Molecular Clues to Bothnia-Type Retinal Dystrophy

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Chapter 75
Molecular Clues to Bothnia-Type Retinal Dystrophy

Xiaoqin He, Joel Lobsiger, and Achim Stocker

Keywords Cellular retinaldehyde-binding protein • 11-*cis*-retinal • Bothnia-type retinal dystrophy • X-ray crystallography structure • R234W

75.1 Introduction

In retinal pigment epithelium (RPE), cellular retinaldehyde-binding protein (CRALBP) is the major acceptor of 11-*cis*-retinol in the isomerization step of the rod visual cycle. It serves as a substrate carrier for 11-*cis*-retinol dehydrogenase (RDH 5), facilitating the oxidation of 11-*cis*-retinol to 11-*cis*-retinal. CRALBP also protects bound ligands from photoisomerization (Saari et al. 2001). The arginine-to-tryptophan missense mutation in position 234 (R234W) in the *RLBP1* gene is associated with the autosomal recessive disease Bothnia-type retinal dystrophy, and impairs regeneration of visual pigment (Burstedt et al. 1999). The rod–cone dystrophy occurs predominantly in northern Sweden with patients losing peripheral and central vision gradually. In this study, we report the X-ray crystallographic structures of CRALBP and its disease causing mutant R234W and provide a molecular explanation of the disease mechanism.

X. He • A. Stocker (✉)
Department of Chemistry and Biochemistry, University of Bern,
Freiestrasse 3, Bern, Switzerland
e-mail: achim.stocker@ibc.unibe.ch

J. Lobsiger
Institute for Molecular Biology and Biophysics, ETH Zürich, Zürich, Switzerland

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75.2 Materials and Methods

75.2.1 Protein Expression, Purification, and Crystallization

The proteins CRALBP WT and R234W were cloned, expressed, purified, and crystallized as described previously (He et al. 2009). Hexagonal crystals of CRALBP WT in complex with 11-*cis*-retinal were formed in 1.0M K/Na tartrate, 0.1M Tris-HCl (pH 7.0), 0.2M Li_2SO_4 after 3 days. The space group was $P6_522$ with $a = 71.92$ Å, $b = 71.92$ Å, $c = 303.20$ Å, $\alpha = 90°$, $\beta = 90°$, $\gamma = 120°$. Monoclinic crystals of R234W in complex with 11-*cis*-retinal were formed in 20% (wt./vol.) PEG 3000, 0.1M Hepes (pH 7.5), 0.2M NaCl. The crystals have a C2 space group with $a = 87.93$ Å, $b = 57.88$ Å, $c = 75.15$ Å, $\alpha = 90°$, $\beta = 122.846°$, $\gamma = 90°$. The structure of R234W was solved by SAD using selenomethionine-labeled protein; the structure of CRALBP WT was subsequently solved by molecular replacement using the atomic model of R234W and the program Phaser (Qian et al. 2007).

75.2.2 Illumination of 11-cis-Retinal

Illumination of 11-*cis*-retinal and its complexes with CRALBP WT and R234W was performed according to the methods of Saari and Bredberg (1987). The time-dependent photoisomerization of 11-*cis*-retinal in ethanol, CRALBP WT, or R234W was plotted respectively, by recording the remaining amount of 11-*cis*-retinal after being exposed to light every 5 s. The first-order rate constants (k) from the slopes of the plots of log a/a_0 vs. time were calculated. The following values were used to calculate quantum yield ΦR234W: ΦCRALBP = 0.07; εCRALBP = εR234W = 15,400 (M^{-1} cm^{-1}).

75.3 Results

75.3.1 The Structure Determination of CRALBP WT and R234W

The crystallographic structure model of CRALBP WT comprises residues 23–306 of 317 residues; the residues 1–22 are missing due to the different crystal contacts. The N-terminal domain comprises five α helices. The C-terminal αβα sandwich comprises a 5-β-strand sheet forming hydrophobic floor for retinal binding, two α helices packed on one side, and four α helices on the other side forming the ligand pocket with the β-sheet. The electron density map of the C11–C15 terminal tail is poorly defined, suggesting that this part of the ligand is slightly disordered in the less tightly packed ligand-binding pocket (Fig. 75.1).

Fig. 75.1 Closer look at the environment of the ligand 11-*cis*-retinal both CRALBP WT (*gray*) and R234W (*pink*). Retinal is buried deeper into the ligand-binding cavity by a dramatic structural change in the loop region. In CRALBP WT, R234 is part of a cluster of three pairs of positively charged residues (R103–K104, K236–R234, and K261–265) at the protein surface. The mutation to tryptophan in R234W changed the potential distribution at the surface patch, which might mediate interactions with specific acidic lipids

The structure model of the R234W mutant comprises residues 57–306 of 317 and the 11-*cis*-retinal ligand tail is well visible. The one-amino-acid mutation caused structural changes of 1 Å root mean square deviation over all Cα atoms. A rather big conformational transition occurred in the loop where the apolar indole ring of W234 is buried between the N-terminal α helices and the C-terminal αβα sandwich. Extensive van der Waals interactions are formed between tryptophan and nearby residues, and K104 and K261 are pushed over 5.4 and 3.4 Å respectively. Residues 227–238 are moved by an average 2.3 Å deviation comparing to wild type. Side chains of F198, F235, and I238 in the ligand-binding pocket are flipped in a domino-like manner; consequently, the pocket size is reduced (He et al. 2009) (Fig. 75.2).

