FEBS Letters 470 (2000) 336–340 FEBS 23473

Purification and characterization of bovine cone arrestin (cArr)

Tadao Maeda[a], Hiroshi Ohguro[a,*], Hitoshi Sohma[b], Yoshio Kuroki[b], Hiroshi Wada[c], Shigekuni Okisaka[c], Akira Murakami[c]

[a] *Department of Ophthalmology, Sapporo Medical University School of Medicine, S-1 W-16, Chuo-ku, Sapporo 060-8543, Japan*
[b] *Department of Biochemistry (section 1), Sapporo Medical University School of Medicine, S-1 W-16, Chuo-ku, Sapporo 060-8543, Japan*
[c] *Department of Ophthalmology, National Defense Medical College, 3-1 Namiki, Tokorozawa, Saitama, Japan*

Received 13 December 1999; received in revised form 1 March 2000

Edited by Takashi Gojobori

Abstract To elucidate the quenching mechanism of phototransduction in vertebrate cone photoreceptors, a cDNA clone encoding cone specific arrestin (cArr) was isolated from a bovine retinal cDNA library using a human cArr cDNA probe. Affinity-purified anti-peptide antibody specific to cArr was prepared. Immunohistochemical staining displayed specific labeling of cArr in cone photoreceptors and immunoblotting identified a 46 kDa protein band. We purified cArr from bovine retinas by sequential column chromatography using DEAE-cellulose, gel filtration and mono Q columns. Binding studies revealed no binding of cArr to rhodopsin regardless of whether it was bleached and/or phosphorylated. cArr also failed to bind to heparin-Sepharose under conditions which rod arrestin (rArr) bound to the column. The present data suggest that cArr may play a role in the quenching of phototransduction in cone photoreceptors and that its activity therein is different to that of rArr.

Key words: Phototransduction; Cone photoreceptor; Cone arrestin; Arrestin; G protein coupled receptor; Rhodopsin phosphorylation

1. Introduction

Members of the arrestin family are known to be involved in the desensitization of agonist stimulated G protein coupled receptors [1]. In vertebrate rod photoreceptors, upon absorption of a photon by the receptor, Meta II, an active photobleaching intermediate of rhodopsin (Rho*), activates the GTP-binding protein (Gt) amplification cascade, which in turn generates a visually evoked signal. This cascade is shut down by phosphorylation of Rho* by rhodopsin kinase (RK), resulting in blockage of further interaction of Meta II with Gt by binding with rod arrestin (rArr). In addition, an alternative mRNA splicing form of rArr, called p44, has been demonstrated to be involved in the quenching phototransduction through its binding with Meta II [2]. Thus, rArr and p44 are both key molecules in the quenching phototransduction of rod photoreceptors. Meanwhile, in cone photoreceptors, it has been suggested that a similar arrestin mediated mechanism is involved in the termination of the photoexcitation since a cone-specific homologue of arrestin (cArr), which is alternatively called X-arrestin, has been cloned from human cDNA library [3,4]. However, the primary structure of the C-terminal domain in rArr, in which the binding site for phosphorylated rhodopsin (P-Rho*) is involved, [5,6] is distinct from that of cArr. Moreover, cone specific visual pigment kinase has not been identified yet. These observations lead us to speculate that cArr may function differently from rArr.

In the present study, to better understand the functional roles of cArr, bovine cArr was cloned using the human cArr cDNA probe and native protein was prepared from bovine retinas. Then, characterization of cArr was performed by determining its localization within retinal cells and its binding affinity to P-Rho and heparin.

2. Materials and methods

Unless otherwise stated, all procedures were performed using ice-cold solutions. All chemicals used were analytical grade.

2.1. Isolation of a cDNA clone of bovine cArr

A bovine retina cDNA library (purchased from Stratagene) was screened using the human cArr cDNA [3,4] as a probe. The plasmid cDNA clones were excised from the lambda phages using the excision phage. The plasmid inserts were sequenced by the dideoxy chain termination method using a Cycle Sequencing kit (Toyobo, Tokyo, Japan). The nucleic acid data were analyzed with computer software GENETEX (System Soft, Japan) and Clastal W. The nucleotide sequence data reported in this paper has been submitted to the DDBJ, EMBL and GenBank nucleotide sequence database with accession number sequence D85340.

