Structure of RPE65 isomerase in a lipidic matrix reveals roles for phospholipids and iron in catalysis

Philip D. Kiser\(^1\), Erik R. Farquhar\(^b\), Wuxian Shi\(^b\), Xuewu Sui\(^a\), Mark R. Chance\(^b\), and Krzysztof Palczewski\(^a,1\)

\(^a\)Department of Pharmacology, School of Medicine, Case Western Reserve University, Cleveland, OH 44106; and \(^b\)Center for Proteomics and Bioinformatics, Center for Synchrotron Biosciences, School of Medicine, Case Western Reserve University, Cleveland, OH 44106

**AUTHOR SUMMARY**

Vision begins when photons are absorbed and in turn activate light receptors in the retina. Central to the activation process is a photochemical isomerization in a vitamin A-derived chromophore bound to the light receptor, rhodopsin. After this geometrical cis–trans photoisomerization, the receptor is insensitive to further stimulation by light; therefore, the photochemical change in the chromophore must be reversed so that response to light can be restored (1). Retinoid isomerase, RPE65, is a key enzyme responsible for restoring the cis form of the chromophore. The biochemistry of RPE65 retinoid isomerase is incompletely understood (2). In particular, the roles of two key factors, phospholipids and iron, in the enzymatic function of RPE65 remain to be fully clarified. Here, we show that phospholipids directly stabilize RPE65 in a conformation that allows retinyl esters to be taken up from membranes, whereas the iron cofactor, by virtue of its positive charge, polarizes the ester group of the up-taken substrate, allowing ester cleavage and the generation of a putative key intermediate in the retinoid isomerization reaction to occur (Fig. P1).

In their work on the visual cycle carried out in the 1950s, George Wald and his colleagues postulated the existence of a retinoid isomerase that regenerates the visual chromophore (3), but it took another ~50 y before RPE65 was identified as the responsible enzyme. A principle reason underlying the failure of traditional biochemical techniques to identify RPE65 as the isomerase relates to the strong inhibitory effects on retinoid isomerase activity of detergents, which are required for solubilization and purification of RPE65 (4). Phospholipids play a key role in RPE65 activity, suggesting that they might help maintain the structure of RPE65. To determine the influence of phospholipids on RPE65 structure, we crystallized enzymatically active RPE65 in the presence of native phospholipids and determined the structure at a high resolution, using X-ray crystallography.

We obtained crystals in which RPE65, in a dimeric assembly, is embedded in a membrane-like matrix that likely mimics its structure on native membranes. Comparison of this lipid-embedded structure with that of detergent-purified RPE65 (5) revealed a key region of RPE65 located near the entrance to the active site that is stabilized by phospholipids. This region undergoes large conformational changes when native phospholipids are replaced by detergent. This finding provides key insights into the stabilizing effects of phospholipids on RPE65 and provides a mechanistic explanation for the inhibitory effects of detergents on its enzymatic activity.

All structures of RPE65, including the lipid-embedded structure, featured residual electron density in the active site cavity suggestive of a fatty-acid molecule coordinated to the iron cofactor (4). However, definitive identification of the ligand has not been possible using X-ray crystallographic methods. Confirmation of binding of a fatty acid to the iron cofactor would support the hypothesis that iron is directly involved in the ester hydrolysis function of RPE65 and that the iron–fatty acid complex represents a reaction product (2, 5). This proposed function contrasts with the role of iron in the enzymology of a group of enzymes that are evolutionarily related to RPE65, known as carotenoid oxygenases. In these enzymes, iron serves to activate molecular oxygen for oxidative cleavage of carotenoids. X-ray absorption spectroscopy is a sensitive technique that provides high-resolution information on metal–ligand bond lengths as well as the symmetry and oxidation state of the element being probed. We used this technique to confirm the presence of an iron-bound carboxylate ligand in the RPE65 active site and showed that the iron cofactor is in the divalent oxidation state. These studies indicate that RPE65 uses its divalent iron cofactor to catalyze the ester cleavage step of the retinoid isomerization reaction.

---

**Author contributions:** P.D.K., E.R.F., M.R.C., and K.P. designed research; P.D.K., E.R.F., W.S., and X.S. performed research; P.D.K., E.R.F., W.S., M.R.C., and K.P. analyzed data; M.R.C. contributed new reagents/analytic tools; and P.D.K. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Data deposition: The atomic coordinates and structure factor amplitudes have been deposited in the Protein Data Bank, www.pdb.org (PDB ID codes 4F2Z, 4F3A, 4F30, and 4F3D).

To whom correspondence may be addressed. E-mail: kxp65@case.edu or pdk7@case.edu.

See full research article on page E2747 of www.pnas.org.

Cite this Author Summary as: PNAS 10.1073/pnas.1212025109.
RPE65 depends on iron and phospholipids for its catalytic activity. Our high-resolution structural data provide evidence that RPE65 uses its iron cofactor to catalyze retinyl ester hydrolysis, a function that was unanticipated on the basis of its close structural and evolutionary relationship to carotenoid oxygenase enzymes. We also have demonstrated that phospholipids directly stabilize the active conformation of RPE65, thus explaining the inhibitory effects of detergents on the activity of this enzyme that have long confounded the study of the chemistry of visual regeneration. Similar structural changes may underlie the commonly observed inhibitory effects of detergents on the activity of other membrane-bound enzymes. Crystallization of these proteins in the presence of endogenous lipids could allow examination of the membrane–protein interface at high resolution by X-ray crystallography.