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Crystallization and preliminary X-ray analysis of arrestin from bovine rod outer segment

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Abstract We present the first X-ray study of a member of the arrestin family, the bovine retinal arrestin. Arrestin is essential for the fine regulation and termination of the light-induced enzyme cascade in vertebrate rod outer segments. It plays an important role in quenching phototransduction by its ability to preferentially bind to phosphorylated light-activated rhodopsin. The crystals diffract between 3 Å and 3.5 Å (space group $P2_12_12$, cell dimensions $a = 169.17$ Å, $b = 185.53$ Å, $c = 90.93$ Å, T = 100 K). The asymmetric unit contains four molecules with a solvent content of 68.5% by volume.

Key words: Arrestin; Bovine rod outer segment; Crystallization; X-ray diffraction

1. Introduction

In vertebrate photoreceptors, a light signal initiates an enzyme cascade leading to a decrease in concentration of the internal transmitter cGMP and subsequently to the closure of cation channels in the plasma membrane, which are kept open by cGMP in the dark.

Besides the visual pigment rhodopsin (R), four cytoplasmic proteins play a key role in the activation and inactivation of the light-induced enzyme cascade. These proteins are the guanine nucleotide binding protein (G-protein) called transducin ($T_{\alpha\beta\gamma}$), the rhodopsin kinase, the regulatory protein arrestin (also termed S-antigen or 48 kDa protein), and the phosphodiesterase (PDE).

In its light-activated conformation (R*) the receptor protein rhodopsin interacts with transducin ($T_{\alpha\beta\gamma}$) inducing thereby the exchange of GDP bound to T_α for a GTP molecule [1]. The T_α-GTP subunit dissociates from $T_{\beta\gamma}$ and from R* [2] and activates the PDE [3,4] by removing the inhibitory subunit PDE_γ [5–7]. The resulting decrease in cGMP concentration causes the closure of cation channels in the plasma membrane [8–10].

Since binding and dissociation of transducin takes only about 1 ms [11], a single R* can readily interact with hundreds of transducin molecules in less than a second, thereby leading to the activation of hundreds of PDE molecules. PDE is an enzyme with a very high turnover number (up to 2000 cGMP/PDE s^{-1}). Therefore, a single photon of light, if leading to hundreds of activated PDE molecules, would be able to hydrolyze the entire cGMP pool of a photoreceptor cell within a second. To limit the amplitude of light response, only a few PDE molecules should be allowed to become activated in response to dim light, i.e. R* has to be rapidly blocked well before it can interact with too many transducin molecules. Inactivation of R* can be performed in two steps: phosphorylation of R* at multiple sites by the rhodopsin kinase reduces its ability to activate transducin [12–15] and subsequent binding of arrestin completely blocks it from further interaction with transducin [16]. Thus, arrestin is essentially involved in terminating the light response.

Soon after the clarification of the function of arrestin in vision, a protein was discovered and called β-arrestin, which shields the agonist occupied, phosphorylated β-adrenergic receptor from binding to G-proteins in a similar way [17]. Meanwhile, arrestin-like proteins have been discovered in several other species and tissues: in various bovine tissues [18], in turkey erythrocytes [19], in the photoreceptor of *Drosophila* [20,21], in human thyroid [22], in yeast cells [23], and many others. Arrestin-like proteins are expected to be common to G-protein coupled receptors and essentially required for the receptor down regulation and adaptation.

Arrestin is one of the most abundant soluble proteins of the vertebrate photoreceptor cell [24]. The cloning of a bovine retinal cDNA revealed that arrestin has 404 amino acids (45.3 kDa).

Here we report the first crystallization and preliminary X-ray analysis of a member of the arrestin family, the bovine retinal arrestin. Elucidation of this protein structure by X-ray crystallography will give insight into the three-dimensional architecture and provide a basis to clarify the interaction between phosphorylated, activated rhodopsin and arrestin.

2. Materials and methods

Rod outer segments (ROS) were purified from fresh bovine eyes as described by Wilden and Kühn [12]. They were kept in the dark and stored in aliquots at −80°C.

