Self-Controlled Ligand Release Mechanism as Determinant of the Selective Retinol Binding to Cellular Carriers

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Retinol is essential for many physiological processes like cell growth and differentiation, morphogenesis, and vision.[1] However, the lack of appreciable solubility in aqueous solution makes it necessary the assistance of carriers to facilitate the transport to the cellular target.[2-4] Cellular retinol-binding proteins (CRBPs) assist the transport to appropriate sites in the cell. The two most abundant CRBP isoforms (I and II) have distinct tissue distribution and roles in the maintenance of retinol homeostasis. These isoforms also exhibit a marked affinity difference for retinol (100-fold difference),[4] but the origin of the binding selectivity and its physiological implications remain to be elucidated. Furthermore, while the different selectivity between isoforms may be ascribed to specific evolution-based residue substitutions, it is unclear whether they influence the ligand uptake and release by altering the retinol affinity to CRBP-I and II, or rather reflect intrinsic differences in the ligand accessibility to the two isoforms, that is, the passage from/to the aqueous environment to/from the inner cavity.[5,6]

The structural fold of CRBP-I and CRBP-II consists in a β-barrel formed by two almost orthogonal five-stranded β-sheets (A-E and F-J), and two short helices (αI and αII) inserted between βA and βB strands. The entry portal site that enables the ligand to enter the cavity is formed by helices αI and αII, and the turns βC-βD and βE-βF. The three-dimensional structure of rat CRBP-I and CRBP-II have been solved by NMR[7] and X-ray[8] crystallography, respectively. Despite the high structural identity, the dissociation constant (K_d) for CBRP-I has been reported to be 0.1 nM, whereas CRBP-II binds retinol with approximately 100-fold lower affinity.[1] NMR studies[9] have revealed differences in the dynamical behavior of CRBP-I and II not only between the *apo* isoforms, but also between *apo* and *holo* states. Therefore, one may argue whether the distinct structural plasticity of the two isoforms controls the entry/release of retinol to/from the binding cavity.

With the aim to disclose the molecular determinants of the binding selectivity between CRBP-I and II and to gain insight into the functional role of these isoforms in the cell, a detailed analysis of their apo and holo forms was accomplished by combining extended atomistic molecular dynamics (MD) simulations and parallel-tempering metadynamics (PT-metaD). We have characterized the conformational flexibility of the two isoforms as well as the free energy surfaces of the processes implicated in retinol binding: opening/closing of the portal-site in both apo and holo forms, and the formation/breaking of interactions between retinol and protein in the holo species. Overall, the results provide a complete picture of the access of retinol to the protein interior that is in agreement with experimental readouts, while affording a linkage between the flexibility of the entry portal and the retinol binding affinity.

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