2.2. Preparation of antibodies against bovine cArr peptide and bovine rArr antibodies

The bovine cArr sequence was analyzed for a unique region and a 15 amino acid sequence, ^{354}PKPSNEAASSEDIVI, was chosen from near the C-terminus. The peptide conjugated with MAP resin (Applied Biosystems) was synthesized and used to prepare an anti-peptide antibody. The peptide was injected into a New Zealand white rabbit. The antiserum was purified by affinity chromatography using the peptide coupled with CSNB-activated Sepharose 4B (Pharmacia Biotech) [4]. A rat was immunized with purified bovine rArr [7] with complete Freund's adjuvant.

The antisera obtained were each subjected to IgG isolation by a protein G column and affinity purification using antigen conjugated column chromatography. IgG concentration was estimated by spectrophotometry (OD_{280}).

2.3. Purification of bovine cArr

Fifty frozen dark-adapted bovine retinas were homogenized with 50 ml of 10 mM HEPES buffer, pH 7.5, containing 1 mM benzamidine in a glass–teflon homogenizer under a dim red light, followed by centrifugation at $46\,000 \times g$ for 60 min. The supernatant was applied

*Corresponding author. Fax: (81)-11-613 6575.
E-mail: ooguro@sapmed.ac.jp

Abbreviations: rArr, rod arrestin; cArr, cone arrestin; Gt, GTP-binding protein; Rho, rhodopsin; Rho*, photolyzed rhodopsin; P-Rho, phosphorylated rhodopsin; RK, rhodopsin kinase; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; BTP, bis–tris propane; FPLC, fast protein liquid chromatography