Retina extract was prepared as follows: 70 retinae that had been frozen in liquid N_2 were thawed in 70 ml of sodium phosphate buffer (pH 7.2). They were shaken vigorously by hand for 90 s and then gently stirred for 30 min on ice. After centrifugation for 30 min at 20 000 rpm in a Beckman JA-20 rotor, the supernatant was collected and stored at −80°C in aliquots of 20 ml until use.

Arrestin was purified using its light-dependent binding to phosphorylated rhodopsin as described earlier [25] with some modifications. Briefly, a ROS pellet containing ca. 30 mg of rhodopsin was homogenized in 30 ml of 70 mM sodium phosphate (pH 7.2), 2 mM $MgCl_2$/0.1 mM EDTA/3 mM ATP/1 mM GTP and illuminated at 30°C with white light. After 20 min 40 ml retina extract was added, and the mixture was illuminated for another 10 min at 30°C. The suspension was centrifuged for 30 min at 20 000 rpm. The supernatant was discarded and the pellet washed three times by resuspending it each time with 60 ml of 10 mM HEPES (pH 7.2)/0.1 mM EDTA and centrifuging for 40 min at 20 000 rpm. In the first wash, 0.1 mM GTP

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was added. The pellet was then washed gently with 8 ml of 10 mM HEPES (pH 7.2)/400 mM KCl in order to remove unspecific bound proteins. Finally the pellet was suspended in 10 ml of 10 mM HEPES (pH 7.2)/800 mM KCl and incubated at 20°C in the dark for ca. 16 h. The suspension was then centrifuged at 20000 rpm for 30 min. The supernatant containing the purified arrestin was centrifuged again to remove residual membrane particles and stored at −80°C in aliquots.

For crystallization purposes, arrestin was concentrated to approx. 36 mg/ml using a Centricon-30 concentrator. 10 μl of concentrated arrestin was mixed with 7–10 μl of 18 mM PIPES pH 7.2/0.5 M KCl/30% ethylene glycol/8% polyethylene glycol 6000/18% polyethylene glycol 200/6% polyethylene glycol 1000. Crystals were grown in hanging drops by vapor diffusion against 40 mM PIPES pH 7.2/0.6 M KCl/22% ethylene glycol/5.7% polyethylene glycol 6000/13% polyethylene glycol 200/4% polyethylene glycol 1000 at room temperature.

Protein from solubilized crystals as well as protein used for crystallization was verified as full-length bovine arrestin by SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) [26] and Western blot (data not shown).

Crystals with dimensions of approximately $0.3\times0.2\times0.15$ mm grew within 2–3 weeks. Their shape was pinacoid with commonly prismatic surfaces of {100}, {010} and {001} (Fig. 1).

Native and first derivative datasets were collected using synchrotron radiation sources at the Science and Energy Research Council (SERC, UK) Daresbury Laboratory, European Molecular Biology Laboratory (EMBL) outstation at the Deutsches Electronen Synchrotron (DESY, Germany) Hamburg and at LURE (Orsay, France).

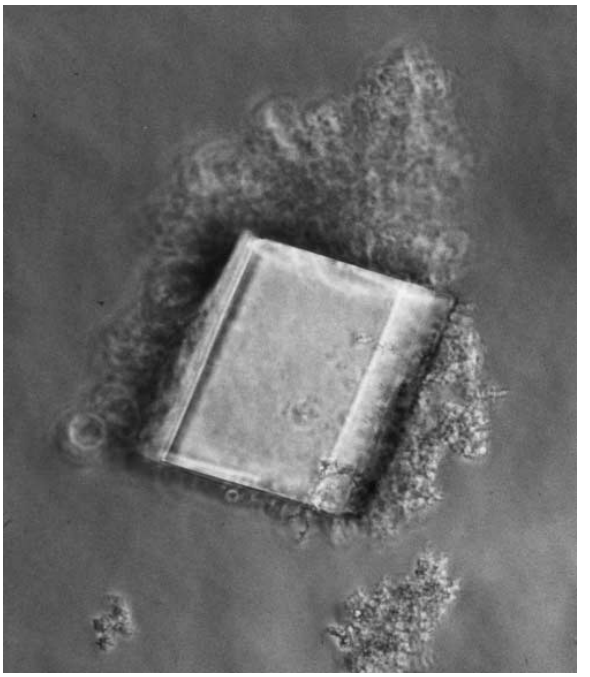

Fig. 1. Crystal of arrestin. The crystal size corresponds to approximately $0.18\times0.17\times0.15$ mm.

