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Membrane-binding and enzymatic properties of RPE65

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A B S T R A C T

Regeneration of visual pigments is essential for sustained visual function. Although the requirement for non-photochemical regeneration of the visual chromophore, 11-*cis*-retinal, was recognized early on, it was only recently that the *trans* to *cis* retinoid isomerase activity required for this process was assigned to a specific protein, a microsomal membrane enzyme called RPE65. In this review, we outline progress that has been made in the functional characterization of RPE65. We then discuss general concepts related to protein–membrane interactions and the mechanism of the retinoid isomerization reaction and describe some of the important biochemical and structural features of RPE65 with respect to its membrane-binding and enzymatic properties.

Contents

Abbreviations: ACO, Apocarotenoid oxygenase; CCO, Carotenoid cleavage oxygenase; CHAPS, 3-[(3-cholamidopropyl)-dimethylammonio]-1-propane sulfonate; CRALBP, Cellular retinaldehyde-binding protein; C_8E_4, Octyltetraoxyethylene; ER, Endoplasmic reticulum; GPI, Glycosyl phosphatidylinositol; LCA, Leber congenital amaurosis; LRAT, Lecithin:retinol acyltransferase; MALDI, Matrix-assisted laser desorption/ionization; PBS, Phosphate-buffered saline; PLA_2, Phospholipase A_2; RBP, Retinol-binding protein; RDH, Retinol dehydrogenase; RGR, retinal G protein-coupled receptor; RPE, Retinal pigment epithelium; RPE65, Retinal pigment epithelium-specific 65 kDa protein; sER, smooth endoplasmic reticulum; S_N1, Unimolecular nucleophilic substitution; S_N2, Bimolecular nucleophilic substitution; VP14, Viviparous 14.

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1. Introduction

1.1. Structure and function of the photoreceptor and pigment epithelial layers of the retina

Vision is a physiological process involved in nearly every aspect of human life. The light-sensing tissue in humans is the retina, which is located in the posterior portion of the eye. The main light-sensitive cells of the retina, called photoreceptor cells, consist of two different types; rod cells for vision under low-illumination conditions and cone cells for color vision in well-illuminated environments (Rodieck, 1998). Visual pigments are the light-sensitive molecules found in photoreceptor cells. Visual pigments consist of a protein moiety called opsin and a vitamin A-derived chromophore called 11-*cis*-retinal that is covalently bound to a Lys side chain amino group of the opsin via a protonated Schiff base linkage (Palczewski, 2006). The covalently bound retinoid is called 11-*cis*-retinylidene. Visual pigments are seven-pass trans-membrane proteins that are tightly packed together in pancake-like membrane structures known as disks, which are located in the outer segments of photoreceptor cells (Palczewski, 2006). Adjacent to the photoreceptor outer segments is a monolayer of epithelial cells known as the retinal pigment epithelium (RPE). The RPE transports nutrients to and removes waste products from the photoreceptors, absorbs excess light through melanin granules, phagocytoses fragments of shed outer segments and recycles chromophore for visual pigments. In addition, all-*trans*-retinol is bi-directionally transferred between the systemic circulation and the RPE via a membrane transporter found on the basolateral plasma membrane of RPE cells called STRA6 (Kawaguchi et al., 2007; Qtaishat et al., 2003). Therefore, the RPE is vital for the health of the retina.