PII: S0014-5793(00)01334-X

to a column of DEAE-cellulose (1.6×17 cm) which had been equilibrated with the 10 mM HEPES buffer, pH 7.5. The column was washed with 10 mM HEPES buffer, pH 7.5, containing 15 mM NaCl until the absorbance at 280 nm dropped below 0.1. Proteins bound to the column were eluted with a gradient of NaCl from 0 to 150 mM in a total of 240 ml of the same buffer. Aliquots (3 ml each) were collected and subjected to immunoblot analysis using cArr IgG. The fractions containing cArr were pooled, concentrated to approximately 1 ml by centricon 30 (Amicon) and applied onto a Hiprep Sephacryl High Resolution gel filtration column (2.6×60 cm) equipped with fast protein liquid chromatography (FPLC), which had been equilibrated with 10 mM HEPES buffer, containing

```
HUMB1 1    --------MGDKGTRVFKKASPNGKLTVYLGKRDFVDHIDLVDPVDGVVLVDPEYLKERRVYVTLTCAFRYGREDLDVLGLTFRKDLFVANVQSFPPAPE
BOVB1 1    --------MGDKGTRVFKKASPNGKLTVYLGKRDFVDHIDLVEPVDGVVLVDPEYLKERRVYVTLTCAFRYGREDLDVLGLTFRKDLFVANVQSFPPAPE
HUMB2 1    -------MGEKPGTRVFKKSSPNCKLTVYLGKRDFVDHLDKVDPVDGVVLVDPDYLKDRKVFVTLTCAFRYGREDLDVLGLSFRKDLFIATYQAFPPVPN
BOVB2 1    -------MGEKPGTRVFKKSSPNCKLTVYLGKRDFVDHLDKVDPVDGVVLVDPDYLKDRKVFVTLTCAFRYGREDLDVLGLSFRKDLFIANYQAFPPTPN
HUMSA 1    MAASGKTSKSEPNHVIFKKISRDKSVTIYLGNRDYIDHVSQVQPVDGVVLVDPDLVKGKKVYVTLTCAFRYGQEDIDVIGLTFRRDLYFSRVQVYPPVGA
BOVSA 1    ----MKANKPAPNHVIFKKISRDKSVTIYLGKRDYIDHVERVEPVDGVVLVDPELVKGKRVYVSLTCAFRYGQEDIDVMGLSFRRDLYFSQVQVFPPVGA
HUMCA 1    ------------MSKVFKKTSSNGKLSIYLGKRDFVDHVDTVEPIDGVVLVDPEYLKCRKLFVMLTCAFRYGRDDLEVIGLTFRKDLYVQTLQVVPAESS
BOVCA 1    ---------MANMSRVFKKTCSNGKLSIYLGKRDFVDHVDMVEPIDGVVLVDPEYLKGRKMFVMLTCAFRYGHDDLDVIGLTFRKDLYVQVQQVVPAESS

HUMB1  93  DKK-PLTRLQERLIKKLGEHAYPFTFEIPPNLPCSVTLQPGPEDTGKACGVDYEAKAFCAENLEE---KIHKRNSVGLVIRKVQYAPERPGPQPTAETTR
BOVB1  93  DKK-PLTRLQERLIKKLGEHAYPFTFEIPPNLPCSVTLQPGPEDTGKACGVDYEVKAFCAENLEE---KIHKRNSVRLVIRKVQYAPERPGPQPTAETTR
HUMB2  94  PPR-PPTRLQDRLLRKLGQHAHPFFFTIPQNLPCSVTLQPGPEDTGKACGVDFEIRAFCAKSLEE---KSHKRNSVRLVIRKVQFAPEKPGPQPSAETTP
BOVB2  94  PPR-PPTRLQERLLRKLGQHAHPFFFTIPQNLPCSVTLQPGPEDTGKACGVDFEIRAFCAKSLEE---KSHKRNSVRLVIRKVQFAPEKPGPQPSAETTR
HUMSA 101  AST--PTKLQESLLKKLGSNTYPFLLTFPDYLPCSVMLQPAPQDSGKSCGVDFEVKAFATDSTDAEEDKIPKKSSVRLLIRKVQHAPLEMGPQPRAEAAW
BOVSA  97  SGA--TTRLQESLIKKLGANTYPFLLTFPDYLPCSVMLQPAPQDVGKSCGVDFEIKAFATHSTDVEEDKIPKKSSVRLLIRKVQHAPRDMGPQPRAEASW
HUMCA  89  SPQGALTVLQERLLHKLGDNAYPFTLQMVTNLPCSVTLQPGPEDAGKPCGIDFEVKSFCAENPEE---TVSKRDYVRLVVRKVQFAPPEAGPGPSAQTIR
BOVCA  92  SPRGSLTVLQERLLHKLGDNAYPFTLQMVVNLPCSVTLQPGPDDTGKACGVDFEVKSFCAENLEE---KVSKRDSVRLVIRKIQFAPLEPGPGPWARLCR

HUMB1 190  QFLMSDKPLHLEASLDKEIYYHGEPISVNVHVTNNTNKTVEKIKISVRQYADICLFNTAQYKCPVAMEEADDTVAPSSTFCKVYTLTPFLANNREKRGLA
BOVB1 190  QFLMSDKPLHLEASLDKEIYYHGEPISVNVHVTNNTNKTVKKIKISVRQYADICLFNTAQYKCPVAMEEADDTVAPSSTFCKVYTLTPFLANNREKRGLA
HUMB2 191  HFLMSDRSLHLEASLDKELYYHGEPLNVNVHVTNNSTKTVKKIKVSVRQYADICLFSTAQYKCPVAQLEQDDQVSPSSTFCKVYTITPLLSDNREKRGLA
BOVB2 191  HFLMSDRSLHLEASLDKELYYHGEPLNVNVHVTNNSTKTVKKIKVSVRQYADICLFSTAQYKCPVAQVEQDDQVSPSSTFCKVYTITPLLSNNREKRGLA
HUMSA 199  QFFMSDKPLHLAVSLNKEIYFHGEPIPVTVTVTNNTEKTVKKIKAFVEQVANVVLYSSDYYVKPVAMEEAQEKVPPNSTLTKTLTLLPLLANNRERRGIA
BOVSA 195  QFFMSDKPLRLAVSLSKEIYYHGEPIPVTVAVTNSTEKTVKKIKVLVEQVTNVVLYSSDYYIKTVAAEEAQEKVPPNSSLTKTLTLVPLLANNRERRGIA
HUMCA 186  RFLLSAQPLQLQAWMDREVHYHGEPISVNVSINNCTNKVIKKIKISVDQITDVVLYSLDKYTKTVFIQEFTETVAANSSFSQSFAVTPILAASCQKRGLA
BOVCA 189  RFLLSAQPLLLQAWMDKEVNYHGQPISVNVSINNSTNKVIKKIKISVDQITDVVLYSLDKYTKTVFVQEFTETIAANSTFSKSFAVTPLLADNCHKQGLA

HUMB1 290  LDGKLKHEDTNLASSTLLREGANREILGIIVSYKVKVKLVVSRGGLLGDLASSDVAVELPFTLMHPKPKEEPPHREVP---------------ENETPVD
BOVB1 290  LDGKLKHEDTNLASSTLLREGANREILGIIVSYKVKVKLVVSRGGLLGDLASSDVAVELPFTLMHPKPKEEPPHREVP---------------EHETPVD
HUMB2 291  LDGKLKHEDTNLASSTIVKEGANKEVLGILVSYRVKVKLVVSRG--------GDVSVELPFVLMHPKPHDHIPLPRPQ-----------SAAPETDVPVD
BOVB2 291  LDGKLKHEDTNLASSTIVKEGANKEVLGILVSYRVKVKLVVSRG--------GDVSVELPFVLMHPKPHDHIALPRPQSAATHPPTLLPSAVPETDAPVD
HUMSA 299  LDGKIKHEDTNLASSTIIKEGIDRTVLGILVSYQIKVKLTVSGFLG--ELTSSEVATEVPFRLMHPQPEDP----------------------AKESYQD
BOVSA 295  LDGKIKHEDTNLASSTIIKEGIDKTVMGILVSYQIKVKLTVSGLLG--ELTSSEVATEVPFRLMHPQPEDPDT--------------------AKESFQD
HUMCA 286  LDGKLKHEDTNLASSTIIRPGMDKELLGILVSYKVRVNLMVSCGGILGDLTASDVGVELPLVLIHPKPSH------------------------EAASSE
BOVCA 289  LDRKLKQGDTNLASSTILRPGVDKELLGILVSYKVRVNLMVSCEGILGDLTASDVGVELPLILMHPKPSN------------------------EAASSE

HUMB1 375  TNLIELDTN---DDDIVFEDFARQRLKGMEDDKEEEEDGTGSPRLNDR
BOVB1 375  TNLIELDTN---DDDIVFEDFARQRLKGMKDDKEEEEDGTGSPRLNDR
HUMB2 372  TNLIEFDTNYATDDDIVFEDFARLRLKGMKDDDYDDQLC
BOVB2 383  TNLIEFETNYATDDDIVFEDFARLRLKGLKDEDYDDQFC
HUMSA 377  AN-------------LVFEEFARHNLKDAGEAEEGKRDKNDADE
BOVSA 375  EN-------------FVFEEFARQNLKDAGEYKEEKTDQEAAMDE
HUMCA 362  D-------I------VIEE-FTRKGEE-ESQKAVEAEGDEGS
BOVCA 365  D-------I------VIEE-FAQQEPSGESQEALAAEGNEGS
```

