengths.

*Okano et al. (30).
†Crescitelli (23).
‡Wald and Brown (33).

Table 3. Divergent amino acid residues affecting spectral properties of visual pigments in the second subgroup of the middle-wavelength group

<table>
<thead>
<tr>
<th>Visual pigment</th>
<th>( \lambda_{\text{max}} ) nm</th>
<th>Amino acid position</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken green</td>
<td>508* C T Q S</td>
<td></td>
</tr>
<tr>
<td>Gecko P467</td>
<td>467† F A Q A</td>
<td></td>
</tr>
<tr>
<td>Bovine rhodopsin</td>
<td>498‡ A L E A</td>
<td></td>
</tr>
</tbody>
</table>

Numbers of amino acid positions refer to those of bovine rhodopsin (17, 18). Single-letter codes for amino acid residues are used. In each column, replacement of an amino acid residue with another (boldface type) could shift \( \lambda_{\text{max}} \) to longer wavelengths.

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