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The primary structure of iodopsin, a chicken red-sensitive cone pigment

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A purified iodopsin was digested by CNBr or several proteolytic enzymes into fragments, the amino acid sequences of which were determined. A partial sequence of the C-terminal fragment was utilized for synthesizing an oligonucleotide probe which identified the iodopsin cDNA (1339 bases). The deduced amino acid sequence (362 residues) had 80%, 42% or 43% homology to that of human red-sensitive cone pigment, cattle or chicken rhodospin, respectively. Although the hydropathy profile implies that iodopsin, like rhodopsin, has 7 transmembrane α-helical segments, iodopsin may have a hydrophilic pocket near the seventh segment on the basis of the unexpected cleavages in the middle of the segment VII by chymotrypsin under nondenaturing conditions.

Iodopsin; Cone visual pigment; Color vision; Primary structure; cDNA cloning; Chicken retina

1. INTRODUCTION

Color vision is initiated by light absorption of several kinds of cone cells having their own spectral photosensitivities. For elucidating the visual transduction process in the cone cells, biochemical and spectroscopic properties of chicken red-sensitive cone pigment, iodopsin [1,2], have been extensively investigated in our laboratory [3-9], including the interaction with a GTP-binding protein [5], the photochemical reactions dependent on and independent of chloride ion [7], and the chromophore-protein interaction [8] modulated by chloride ion [9]. These studies revealed that iodopsin has unique characteristics different from rhodopsin, rod visual pigment. In order to determine which amino acid residues are responsible for these characteristics of iodopsin, we have deduced the primary structure of iodopsin from its complementary DNA (cDNA).

2. EXPERIMENTAL

2.1. *Peptide sequencing and probe synthesis*

Iodopsin was purified from chicken retinas as described [6]. The purified iodopsin was digested as follows: (i) by cyanogen bromide (CNBr) after carboxymethylation with monoiodoacetic acid; (ii) by *A. lyticus* protease I (API) in the presence of 4 M urea; (iii) by *S. aureus* V8 protease (V8) in 10 mM Tris-HCl buffer (pH 8.0) containing 0.75% Chaps; or (iv) by tosyl-lysine chloromethyl ketone-treated chymotrypsin (Ch) after dialysis against 0.1 M NH_4HCO_3 (pH 8.0) containing 0.75% Chaps. The digests were fractionated by a reversed-phase HPLC (Waters; Model 600E), and sequenced by an automated gas-phase amino acid sequencer (Applied Biosystems; Model 477A). The oligonucleotide G1A56 (5'-ACCTCGGAGCCATCATCCACCTTCTTGCCAAACAGCTGCAGGATGCAGTTCCGGAA-3'; 56-mer) was synthesized by an automated DNA synthesizer (Applied Biosystems; Model 380B) in accordance with the C-terminal amino acid sequence of iodopsin (FRNCILQLFGKKVDDGSEV).

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2.2. *cDNA cloning and northern hybridization analysis*

Plaques of 1.2×10^5 recombinant phages in a λgt11 cDNA library derived from chick retinal poly(A)$^+$ RNA were transferred to nylon membranes (Amersham; Hybond-N$^+$), which were hybridized with ^{32}P-end-labeled G1A56 at 42°C in 5 × SSC (1 × SSC = 0.3 M sodium chloride, 0.03 M sodium citrate, pH 7.0) containing 30% formamide, washed in 0.25 × SSC containing 0.1% sodium dodecyl sulfate (SDS) at 50°C and autoradiographed. cDNA inserts of hybridization-positive phages were subcloned into pUC118/119 plasmid vector and sequenced [10]. Isolated cDNA clones were used as hybridization probes for further screening and the longest cDNA clone was designated NECO. Comparison of sequences and calculation of hydropathy profiles [11] were conducted by DNASIS program (Hitachi Software Engineering). Blots of glyoxylated poly(A)$^+$ RNA (4 μg) from chick retina, brain, kidney and liver were hybridized with the ^{32}P-labeled NECO at 42°C in 5 × SSC and 50% formamide, washed in the 0.1 × SSC containing 0.1% SDS at 50°C and autoradiographed.