Fig. 1. Multiple alignment of proteins belonging to the arrestin family. An alignment was made using CLUSTAL W computer program. The proteins aligned are (in parentheses known in protein name and Swiss-protein accession No.): HUMB1 (human β-arrestin 1, P49407), BOVB1 (bovine β-arrestin 1, P17870), HUMB2 (human β-arrestin 2, P32121), BOVB2 (bovine β-arrestin 2, P32120), HUMSA (human rArr, P10523), BOVSA (bovine rArr, P08168), HUMCA (human cArr, P36575) and BOVCA (bovine cArr). The selected amino acid peptide sequence for the antigen preparation is underlined.

150 mM NaCl, pH 7.5. Aliquots (2 ml each) were collected and analyzed by immunoblotting as above. Fractions containing cArr were diluted 6 times with 20 mM bis–tris propane (BTP) buffer, pH 8.4, and applied onto a mono Q column (Pharmacia Biotech; HR 5/5) equipped with FPLC, which had been equilibrated with 20 mM BTP buffer, pH 8.4. After washing the column extensively with the same buffer, proteins were eluted using a linear gradient of NaCl from 50 to 300 mM in the same buffer at a flow rate of 0.5 ml/min. Absorbance at 280 nm was monitored (Fig. 3A).

2.4. Light microscopy and immunohistochemistry

Fresh bovine eyes fixed at 4°C for 3 h in 4% paraformaldehyde in 82 mM phosphate buffer, pH 7.4, and the parafoveal region (4×4 mm) were excised and embedded in paraffin. Cross-sections of retina (6 μm thickness) were mounted on slides and subjected to hematoxylin and eosin staining (Fig. 2A) or immunostaining (Fig. 2B) after de-paraffinization by graded alcohol.

For immunostaining, sections were incubated for 1 h at room temperature with affinity-purified rabbit anti-bovine cArr peptide IgG (50 μg/ml). After washing with phosphate buffered saline (PBS), sections were incubated with Cy3-labeled goat anti-rabbit IgG at 1:400 dilutions in PBS with 0.3% Tween 20 at room temperature for 1 h. The sections were then rinsed three times with PBS for 5 min and coverslipped in 90% glycerol in PBS containing 2% 1,4-diazabicyclo (2,2,2) octane. The sections were photographed using a Cy3 filter set.

