HUMAN PLASMA RETINOL-BINDING PROTEIN CAN PHYSIOLOGICALLY BE BOUND TO PALMITIC ACID; NEW INFORMATION FROM OLD CRYSTALS

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RBP4 (plasma retinol-binding protein) is the 21 kDa transporter of all-trans retinol that circulates in serum as a moderately tight 1:1 molar complex of the vitamin with the protein. RBP4 is primarily synthesised in the liver but is also produced by adipose tissue (about 20-40 % of the amounts released by the liver) and circulates bound to a larger protein, transthyretin, TTR, that serves to increase its molecular mass to about 80,000 and thus avoid its elimination by glomerular filtration. The RBP-TTR complex dissociates readily upon interaction with the RBP receptor, STRA6, that removes the vitamin from the transporter and facilitates its entrance into the cell. When retinol is not present in the complex, RBP dissociates from TTR and is eliminated in urine. We previously reported the X-ray structure of human holo RBP4 and what we expected to be the apo form, i.e. after the loss of retinol, to 2.5 Å resolution. Our most important finding was the observation of a well-defined conformational transition involving a loop at the entrance of the ligand binding site. We also reported that the protein molecule without retinol contained residual electron density in the central cavity that we interpreted as ordered solvent molecules. This job reports the three-dimensional structure of human holo-RBP and of the protein naturally deprived from retinol purified from plasma, urine and amniotic fluid determined to resolutions of 1.5, 2.0, 1.5 and 1.7 Å respectively. It is remarkable that the crystals used in this study of the plasma RBP4 holo and deprived from the retinol molecule are of the same batch as those used to determine the 2.5 Å resolution structure more than 20 years ago. In all the crystal forms of the RBP4 naturally deprived from the ligand we found palmitic acid bound in the hydrophobic ligand-binding site, a result that we confirmed by mass spectrometry measurements. The interactions of all-trans retinol with the protein at this significantly improved resolution as well as the conformational changes induced by vitamin deprivation are discussed in detail as is the structure of the complex of human RBP4 with palmitic acid.

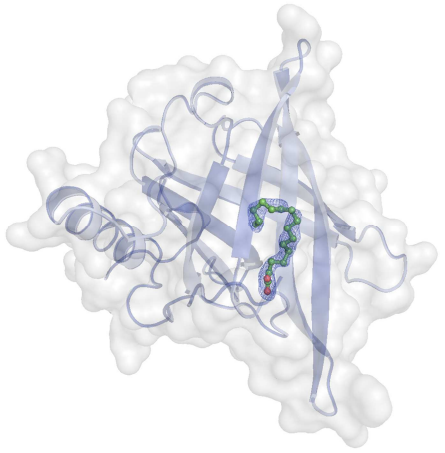

Figure 1. Surface and ligand representation of Human RBP4 with palmitic acid bound.