3. RESULTS

A purified iodopsin was digested by cyanogen bromide (CNBr) and several kinds of proteolytic enzymes. The resulting peptide fragments were isolated and sequenced (Fig. 1). A part of the sequence of the fragment obtained by CNBr treatment (F326-V344 in the C-terminal fragment N323-A362; see Fig. 1) was used to synthesize a single 56meric oligonucleotide probe termed G1A56. A chicken retinal cDNA library was screened by using ^{32}P-end-labeled G1A56. One of the positive cDNA clones termed NECO was confirmed to

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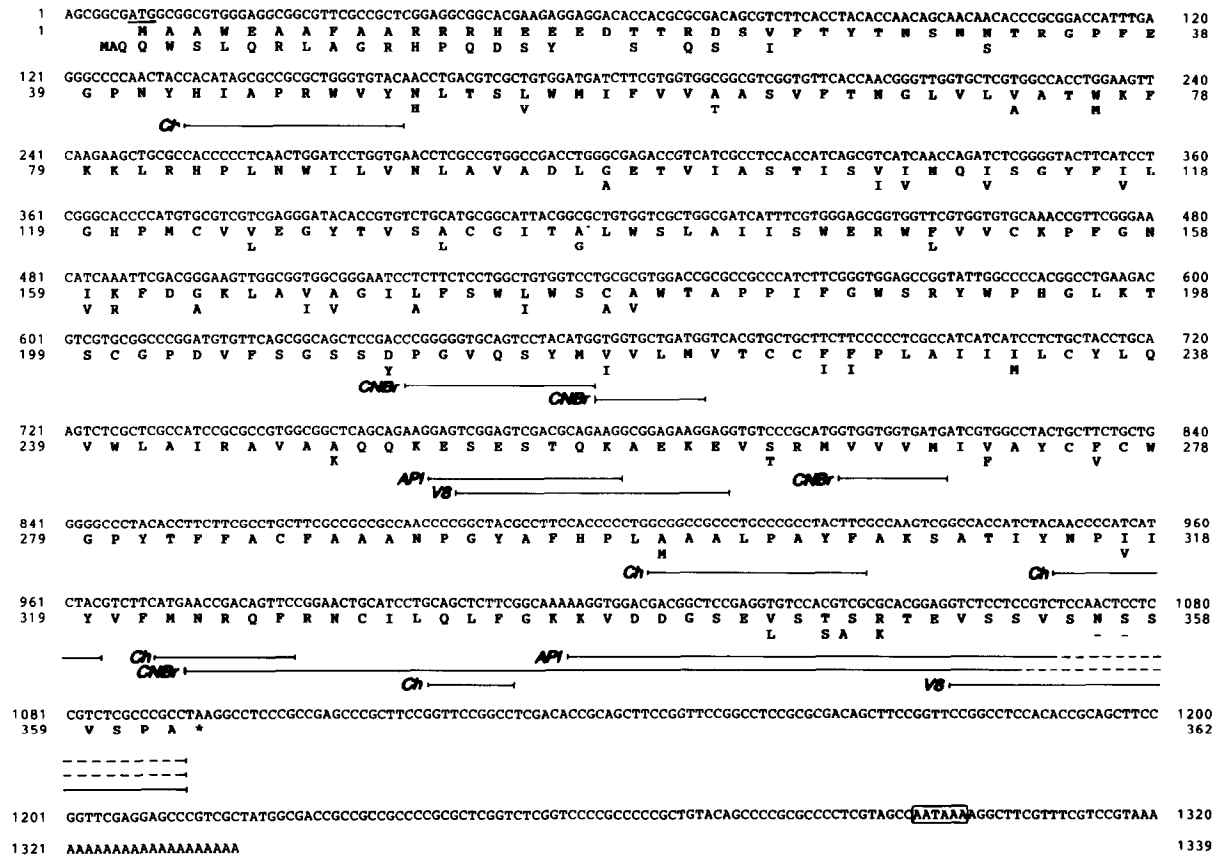

Fig. 1. The nucleotide sequence of cDNA clone NECO aligned with its deduced amino acid sequence. Partial amino acid sequences determined by analyses of iodopsin fragments are underlined. (Broken underlinings indicate sequences which could not be identified exclusively.) Amino acid residues of human red-sensitive pigment [13] are indicated below those of iodopsin only when different. A polyadenylation signal (AATAAA at positions 1294–1299) is boxed.

encode iodopsin because the deduced amino acid sequence comprised all the partial sequences of the purified iodopsin fragments (Fig. 1).