3. Results and discussion

As outlined in Section 1, arrestin is an important modulator for G-protein signal transduction in different cellular processes. So far no crystal structure of a member of the arrestin family has been published or its crystallization reported. The reason for this may lie in the unusual behavior of arrestin we observed during our crystallization procedures. A rapid initial screening of crystallization conditions was conducted using a sparse matrix without any success. After a long search only a mixture of different molecular weight species of polyethylene glycols, as described in Section 2, was successful.

Arrestin crystallizes in the orthorhombic space group $P2_12_12$, two screw axes and one twofold axis were verified by native Patterson synthesis and its possible Harker sections. The unit cell dimensions are $a=169.17$ Å, $b=185.53$ Å, $c=90.95$ Å (T = 100 K). A V_m value of 3.94 Å^3 Da^{-1} is consistent with the presence of four molecules in the asymmetric unit. This value is in the upper range of values tabulated by Matthews [27]. The solvent content is estimated to be 68.5%.

Due to the strong X-ray radiation damage of the crystals all datasets were collected under cryo-conditions. The crystals were soaked prior to flash-freezing to 100 K (nitrogen gas stream) in reservoir solution containing 0.1% glutaraldehyde and subsequently in 2.5 mM imidazole. All X-ray diffraction data were collected on MAR Research image-plate area detector. The measurements were evaluated, scaled and merged by the programs *DENZO* [28], *SCALA* and *AGROVATA* [29]. The actual resolution was 3.0 Å with a data completeness of 90.3% and a $R_{sym}=0.077$.

An examination of the self-rotation function (program *POLARRFN*) [29] reveals one major (85% of origin height) and one minor peak (27% height) in the $\kappa=180°$ section, using data from 20.0 to 6.0 Å and 20 Å radius of integration (rotation function not shown). The major twofold non-crystallographic symmetry (ncs) operation is nearly parallel to the *c*-axis, the minor ncs operation lies perpendicular to the first operation axis. The two different sets of twofold ncs symmetries reflect that the molecules are only identical by pairs. The differences between the pairs may result from crystal packing effects.

A further search for suitable heavy-atom derivatives to solve the structure by multiple isomorphous replacement methods is still in progress, but the first SIRAS (single isomorphous replacement, anomalous scattering) electron-density map is predominantly interpretable. A first model building at 3.5 Å is under way and not yet finished, but we can clearly see that the arrestin structure is dominated by two domains of antiparallel β-sheets connected via a hinge region and one short α-helix on the backside of the N-terminal domain. The overall amounts in the elements of the secondary structure are in accordance with spectroscopic (CD and FTIR) results of Shinohara et al. [30] and Garcia-Quintana et al. [31]. There are no extended coil regions. Therefore we cannot agree with the interpretation of Gurevich and Benovic [32] that the binding domain of arrestin to the activated and phosphorylated rhodopsin has no distinct features in the secondary structure. The postulated region must be located in one of the β-strands.

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References

[1] Fung, B.K.K. and Stryer, L. (1980) Proc. Natl. Acad. Sci. USA 77, 2500–2504.
[2] Kühn, H. (1980) Nature 283, 587–589.
[3] Fung, B.K.K., Hurley, J.B. and Stryer, L. (1981) Proc. Natl. Acad. Sci. USA 78, 152–156.
[4] Uchida, S., Wheeler, G.L., Yamazaki, A. and Bitensky, M.W. (1981) J. Cycl. Nucleotide Res. 7, 95–104.

