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Retinoic Acid Transfer And Protein-Protein Interaction Between The Cellular Retinoic Acid Binding Protein And The Retinoic Acid Receptor Probed By Hydrogen Deuterium Exchange Mass Spectrometry

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Abstract
Retinoic acid (RA) is the most potent metabolite of vitamin A and is utilized in signaling pathways that control many cellular events. Cellular retinoic acid binding proteins I and II are carriers of RA inside the cell and CRABPII transports RA to the nucleus where it binds to the retinoic acid receptors (RARs) and can then exert its effect on the transcription of genes. CRABPI is thought to bind excess RA in the cell cytoplasm to protect it from the oxidizing effect of RA. The interaction between CRABPs and RARs is very transient and such a complex has never been observed. Entry and exit of RA in CRABP occurs through fluctuation of the protein backbone through the portal region. Based on this and the crystal structures of CRABPs, a mutant containing a disulfide bond in the portal region it the holo-form of the protein was designed. This mutant effectively "locks" RA inside its binding cavity in the presence of lipid vesicles, confirming that ligand movement in and out of CRABP necessitates backbone fluctuation. Using a combination of Bioaffinity chromatography and hydrogen deuterium exchange mass spectrometry, the mechanism of transfer of RA during the CRABP/RA/RAR interaction was studied. Holo-CRABP was more protected than the apo-form and the presence of RAR did not result in a change in the protection level of apo-CRABP. However, the exchange kinetics of holo-CRABP in the presence of RAR were different from that of apo- or holo-CRABP alone. The CRABP mutants containing a disulfide bond in the portal region have an initial exchange rate that is higher than for the wild type CRABP. The complexes between those holo-mutants and RAR have a longer half-life than the wild type CRABP. The accelerated hydrogen deuterium exchange kinetics of the holomutant in the presence of RAR could therefore not be the result of faster ligand transfer from CRABP to RAR. These results were observed with both CRABPI and CRABPII. This led to a model in which during RA transfer from CRABP to RAR, the backbone of CRABP is destabilized through its interaction with RAR.