2.5. Heparin binding using a heparin-Sepharose column

The fractions (5 ml, each) containing cArr or rArr from DEAE column, respectively, were dialyzed against 10 mM HEPES buffer, pH 7.5, containing 150 mM NaCl. Then, each dialyzate was applied at a flow rate of 15 ml/h to a column of heparin-Sepharose column (1×3 cm) which had been equilibrated with 10 mM HEPES buffer, pH 7.5, containing 150 mM NaCl and the flow through fractions (5 ml each) were collected. The column was washed with 10 mM HEPES buffer, pH 7.5, containing 150 mM NaCl at a flow rate of 15 ml/h until the absorbance at 280 nm dropped below 0.1. The protein bound to the column was eluted by 10 ml of 6 mM InP_6 and 100 mM NaCl in 10 mM HEPES buffer, pH 7.5. The aliquots (15 μl) of the bound or unbound fractions were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and immunoblotting.

2.6. Binding assay using phosphorylated and unphosphorylated urea-washed Rho

Preparation of phosphorylated and unphosphorylated urea-washed Rho and their binding arrestin were performed as described previously [8] with some modifications. Phosphorylated (1∼2 phosphates/molecules of Rho) or unphosphorylated urea-wash Rho (5 μM) were incubated with purified rArr (2 μM) or purified cArr (approximately 200 nM) in 160 μl of 10 mM HEPES buffer, pH 7.5, containing 100 mM NaCl, under dark or illuminated conditions (100-W lamp from a distance of 10 cm) for 5 min at 30°C. After the incubation, the sample was centrifuged at 15 000 rpm for 15 min. The pellet was washed with 300 μl of the buffer and analyzed by SDS–PAGE and immunoblotting.

2.7. Other analytical methods

SDS–PAGE was performed by the method of Laemmli [9] using a 12.5% SDS–PAGE slab gel and a Hoeffer minigel apparatus. Immunoblot analysis was performed as described by Ohguro et al. [10].

3. Results

To understand quenching phototransduction in vertebrate cone photoreceptor cells, we studied the structure and function of bovine cone-specific arrestin (cArr), since arrestin is known to be a key molecule in the quenching phototransduction of rod photoreceptor cells [1]. As an initial step, we isolated cDNA clone of cArr using the human cDNA probe of cArr. About 0.3% of the clones in the cDNA library cross-hybridized to the human cDNA probe, which contained the entire coding region. Twenty hybridization positive clones were isolated and analyzed. A clone containing the largest cDNA insert of 1340 bp was chosen for sequence analysis. The nucleic acid sequence demonstrated 85% identity to human cArr cDNA. The bovine cArr cDNA contained an open reading frame of 1176 bp, which was 12 bp longer than the human cArr. The deduced amino acid sequence showed 86, 64 and 51% homology to human cArr, bovine β arrestin and bovine rArr, respectively [3] (Fig. 1). The restriction map and sequence analyses of the other hybridization-positive clones showed that they contained the full-length or part of the same open reading frame encoding the bovine cArr homologue.

The multiple alignment study of the arrestin family proteins with Clastal W shows that cArr of human and bovine has the serine rich regions near the C-terminus which are distinct from other vertebrate arrestins (Fig. 1). A synthetic peptide corresponding with this unique region (residues 354–368) was prepared to obtain antiserum by immunizing rabbit. Immunohistochemical study revealed that affinity-purified cArr IgG specifically recognized cone photoreceptors (Fig. 2B). Additionally, cArr IgG recognized only a 46 kDa band of retinal homogenate by immunoblot analysis (Fig. 3B).

To purify cArr, soluble fractions from frozen bovine retinas were loaded onto a column of DEAE-cellulose and proteins were eluted from the column by a NaCl gradient (0–150 mM). The aliquots of the eluted fractions were analyzed by immunoblotting and cArr containing fractions were pooled. The sample was then loaded onto a Hiprep Sephacryl High Res-

