Combined classical MD, hybrid QM/MM simulations, and in vitro tests reveal a novel function for retinal transporter protein CRALBP

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Cellular retinaldehyde-binding protein (CRALBP) is a transporter protein of the Sec 14-like family expressed in the retina. CRALBP helps migration of cis-retinol and cis-retinal in the retinal epithelium, and protects cis retinoids from unwanted photoisomerization to all-trans compounds before they are properly inserted in opsin receptors. Apart from the most common 11-cis retinoids, there is recent evidence that RPE65, the enzyme responsible for the back all-trans-to-cis- retinoid conversion in the visual cycle, is not a specific enzyme, but it can produce the 9-cis isomer apart from the 11-cis one. CRALBP can bind 9-cis retinoids as well as 11-cis isomers. Our in vitro kinetic measurements show that, when bound to CRALBP, 9-cis retinal is quantitatively converted into 9-13-dicis-retinal in the dark. Thanks to our simulations studies, we propose a mechanism for the isomerization reaction characterized by a transient proton attachment to the unsaturated carbons, which enables the trans-cis conversion. In fact, the low-temperature x-ray structure of the CRALBP/9-cis retinal complex shows unexpected structural features for the bound ligand. Hybrid QM/MM calculations demonstrate that the detected ligand is a retinal molecule carrying an extra hydrogen atom at carbon 12, revealing a kinetically trapped intermediate of the isomerization reaction. Classical MD simulations of the reactant state identify a stable H-bond network linking one buried glutamic acid and few water molecules trapped in the pocket, thus providing the natural source of protons for the reaction. In vitro detection of nuclear isotope effect confirms that the mechanism of isomerization is driven by proton transfer. Finally, MD simulations studies, also coupled to metadynamics, explain how the subtle differences of the binding geometries between 9-cis and 11-cis retinal make it possible that the isomerase activity is present only when 9-cis is bound to CRALBP, and provide insights on possible mutations able to completely inhibit light-driven cis to all-trans retinal isomerization.