Northern hybridization analyses confirmed that a poly(A)$^+$ RNA (mRNA) which hybridizes with the iodopsin cDNA was exclusively localized in the retina: ^{32}P-labeled NECO strongly hybridized with a 1.5 kb poly(A)$^+$ RNA derived from chick retinas (Fig. 2, lane 1), whereas no hybridization was detected with RNAs from brain, liver and kidney. A slight difference in size between the poly(A)$^+$ RNA (1.5 kb) and NECO (1339 bases, including 22 bases of poly(A) tail) suggests that iodopsin mRNA may have about 0.2 kb of poly(A) tail.

An initiation site of iodopsin was estimated to be an ATG codon located at positions 8–10 in NECO (Fig. 1) on the basis of the following two reasons. First, this is the only possible initiation site for encoding the whole amino acid sequence determined by means of peptide sequencing, and second, the presence of a purine-base at a position three nucleotides upstream from the ATG codon (G at position 5 in NECO) fulfills the Kozak criteria [12] for eukaryotic initiation sites. Thus the translation of iodopsin starts at this ATG codon and ends at a termination codon (TAA) of positions 1094–1096 (Fig. 1). The translated product was composed of 362 amino acid residues (M1–A362). The molecular mass of iodopsin calculated from the deduced amino acid sequence was 40 338, which was

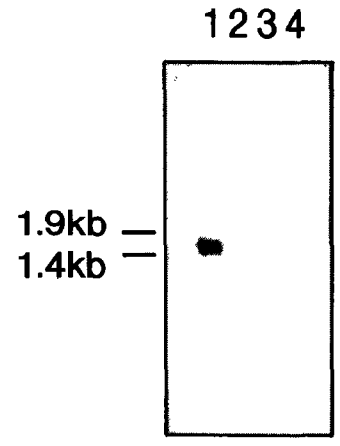

Fig. 2. The Nothern hybridization of the iodopsin cDNA was conducted with poly(A)$^+$ RNA from chick retina (lane 1), brain (2), kidney (3) and liver (4). Denatured fragments of *Bst*PI-digested λDNA were used as size markers. Strong hybridization of retinal poly(A)$^+$ RNA (1.5 kb) was detected (lane 1).

somewhat higher than the value estimated from an SDS-polyacrylamide gel electrophoresis of a purified iodopsin (36000) [6]. This difference could be caused by inaccuracy of the measurement by electrophoresis and/or post-translational modification(s) including a cleavage of amino acid(s) from the N-terminus of iodopsin. Our attempt to determine the N-terminal sequence of the purified iodopsin by Edman degradation was unsuccessful, presumably due to a modification at the N-terminal amino acid.

Iodopsin was 75% identical to human red-sensitive pigment [13] in the nucleotide sequence, and their mutual identity in the deduced amino acid sequence was 80% (Fig. 1). On the other hand, only 42% or 43% of amino acid residues of iodopsin were identical to those of cattle [14] or chicken [15] rhodopsin, respectively (Fig. 3A). However, the notable conservations of amino acids among all the visual pigments indicate that they have been evolutionally derived from a common ancestral gene [16,17].

4. DISCUSSION

Cattle rhodopsin has been inferred to form 7 transmembrane α-helical segments [14]. Iodopsin would have the similar structure because the hydro-