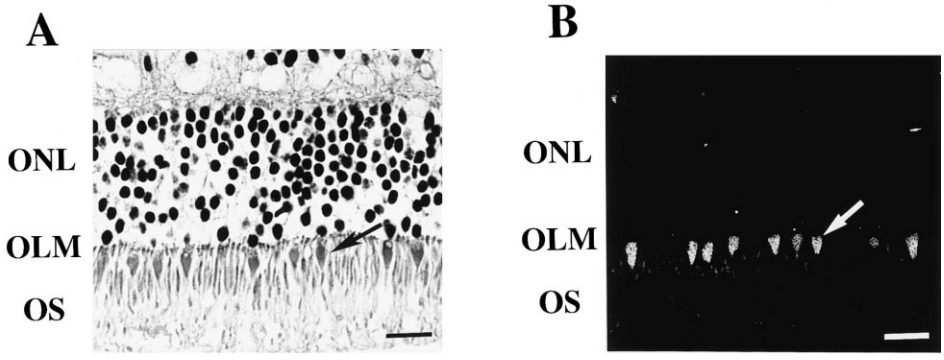

Fig. 2. Histological examination and immunocytochemical demonstration in bovine retina inducible cArr. The sections embedded in paraffin of parafoveal bovine retina were stained with hematoxylin–eosin (A) or immunostained with affinity-purified bovine cArr anti-peptide antibody (B). Arrows (A; black, B; white) point to cone outer segments. ONL, outer nuclear layer; OLM, outer limiting membrane; OS, outer segment. Magnification ×127.5. Scale bar (A, B), 50 μm.

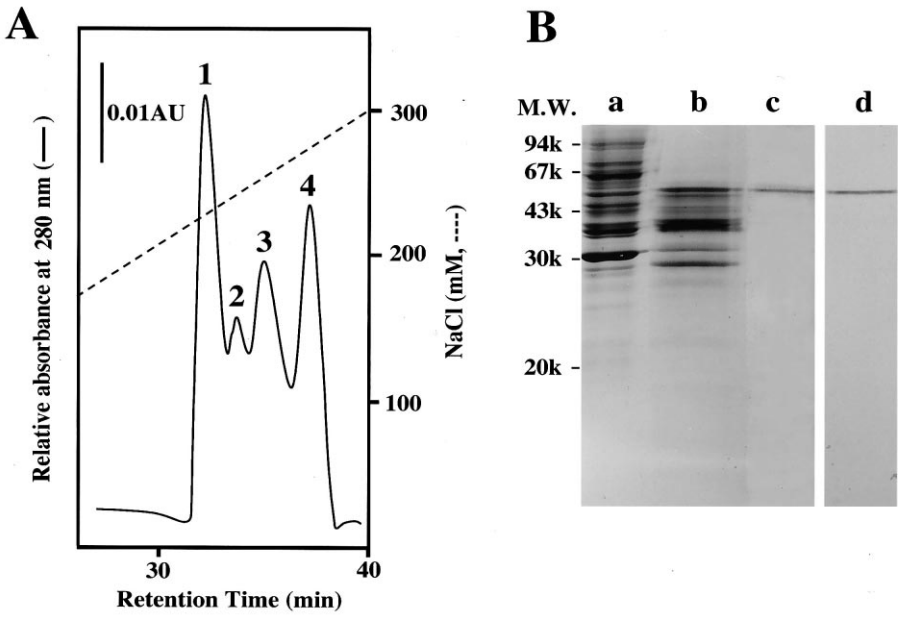

Fig. 3. Purification of bovine cArr. Bovine cArr was purified from frozen bovine retinas by sequential column chromatography using DEAE-cellulose, Hiprep Sephacryl High Resolution gel filtration and mono Q columns. A: An elution profile by a mono Q column employing a liner gradient of NaCl (50–300 mM). Peak 4 contained homogeneous cArr. B: SDS–PAGE patterns of 15 μl aliquot from each step of the purification. a: DEAE-cellulose column; b: Hiprep Sephacryl High Resolution gel filtration column; c: fraction 4 from mono Q column and d: immunoblot of sample c using cArr IgG (200 dil.).

olution gel filtration column, followed by a mono Q column, both equipped with FPLC. As shown in Fig. 3A, peak 4 obtained by elution with linear gradient of a NaCl (50–300 mM) showed a 46 kDa band of cArr which was distinct from 48 kDa rArr on SDS–PAGE gels and immunoblots (data is shown in Fig. 5).

To study the functional property of cArr, its binding ability with Rho or P-Rho was examined. rArr specifically bound with P-Rho in a light dependent manner, whereas, cArr did not bind with either Rho or P-Rho regardless of the light conditions (Fig. 4). rArr is known to specifically bind to heparin, which mimics the phosphorylated form of Rho. To test if cArr bound to heparin or not, partially purified rArr and cArr obtained by a DEAE-cellulose column were subjected to heparin-Sepharose and eluted with IP_6. As shown in Fig. 5, rArr tightly bound to the column but no binding of cArr was observed.