1.2. The retinoid (visual) cycle

Light sensation by photoreceptor cells in the retina begins with a photochemical event in which absorption of a single photon causes the geometrical photoisomerization of the pigment 11-*cis*-retinylidene chromophore to an all-*trans* configuration (Palczewski, 2006). This conversion activates the visual pigment allowing it to trigger a cascade of downstream signaling events that lead to the transmission of electrical signals to the visual cortex and the perception of light by the brain. After a brief period of time, signaling by the pigment is terminated by phosphorylation and subsequent binding of a silencing protein known as arrestin (Polans et al., 1996). Following a light stimulus, the pigment is no longer capable of being photoactivated. Thus, a mechanism for the regeneration of light-sensitive pigments, that is the isomerization of all-*trans*-retinylidene back to the 11-*cis* configuration, is essential for continuity of vision (Wald and Brown, 1953) (Fig. 1). At least in vertebrates, the chromophore is not directly reisomerized while it is bound to opsin. Instead, the labile Schiff base linkage between the chromophore and opsin is hydrolyzed and free all-*trans*-retinal is released in a process known as bleaching (Matthews et al., 1963). all-*trans*-Retinal is subsequently reduced in the photoreceptor cells by NAD(P)H-dependent retinol dehydrogenases (RDHs) to yield all-*trans*-retinol (vitamin A). The vitamin A is transferred to the RPE where it is esterified by lecithin:retinol acyltransferase (LRAT) (Batten et al., 2004)(summarized in (Travis et al., 2007)). It is these retinyl esters that serve as substrates for retinoid isomerization, which occurs in a complex enzymatic reaction involving simultaneous hydrolysis of the ester moiety. The retinoid product of this reaction, 11-*cis*-retinol, is then oxidized to 11-*cis*-retinal and transported back to the photoreceptors where it condenses with opsin to reform a light-sensitive pigment (summarized in (Travis et al., 2007)). This multistep process of converting all-*trans*-retinal to 11-*cis*-retinal is known as the retinoid or visual cycle. A 61 kDa RPE-specific protein called RPE65, because its apparent molecular mass based on SDS-PAGE analysis is 65 kDa, is the enzyme responsible for the light-independent conversion of all-*trans*-retinyl esters, primarily palmitoyl esters, into 11-*cis*-retinol. Although the need for light-independent isomerization activity in the regenerative visual cycle was recognized early, the identification of RPE65 as the responsible enzyme was not straightforward and occurred only recently (Jin et al., 2005; Moiseyev et al., 2005; Redmond et al., 2005). RPE65 is involved not only for regeneration of rhodopsin but also plays an important role in regeneration of cone opsins and is critical for the health of cone photoreceptors (Jacobson et al., 2007). In contrast to the RPE65-dependent chromophore regeneration pathway for rhodopsin, there is substantial evidence that chromophore for cone opsins is at least partially regenerated through an alternative metabolic pathway involving enzymes located in cone photoreceptor and Müller cells (Mata et al., 2002, 2005; Muniz et al., 2009; Wang et al., 2009). This review summarizes the history, biochemical and structural properties of RPE65 with an emphasis on its mode of membrane binding and enzymatic activity, the former being highly critical for the latter.

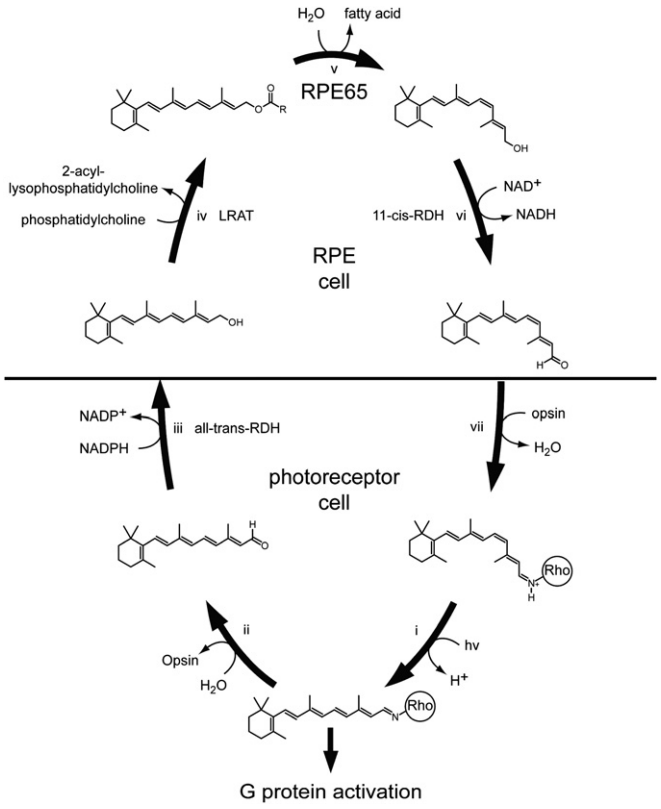

Fig. 1. Schematic representation of the retinoid (visual) cycle—Vision begins when light (hv) causes photoisomerization of the 11-*cis*-retinylidene chromophore of ground-state rhodopsin. Subsequently, the Schiff base linkage loses a proton enabling rhodopsin to activate G proteins (i). After remaining active for a short period of time, the isomerized chromophore is released via hydrolysis, generating free all-*trans*-retinal and opsin (ii). The all-*trans*-retinal is enzymatically reduced (iii) and the resultant all-*trans*-retinol is exported from the rod outer segment to the RPE. Here all-*trans*-retinol is metabolized by LRAT to produce all-*trans*-retinyl esters (iv), which can be either stored in retinosomes or further processed. RPE65 is the key enzyme that catalyzes the conversion of all-*trans*-retinyl esters to 11-*cis*-retinol (v). 11-*cis*-Retinol is enzymatically reduced to 11-*cis*-retinal (vi), which is then transported back to the photoreceptor outer segment where it recombines with opsin to form ground-state rhodopsin (vii). Continuous operation of this cycle is what sustains vision under conditions where rods are primarily active.