PROBING PROTEIN-LIGAND INTERACTIONS BY AUTOMATED HYDROGEN/DEUTERIUM EXCHANG MASS SPECTROMETRY

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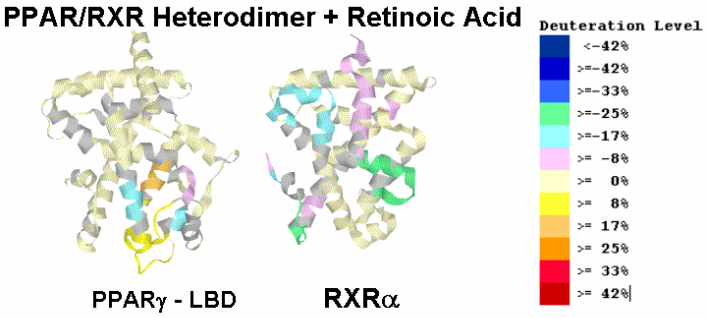
Results and Discussion

Amide hydrogen/deuterium exchange is a powerful biophysical technique for probing changes in protein dynamics induced by ligand interaction. The inherent low throughput of the technology has limited its impact on drug screening and lead optimization. Here we describe the first fully automated H/D exchange system that provides highly reproducible H/D exchange kinetics from 130 ms to 24 h. Throughput is maximized by parallel sample processing in triplicate without user intervention. We differentiate structural perturbations in the ligand-binding domain (LBD) of the nuclear receptor PPARγ upon binding a full agonist and a partial agonist. PPARγ is the target of glitazones, drugs used for treatment of insulin resistance associated with type II diabetes. Recently it has been shown that partial agonists of PPARγ have insulin sensitization properties while lacking several adverse effects associated with full agonist drugs. To further examine the mechanism of partial agonist activation of PPARγ, we analyzed ligand interactions with the heterodimeric complex of PPARγ/RXRα LBDs. Changes in H/D exchange rates for the PPARγ LBD/RXRα LBD heterodimer are shown in the Figure, for segments of PPARγ (left) and RXRα (right) upon binding of 9-*cis*-RA to RXRα. These data demonstrate that H/D exchange can detect allosteric effects of ligand binding to multi-protein complexes. Linear ion trap mass spectrometry was performed with a Thermo Finnigan LTQ instrument at Scripps Florida, fitted with an "ion-max" ESI source. For FT-ICR MS experiments, the H/D exchange system was transported to NHMFL in Tallahassee, FL, and coupled to anLTQ-FT (Thermo Electron Corporation, Bremen, Germany) linear ion trap FT-ICR mass spectrometer modified for use in a 14.5 Tesla superconducting magnet (Magnex Scientific, Oxford, UK.



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References

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