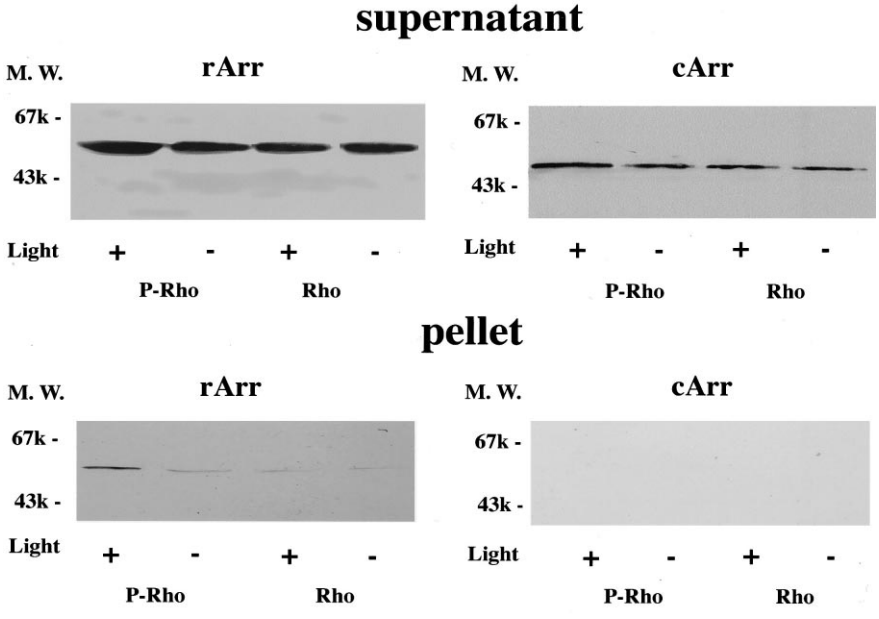

Fig. 4. Binding assay of cArr or rArr to various states of phosphorylated Rho or urea-washed Rho. The reaction mixture composed of Rho (5 μM) or P-Rho (5 μM), rArr (2 μM) or cArr (approximately 200 nM) in 160 μl buffer was incubated at 30°C under dark or illuminated conditions. After the incubation, samples were centrifuged and the supernatant and the pellet were each subjected to immunoblot analysis using rArr or cArr IgG as described in Section 2.

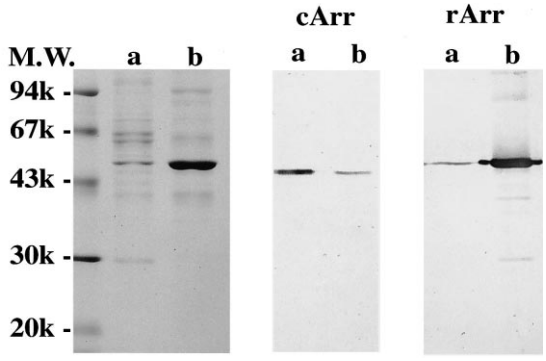

Fig. 5. Heparin binding using heparin-Sepharose chromatography. The fractions (5 ml each) containing rArr or cArr obtained from DEAE-cellulose column were loaded onto a heparin-Sepharose column and the flow through fractions (5 ml each) were collected. After extensive washing of the column, rArr was eluted by 6 mM IP_6. Aliquots from the unbound (a) and bound (b) were analyzed by immunoblotting as described in Section 2.

