Retinol Modulates Site-specific Mobility of apo-Cellular Retinol-Binding Protein to Promote Ligand Binding

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Materials and Methods

Protein titrations and line shape analysis

Influence of methanol on apo-CRBP

CD_3OD was added to apo-CRBP in steps of 5 µl. High resolution $^1H/^{15}N$-HSQC spectra were recorded with the same parameters as for the titration with retinol. Line shapes were extracted for each residue and compared to the line shapes obtained for the titration with retinol. If the direction and extent of chemical shift changes were similar in both titrations, the effect was attributed to CD_3OD rather than retinol.

Influence of retinol on holo-CRBP

The line shapes of a subset of residues revealed a third ligand dependent step with a high off-rate beginning at a retinol/protein ratio of 0.8. To investigate the nature of this third step, excess retinol was removed from holo-CRBP by passing the solution over a delipidating column. After a subsequent UV spectrum the solution still revealed total saturation of CRBP with retinol.

Further addition of retinol in CD_3OD to holo-CRBP in similar steps as in the original titration resulted in identical small chemical shift changes as for low concentrations of retinol added to apo-CRBP.

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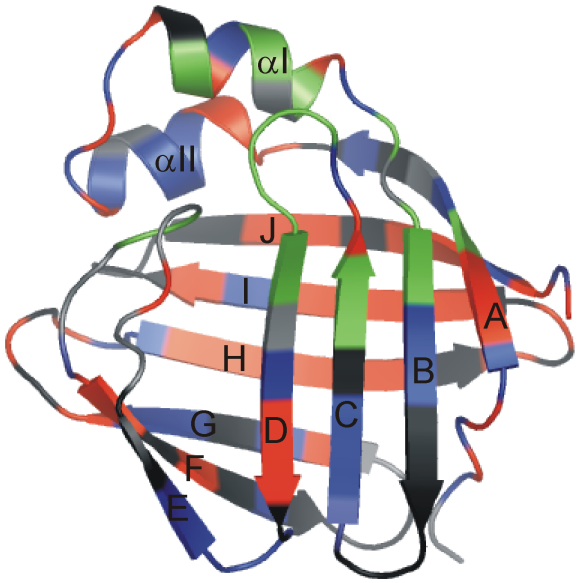

Figure 1: Ribbon diagram of apo-CRBP grouping residues according to their binding mechanism as revealed by line shape analysis. Black: no perturbation of the chemical shift upon addition of retinol; blue: one-step binding mechanism (eqn. 1) with signals in slow exchange; light blue: one-step binding mechanism (eqn. 1) with signals in fast exchange; red: two-step binding mechanism (eqn. 2); green: complex line shape with shoulders (eqn. 3); gray: no mechanism assignable due to missing assignment in one state or severe overlap.