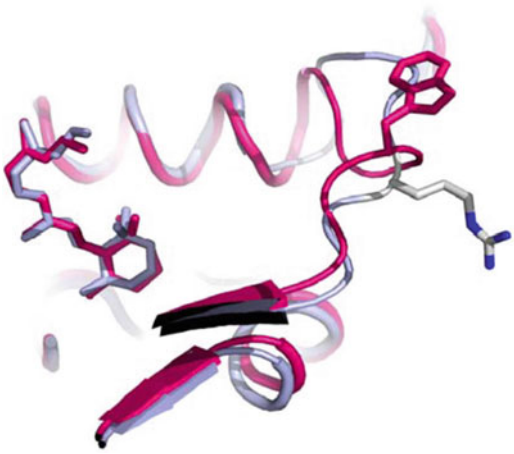

Fig. 75.2 Comparison of the ligand-binding cavity of CRALBP WT (*gray*) and R234W (*pink*). The pocket volumes were calculated using the program VOIDOO and a rolling probe with a radius of 1.0 Å. The R234W volume is 5.989×10^2 Å^3, CRALBP WT volume is 6.454×10^2 Å^3. Pictures are drawn using PyMol (Delano 2002)

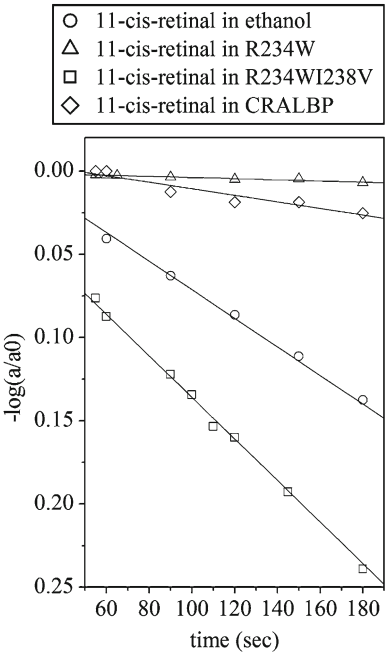

Fig. 75.3 Time courses of the photoisomerization of 11-*cis*-retinal in the presence of ethanol, wild-type CRALBP, or R234W. The amount present at $t=0$ is a_0; the amount present after illumination is a. For a first-order decay process, $a=a_0e^{-kt}$. The first-order rate constants calculated from this experiment are $k_{ethanol}=0.859\times10^{-3}$ s^{-1}; $k_{CRALBP}=0.197\times10^{-3}$ s^{-1}; $k_{R234W}=0.035\times10^{-3}$ s^{-1}

75.3.2 Photoisomerization of 11-cis-Retinal

We determined first-order rate constants for the photoisomerization of 11-*cis*-retinal in ethanol, CRALBP WT, or R234W using the method described by Saari and Bredberg (Fig. 75.3). The measurements revealed a fivefold reduction of R234W photosensitivity (191.5 M^{-1} cm^{-1}) relative to CRALBP WT. The reduction must be

Table 75.1 Crystallographic statistics

	Wild-type CRALBP	R234W Se-Met
Crystal parameters	$P6_522$	C121
	a=71.9 Å, b=71.9 Å, c= 303.2 Å	a=87.9 Å, b=57.9 Å, c=75.6 Å
	α=90°, β=90°, γ=120°	α=90°, β=122.85°, γ=90°
Molecules/asymmetric unit	1	1
Data collection		
Wavelength (Å)	0.9762	0.9793
No. crystals	1	1
Resolution (Å) (outer shell)	50.0–3.0 (3.2–3.0)	50–1.7 (1.8–1.7)
No. observations	10,3496 (14,433)	38,0336 (46,406)
No. unique reflections	9,692 (1,499)	67,824 (9,663)
Mean redundancy	10.7 (9.6)	5.6 (4.8)
Completeness (%)	99.5 (99.6)	97.4 (86.1)
R_{sym} (%)	0.059 (0.53)	0.048 (0.58)
$I/\sigma(I)$	22.8 (4.1)	19.8 (2.7)
Phasing		
Se sites		5
FOM (SAD phases)		0.49
Refinement		
Resolution range (Å)	48.1–3.0	45.6–1.7
No. reflections working set	9,690	67,814
No. reflections test set	970	6,775
R_{work}/R_{free} (%)	23.9/27.2	16.7/18.4
RMS bonds (Å)	0.004	0.005
RMS angles (°)	0.74	1.18
Residues included	23–306	57–306
Ramachandran statistics		
Generously allowed (%)	99.65	100.00
Not allowed (%)	0.35	n.a.

attributed to the local increase in packing density through the I238 Cδ methyl group, because the extinction coefficient of CRALBP and R234W decreased equally from 25,000 to 15,400 M^{-1} cm^{-1} after binding 11-*cis*-retinal. The result is consistent with crystallographic data (Table 75.1).

75.4 Discussion

The molecular basis of retinoid transportation by CRALBP was not well understood due to the lack of structural data. The crystal structure of CRALBP here provides insight into stereospecific binding and into chemical protection of 11-*cis*-retinal in the human eye. The clinical phenotype of Bothnia-type Retinal Dystrophy is caused by the R234W mutation in CRALBP, and leads to a defective retinal metabolism. The crystal structure of the pathologic R234W mutation of CRALBP reveals impaired 11-*cis*-retinal release through stabilization of the ligand complex and

disruption of a conserved basic surface patch with putative loss of lipid stimulation of retinoid transfer. The results of our study suggest that impaired 11-*cis*-retinal release may be a major cause of night blindness and retinal pathology in patients carrying the R234W missense mutation in the *RLBP1* gene. This study may help elucidate CRALBP's role in visual cycle regulation, and provide further information in search of Bothnia-type retinal dystrophy treatment.

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