4. Discussion

Based on the following evidence, it was suggested that phototransduction and its quenching mechanisms in cone photoreceptors closely resemble those of rod photoreceptors in vertebrates; (1) most of the cone specific components participating in these cascades have been cloned, including cone visual pigments [11], Gt [12], cGMP phosphodiesterase [13] and cArr [3,4] and (2) these components are homologous with those of rod photoreceptors. In addition, some of these components have been demonstrated to possess functional homology, i.e. red sensitive cone pigment (iodopsin) purified from chicken retinas was able to activate rod Gt [14] and to be phosphorylated by RK in a light-dependent manner [15]. In contrast, electrophysiological studies revealed that photoexcitation and its quenching of cone photoreceptors are much faster than those of rod photoreceptors [16]. Therefore, the possibility that some unknown mechanisms are involved in these cascades in cone photoreceptors is suggested. In the present study, to understand more about the functional difference among these photoreceptors, we cloned and purified cArr, which seems to be a key molecule in the quenching phototransduction, from bovine retinas using a specific antibody against the C-terminus of cArr, which is not cross-reacted with rArr. cArr showed no binding to Rho in any conditions, bleached and/or phosphorylated, suggesting that cArr may exclusively require cone visual pigments for the receptor binding. It has been demonstrated that rArr specifically bound with P-Rho*. Palczewski et al. reported that rArr binding to P-Rho* mimics binding to heparin since (1) heparin inhibited rArr binding with P-Rho* and (2) limited proteolysis of rArr by trypsin was identical to that when rArr was incubated with P-Rho* or heparin [17]. Therefore, he suggested that negative charges by P-Rho* or heparin induced specific structural change possibly in the C-terminus of rArr resulting in forms suitable for binding with the photolyzed receptor. This scheme was also proposed by Benovic and his associates by binding assay using several kinds of mutants of rArr [6,18]. In addition, p44, an alternative spliced form of rArr deficient in the C-terminal portion of rArr, shows high affinity toward Rho* [2]. In the present study, we found a much lower affinity of cArr with heparin-Sepharose column as compared with that of rArr. These observations lead us to speculate that cArr may bind with the photolyzed form of the cone visual pigments and may not be necessary for their phosphorylation. Assuming this hypothesis, the skipping of the phosphorylation step may facilitate prompt termination of the photoexcitation cascade. In fact, the deduced amino acid sequence revealed that the C-terminus of cArr is distinct from that of rArr, which is believed to be important for P-Rho* or heparin binding to rArr. However, this mechanism is still not clearly understood. Therefore, an additional binding study using cone visual pigments and mutagenesis of cArr is our next project.

Acknowledgements: This work was supported by grants from the Japanese Ministry of Health, Naito Memorial Foundation, Ciba-Geigy Foundation for the Promotion of Science, The Mochida Memorial Foundation for Medical and Pharmaceutical Research, Uehara Memorial Foundation and JRPS Research Foundation.

References

[1] Palczewski, K. and Saari, J.C. (1997) Curr. Opin. Neurobiol. 7, 500–504.
[2] Pulvermüller, A., Maretzki, D., Rudnicka-Nawrot, M., Smith, W.C., Palczewski, K. and Hofmann, K.P. (1997) Biochemistry 36, 9253–9260.
[3] Murakami, A., Yajima, T., Sakuma, H., McLaren, M.J. and Inana, G. (1993) FEBS Lett. 334, 203–209.
[4] Sakuma, H., Inana, G., Murakami, A., Higashide, T. and McLaren, M.J. (1996) FEBS Lett. 382, 105–110.
[5] Palczewski, K., Buczyłko, J., Imami, N.R., McDowell, J.H. and Hargrave, P.A. (1991) J. Biol. Chem. 266, 15334–15339.
[6] Gurevich, V.V. (1998) J. Biol. Chem. 273, 15501–15506.
[7] Buczyłko, J. and Palczewski, K. (1993) in: Methods Neurosci. (Hargrave, P.A., Ed.), Vol. 15, pp. 223–236. Academic Press, Orlando.
[8] Ohguro, H., Johnson, R.S., Ericsson, L.H., Walsh, K.A. and Palczewski, K. (1994) Biochemistry 33, 1023–1028.
[9] Laemmlli, U.K. (1970) Nature 227, 680–685.
[10] Ohguro, H., Ogawa, K. and Nakagawa, T. (1999) Invest. Ophthalmol. Vis. Sci. 40, 82–89.
[11] Nathans, J., Thomas, D. and Hogness, D.S. (1986) Science 232, 193–202.
[12] Lerea, C.L., Somers, D.E., Hurley, J.B., Klock, I.B. and Bunt-Milam, A.H. (1986) Science 234, 77–80.
[13] Hurwitz, R.L., Bunt-Milam, A.H., Chang, M.L. and Beavo, J.A. (1985) J. Biol. Chem. 260, 568–573.
[14] Okada, T., Matsuda, T., Kandori, H., Fukada, Y., Yoshizawa, T. and Shichida, Y. (1994) Biochemistry 33, 4940–4946.
[15] Fukada, Y., Kokame, K., Okano, T., Shichida, Y., Yoshizawa, T., McDowell, J.H., Hargrave, P.A. and Palczewski, K. (1990) Biochemistry 29, 10102–10106.
[16] Yau, K.W. (1994) Invest. Ophthalmol. Vis. Sci. 35, 9–32.
[17] Palczewski, K., Pulvermüller, A., Buczyłko, J. and Hofmann, K.P. (1991) J. Biol. Chem. 266, 18649–18654.
[18] Gray-Keller, M.P., Detwiler, P.B., Benovic, J.L. and Gurevich, V.V. (1997) Biochemistry 36, 7058–7063.