Experimental Eye Research 170 (2018) 51–57

Contents lists available at ScienceDirect

Experimental Eye Research

journal homepage: www.elsevier.com/locate/yexer

Tauroursodeoxycholic acid binds to the G-protein site on light activated rhodopsin

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ARTICLE INFO

Keywords:
Bile acid
Chemical biology
G protein
G protein-coupled receptor (GPCR)
Molecular docking
Photoreceptor
Phototransduction
Retina
Rhodopsin
Signal transduction

ABSTRACT

The heterotrimeric G-protein binding site on G-protein coupled receptors remains relatively unexplored regarding its potential as a new target of therapeutic intervention or as a secondary site of action by the existing drugs. Tauroursodeoxycholic acid bears structural resemblance to several compounds that were previously identified to specifically bind to the light-activated form of the visual receptor rhodopsin and to inhibit its activation of transducin. We show that TUDCA stabilizes the active form of rhodopsin, metarhodopsin II, and does not display the detergent-like effects of common amphiphilic compounds that share the cholesterol scaffold structure, such as deoxycholic acid. Computer docking of TUDCA to the model of light-activated rhodopsin revealed that it interacts using similar mode of binding to the C-terminal domain of transducin alpha subunit. The ring regions of TUDCA made hydrophobic contacts with loop 3 region of rhodopsin, while the tail of TUDCA is exposed to solvent. The results show that TUDCA interacts specifically with rhodopsin, which may contribute to its wide-ranging effects on retina physiology and as a potential therapeutic compound for retina degenerative diseases.

1. Introduction

G-protein-coupled receptors (GPCRs) are versatile transmembrane proteins that are responsible for the detection of extracellular stimuli, such as hormones, neurotransmitters and light and for the transmission of that information inside the cell via interaction with the membrane-associated heterotrimeric G-proteins to regulate various intracellular second messenger pathways (Palczewski and Orban, 2013; Manglik and Kobilka, 2014). Consequently, GPCRs possess two distinct sites for the binding of ligands and for the interactions with G-proteins on its extracellular and intracellular interfaces respectively. The ligand binding sites of GPCRs have received the most attention due to their extreme importance in receptor pharmacology resulting in estimates that 30–50% of drugs currently on the market target GPCRs (Howard et al., 2001; Salon et al., 2011). The G-protein binding site is relatively less explored, but is quickly catching up with regard to the number of recent biochemical and structural studies (Kisselev et al., 2011; Preininger et al., 2013). Most notably, the NMR and X-ray structures of the C-terminal tail of the G-protein α-subunit, Gt_{α}(340–350), one of the main protein domains on G-proteins that binds GPCRs and stabilizes its active conformation, provide solid structure-based framework for possible pharmacological exploration of this site (Kisselev et al., 1998; Choe et al., 2011).

We have used light receptor rhodopsin and its cognate G-protein transducin to identify a number of small molecule compounds that bind to and stabilize the active form of rhodopsin, metarhodopsin II (Meta II) (Taylor et al., 2008, 2010). These molecules can be used to modulate GPCR-G-protein interactions, which can potentially be helpful as therapeutics for various retinal diseases caused by constitutively active GPCRs, such as Leber Congenital Amaurosis (LCA), Congenital Stationary Night Blindness (CSNB) and some forms of Retinitis Pigmentosa (RP) (Park, 2014; Rao and Oprian, 1996; Fain, 2006; Dizhoor et al., 2008). One group of molecules that we identified via in-silico and biochemical screens belongs to natural products, sapogenins, which share triterpenoid scaffolds (Taylor et al., 2008). Sapogenins, such as madecassic acid, also share structural resemblance with bile acids. One

