

NRP Endocrine Disruptors

Final Summary

Original project title Development of a Mass Spectrometry-Based Assay for the Analysis and Screening of Endocrine Disruptors
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Development of a Mass Spectrometry-Based Assay for the Analysis and Screening of Endocrine Disruptors

Electrospray Ionization Mass Spectrometry (ESI-MS), a soft ionization method capable of producing intact protein and protein-ligand complexes in the gas phase has been shown to be a powerful method for studying the interaction of endocrine disruptors with the estrogen receptor (ligand binding domain), ER LBD.

Research questions

Estrogens, a group of steroid hormones, regulate the differentiation and maintenance of a variety of tissues by binding through the estrogen receptor ligand binding domain (ER LBD). ER binds not only the natural hormone, but also to a wide repertoire of non-steroidal compounds such as pharmaceuticals, drugs, and environmental contaminants also known as endocrine disruptors. The characterization of the ligand binding strength and the mode of action of different ligands is of considerable interest for drug development and environmental risk assessment, because through ligand binding, ER regulates the differentiation and maintenance of a variety of tissues. Because of its speed, sensitivity and ability to transfer intact noncovalent complexes, electrospray ionization mass spectrometry (ESI-MS) has the potential to become a powerful screening method to identify and distinguish ER ligands. This soft ionization method allows measuring the mass of intact proteins as well as protein-ligand complexes in the gas phase.

Results

Using proper experimental conditions, ESI-MS allowed the detection of specific ligand interactions with native hER(alpha)LBD. The best approach to evaluate relative solution-phase binding affinity by ESI-MS was to perform competitive binding experiments with 17(beta)-estradiol (E2) as

a reference ligand. Among the ligands tested, the relative binding affinity for hER(alpha) LBD measured by nanoESI-MS was 4-hydroxtamoxifen \approx diethylstilbestrol > E2 >> genistein >> bisphenol A, in agreement with reported solution-binding affinities. hER(alpha) LBD samples were then incubated with a peptide containing the binding sequence of a coactivator protein (CAP). Since CAP is selectively stabilized in agonist-bound ER, we used this property to distinguish by MS agonist from antagonist ligand. Intact hER(alpha)LBD bound to CAP was detected by our ESI-MS method only in the presence of an agonist ligand.

Perspectives

The specificity of the ESI-MS combined with its speed (1 min/ligand) and low sample consumption (90 pmol protein/ligand) demonstrates that this technique is promising for screening and discerning suspected endocrine disrupting compounds.