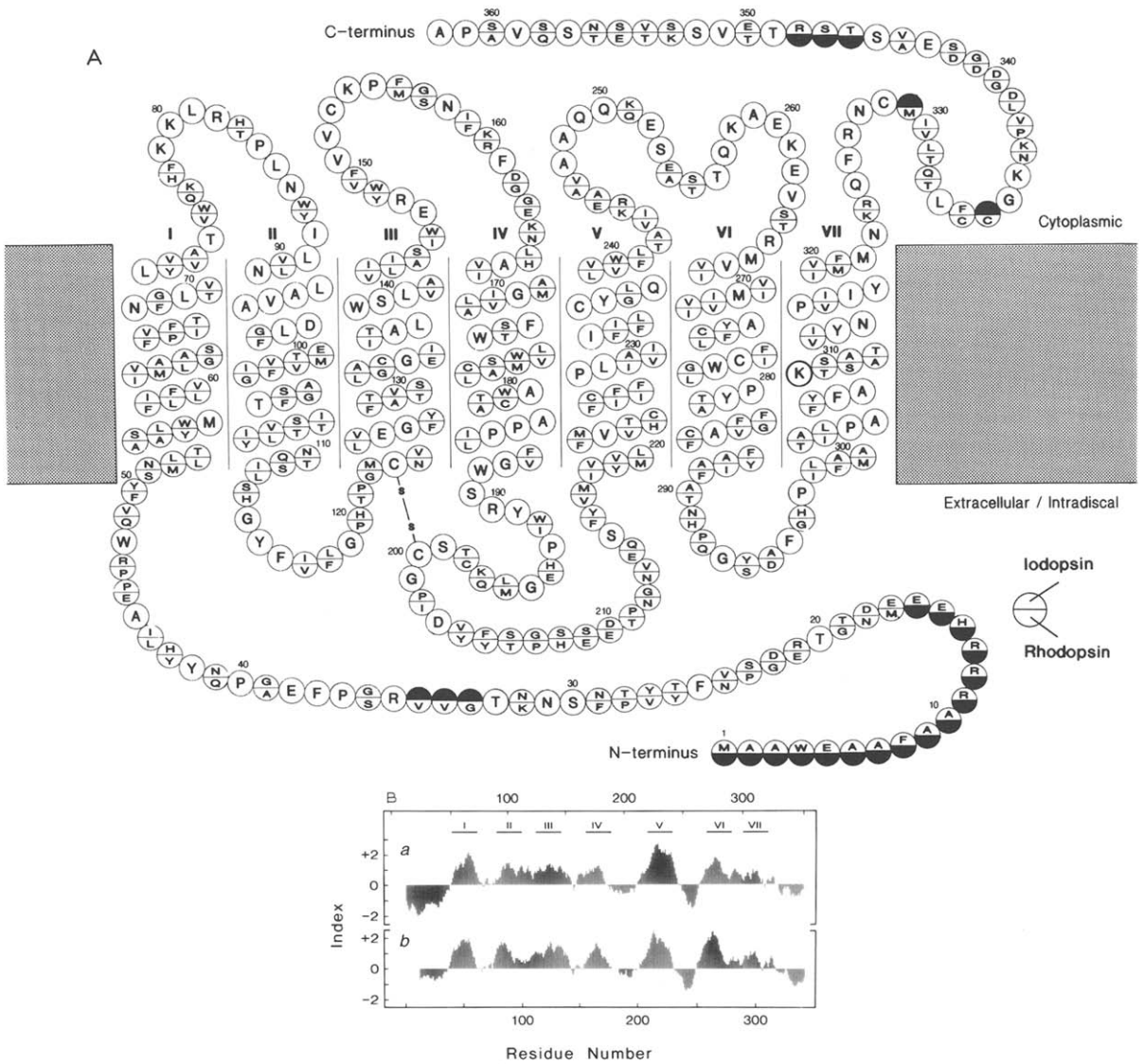

Fig. 3. The structural model and the hydropathy profile of iodopsin compared with those of cattle rhodopsin. (A) Structural model for iodopsin is constructed on the basis of the proposal for cattle rhodopsin [14]. Residues of cattle rhodopsin are shown in the lower half of each circle when different from corresponding residues of iodopsin. Several blank residues indicated by closed semicircles are inserted to make a good match in sequence between iodopsin and rhodopsin. The residues of iodopsin are numbered beginning with the N-terminal methionine. Seven putative transmembrane α-helical segments are numbered as I–VII. The chromophore-binding site (K309) is highlighted in the middle of segment VII. A disulfide bond (–S–S–) is assumed between loop II-III (inter-helical domain between segments II and III) and loop IV-V. (B) Hydropathy profiles [11] of iodopsin (a) and cattle rhodopsin (b) were calculated. The residue window size used for smoothing is 20. A mean value of hydrophobicity index was 0.36 or 0.48 for iodopsin or rhodopsin, respectively. Predicted transmembrane segments are indicated by horizontal bars.

pathy profiles [11] of iodopsin and cattle rhodopsin resemble each other (Fig. 3B). Cleavage sites of iodopsin under nondenaturing conditions by V8 protease (at E252-S253, E262-V263 and E350-V351) or by chymotrypsin (at Y42-H43, Y50-N51, L299-A300, F321-M322, F326-R327, L331-Q332 and F334-G335) helped us to estimate the inter-helical loop domains (Fig. 3A).