Abbreviations: GPCR, G-protein coupled receptor; G-protein, heterotrimeric GTP-binding protein; R, dark-adapted rhodopsin; R*, photoactivated rhodopsin; Meta II, metarhodopsin II; NMR, nuclear magnetic resonance; TrNOE, transferred nuclear Overhauser effect; IC, intracellular; EC, extracellular; TM, transmembrane; UM, urea-washed rod outer-segment membranes; ROS, rod outer segment; RMSD, root mean square deviation; Gt, transducin; Gt_{α}(340–350), transducin alpha subunit C-terminal region; TUDCA, tauroursodeoxycholic acid; DOC, sodium deoxycholate

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https://doi.org/10.1016/j.exer.2018.02.015
Received 13 November 2017; Received in revised form 14 February 2018; Accepted 14 February 2018
Available online 16 February 2018

of the bile acids that has recently been gaining significant interest due to broad medicinal properties is tauroursodeoxycholic acid (TUDCA), which is a natural hydrophilic molecule containing taurine conjugated with the ursodeoxycholic acid (UDCA). While a major constituent of bear bile (Luo et al., 2010), TUDCA is produced in humans at relatively low levels. Used as a therapeutic compound it has been shown to prevent hepatic cytotoxicity and have neuroprotective properties (Keene et al., 2002; Oveson et al., 2011; Romero-Ramirez et al., 2017). Interestingly, TUDCA has been in use since ancient times as part of the traditional Chinese medicine toolbox (Boatright et al., 2006). The mechanisms of TUDCA effects have been under active investigations. They are currently believed to be mediated via effects on mitochondrial toxicity and inhibition of apoptotic and anti-inflammatory pathways (Romero-Ramirez et al., 2017).

There is accumulating evidence that TUDCA has wide-ranging effects on retina physiology. Positive effects on the retina morphology and functions were noted for Diabetic Retinopathy (DR) (Gaspar et al., 2013; Wang et al., 2016; Fernandez-Sanchez et al., 2015), RP (Phillips et al., 2008; Drack et al., 2012; Fu and Zhang, 2014), and LCA (Fu and Zhang, 2014). Considering structural similarity to the small molecule compounds we identified in earlier studies, we investigated whether TUDCA may potentially exert some of its effects on retinal photoreceptors by binding to the transducin site on light-activated rhodopsin. We used an assay that quantitatively measures stabilization of the active form of rhodopsin, Meta II by UV–Visible spectroscopy. We also used computer simulations to determine whether TUDCA would dock at the transducin binding site on Meta II. Both methods strongly argue for the specific binding of TUDCA to light activated rhodopsin.

2. Experimental procedures

2.1. Isolation of rhodopsin

Dark adapted frozen bovine retinas are obtained from W.L. Lawson, Co. (NE). Rod outer segments (ROS) are prepared by the method of Papermaster and Dreyer (1974). Urea-washed ROS membranes (UM) are prepared using the procedure adapted from Yamazaki et al. (1982), and Willardson et al. (1993), essentially as we described earlier (Kisselev et al., 1999a, 2007). Rhodopsin concentration is measured as ΔA498 before and after bleaching in the presence of 20 mM hydroxylamine, based on the molar extinction coefficient at 498 nM of 42,700 M-1 cm-1 (Hong and Hubbell, 1972).

2.2. UV/Visible spectroscopy

The amount of extra Meta II was measured on a Cary-50 UV/Visible spectrophotometer (Varian, CA), at 4 °C, cuvette path-length 10 mm, essentially as we described before (Kisselev et al., 1994, 1999b). Specific temperatures were maintained using Peltier-controlled cuvette holder. The sample compartment was continuously infused with dry air. Photoactivation of rhodopsin was achieved by illumination of samples for 20 s with a 150-Watt fiber optic light source passed through a 490 ± 5 nm bandwidth interference filter. Samples contained 2.5 μM of urea-washed ROS membranes in buffer Meta II (20 mM Tris-HCL pH 8.0, 130 mM NaCl, 1 mM $MgCl_2$, 1 mM EDTA) and various amounts of tauroursodeoxycholic acid or deoxycholic acid (Sigma, MO). 700 nm–250 nm spectra were recorded before and after activation of the sample with a 490 ± 5 nm light. The amount of Meta II was calculated as the absorbance difference A380-A417 before and after photoactivation. The amount of Meta II calculated in reaction buffer was taken as zero. Sample turbidity was measured as the absorbance difference A280-A700 after photoactivation. The data were processed offline using KaleidaGraph 3.6.2. Full spectra scans were normalized to zero at 700 nm.