[5] Hurley, J.B. and Stryer, L. (1982) J. Biol. Chem. 257, 11094–11099.
[6] Deterre, P., Bigay, J., Forquet, F., Robert, J. and Chabre, M. (1988) Proc. Natl. Acad. Sci. USA 85, 2424–2428.
[7] Catty, P., Pfister, C., Bruckert, E. and Deterre, P. (1992) J. Biol. Chem. 267, 19489–19493.
[8] Fesenko, E.E., Kolesnikov, S.S. and Lyubarsky, A.L. (1985) Nature 313, 310–313.
[9] Zimmermann, A.L., Yamanaka, G., Eckstein, F., Baylor, D.A. and Stryer, L. (1985) Proc. Natl. Acad. Sci. USA 82, 8813–8817.
[10] Haynes, L.W., Kay, A.R. and Yau, K.W. (1986) Nature 321, 66–70.
[11] Young, T.M., Chabre, M. and Stryer, L. (1984) Nature 311, 659–661.
[12] Wilden, U. and Kühn, H. (1982) Biochemistry 21, 3014–3022.
[13] Miller, J.L. and Dratz, E.A. (1984) Vision Res. 24, 1509–1521.
[14] Wilden, U., Hall, S.W. and Kühn, H. (1986) Proc. Natl. Acad. Sci. USA 83, 1174–1178.
[15] Wilden, U. (1995) Biochemistry 34, 1446–1454.
[16] Schleicher, A., Kühn, H. and Hofmann, K.P. (1989) Biochemistry 28, 1770–1775.
[17] Lohse, M.J., Benovic, J.L., Codina, J., Caron, M.G. and Lefkowitz, R.J. (1990) Science 248, 1547–1550.
[18] Scheuring, U., Franco, M., Fievet, B., Guizouarn, H., Mirashahi, M., Faure, J.P. and Motais, R. (1990) FEBS Lett. 276, 192–196.
[19] Mirshahi, M., Borgese, F., Razaghi, A., Scheuring, U., Garcia-Romeu, F., Faure, J.P. and Motais, R. (1989) FEBS Lett. 258, 240–243.
[20] Smith, D.P., Shieh, B.H. and Zuker, C.S. (1990) Proc. Natl. Acad. Sci. USA 87, 1003–1007.
[21] Matsumoto, M. and Yamada, T. (1991) Biochem. Biophys. Res. Commun. 177, 1306–1312.
[22] Rapoport, B., Kaufmann, K.D. and Chazenbalk, G.D. (1992) Mol. Cell. Endocrinol. 84, R39–R43.
[23] Jeansonnet, N.E., Jazwiski, S.M. and Donoso, L.A. (1991) J. Biol. Chem. 266, 1–6.
[24] Broekhuyse, R.M., Janssen, A.P.M. and Tolhuizen, E.F.J. (1987) Curr. Eye Res. 6, 607–610.
[25] Wilden, U., Wüst, E., Weyand, I. and Kühn, H. (1986) FEBS Lett. 207, 292–295.
[26] Laemmli, U.K. (1970) Nature 227, 680–685.
[27] Matthews, B.W. (1968) J. Mol. Biol. 33, 491–497.
[28] Otwinowski, Z. (1993) in: Proceedings of the CCP4 Study Weekend: Data Collection and Processing, 29–30 January 1993, compiled by L. Sawyer, N. Isaacs and S. Bailey, pp. 56–62. Daresbury Laboratory, Warrington.
[29] The CCP4 Suite (1994) Acta. Crystallogr. D50, 760–763.
[30] Shinohara, T., Dietzhold, B., Craft, C.M., Wistow, G., Early, J.J., Donoso, L.A., Horwitz, J. and Tao, R. (1987) Proc. Natl. Acad. Sci. USA 84, 6975–6979.
[31] Garcia-Quintana, D., Garriga, P. and Manyosa, J. (1992) Biochim. Biophys. Acta 1122, 269–272.
[32] Gurevich, V.V. and Benovic, J.L. (1997) Mol. Pharmacol. 51, 161–169.