A chromophore of iodopsin, 11-*cis* retinal, should be attached to K309 through a Schiff-base linkage, which was proved in case of cattle rhodopsin [18]. The corresponding lysine residue is conserved among all the known sequences of visual pigments. In Fig. 3A, K309 is positioned in the middle of the putative transmembrane segment VII as was proposed in cattle rhodopsin. It should be noted, however, that chymotrypsin cleaved iodopsin at 3 positions, F307-A308, Y314-N315 and Y319-V329, within the segment VII. This fact suggests that iodopsin has a hydrophilic pocket near the segment VII, which may share the chloride-binding site [7,9] characteristic to iodopsin. This idea is consistent with the previous observations that the Schiff-base linkage of the chromophore of iodopsin, unlike rhodopsin, was attacked by chemical reagents such as NH_2OH [1] and $NaBH_4$ [3].

One of the 3 possible glycosylation sites (N-X-S/T) in iodopsin N31, N51 and N356) is located in the N-terminal tail (N31) where rhodopsin has two sites [19]. At least one of them should be glycosylated because of the binding of iodopsin to the concanavalin A-Sepharose column [4,6].

Two cysteine residues in the loop II-III and IV-V (C123 and C200, respectively, in iodopsin) are conserved among all the visual pigments [13], and may form a disulfide bond [20] (Fig. 3A). On the other hand, two adjacent cysteine residues in the C-terminal region found in rhodopsin are replaced by a single phenylalanine residue (F334). In the case of cattle rhodopsin, the two residues are covalently modified with palmitates [21], which are believed to be attached to the membrane to form an extra cytoplasmic loop. The absence of those cysteine residues in iodopsin and all the human cone pigments [13] might represent some functional differences of the C-terminal domain between cone and rod pigments.

It has been demonstrated that a lysine residue (corresponding to K261 of iodopsin) in the cytoplasmic loop domain V-VI of photoexcited cattle rhodopsin was implicated in activation of transducin (a retinal GTP-binding protein) [22]. Since an irradiated iodopsin was able to activate cattle transducin [5], the corresponding domain of iodopsin having homologous sequence to rhodopsin would be a candidate for the interaction site(s) with cone transducin.

The C-terminal tail of iodopsin shows a serine- and threonine-enriched sequence. The hydroxyl groups in the C-terminal tail of cattle rhodopsin are phosphorylated in a light-dependent manner by opsin kinase [23], which is involved in the deactivation process of transducin. Thus the corresponding region of iodopsin would have a similar function in the shut-off process of the visual transduction in cone. In fact, we have observed the light-dependent phosphorylation of iodopsin by cattle opsin kinase [24].

The characteristic absorption spectra of visual pigments are originated by electrostatic and/or steric interactions between the common chromophore, 11-*cis* retinal, and their apoproteins, opsin. In cattle rhodopsin, the glutamate at position 113 has been shown to be a candidate for the counterion to the protonated Schiff-base of the chromophore [25,26]. Since the corresponding residue (E126) was found in iodopsin, this residue may also serve as the counterion.

It was also suggested that the glutamate at position 122 of rhodopsin would be a putative second point charge which interacts with the chromophore [27], although a recent investigation suggests that it is not dissociated [25]. Interestingly, this residue was replaced by I135 in iodopsin. However, our recent experiments [8] suggested the presence of the corresponding second point charge in iodopsin at a position similar to that in rhodopsin. These results, taken together, may suggest that the replacement would be compensated by the occurrence of E99 (that is absent in rhodopsin) in the segment II which can be placed at a similar position to that of the glutamate-122 in cattle rhodopsin. Therefore, the unique absorption maximum of iodopsin which is 70 nm red-shifted from that of rhodopsin is likely due to the binding of chloride in the hydrophilic pocket near the Schiff-base linkage of the chromophore.

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