2.3. Acid trapping

Acid trapping was used to verify the Meta II state (Kisselev et al., 1998; Kito et al., 1968) of R*. Essentially, the extra MII-stabilization protocol was used with some minor modifications. The UV/Vis absorbance spectra of dark-adapted rhodopsin mixed with TUDCA was taken in the dark and then after light activation. The final concentration of TUDCA was 5 mM. Immediately following the light activation scan, 1% HCl (v/v) was added and mixed. The sample was incubated in the spectrophotometer at 4 °C for 5 min, then the absorbance spectra was scanned an additional time.

2.4. Generating conformations of TUDCA

TUDCA was drawn and minimized (Tripos forcefield) in Sybyl 7.3. Gasteiger-Huckel charges were added to TUDCA using Sybyl 7.3. Because Gt_α(340–350) is a peptide, the charges were calculated by RosettaLigand, as RosettaLigand is optimized for calculating charges on proteins (Davis and Baker, 2009). The Omega package from Open Eye was used to generate a series of 200 low-energy conformations of TUDCA (OpenEye Scientific Software, 2005). These conformations were sampled by RosettaLigand to provide ligand flexibility during docking.

2.5. Preparing the receptor

A model of R* and the X-ray crystal structure of opsin bound to Gt_α(340–350)K341L (3DQB) (Scheerer et al., 2008) were used for docking studies. The 3D intracellular (IC) loop model of R* resulted from a previous study (Taylor et al., 2007) in which experimental TrNOE structures of Gt_α(340–350) and its analogs (Kisselev et al., 1998; Anderson et al., 2006a, 2006b) were docked onto the IC loops. These docked structures revealed a common binding mode with similar residue-residue interactions that were potentially important for complex formation between R* and Gt_α(340–350). The other docking target used was the structure of opsin bound to Gt_α(340–350) (Scheerer et al., 2008). The two different models were utilized for validation of Gt_α(340–350) docking to Meta II. Both structures were repacked using ligand_rpkmin with default parameters in the RosettaLigand package. RosettaLigand repacks the side chains in a stochastic manner to remove any clashes that exist using RosettaLigand's energy function. A total of 10 structures were output, and the minimum energy repacked structure was used for docking calculations. For the model of the R* loops, capping on loop termini had to be removed to run RosettaLigand.

2.6. Docking

RosettaLigand was used for all docking calculations. The standard flags were used for RosettaLigand as outlined in the Rosetta 3.0 software (Davis and Baker, 2009). A random perturbation of up to 5 Å in the X, Y, and Z dimensions from the center of mass was implemented. However, points outside a 5 Å sphere were not considered, yielding uniform sampling within the sphere. The starting position for the docking calculation was the center of mass of the Gt_α(340–350) from either the X-ray crystal structure of opsin (for docking to opsin) or from the model of R*. For each compound docked, a total of 10,000 poses were generated.

As a proof-of-principle experiment, Gt_α(340–350)K341L from the X-ray crystal structure was re-docked onto the opsin crystal structure to determine if the binding pose would recapitulate the crystal structure. The Gt_α(340–350) conformation that resulted from a previous study (Taylor et al., 2007) on R* loops was re-docked onto the R* loops to determine if its pose could be recapitulated. TUDCA was docked onto the opsin crystal structure and onto the 3D IC loop model of rhodopsin in a conformation bound to Gt_α(340–350).

The scoring scheme of Davis et al. in RosettaLigand was used (Davis and Baker, 2009). First, any pose where the ligand was not interacting