562f Determining Ligand/Receptor Affinities with an Electrochemical Detection Scheme Based on a β-Galactosidase Conjugate

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A novel enzyme-multiplied assay technique (EMAT) incorporating β-galactosidase (β-gal) as the reporter enzyme with electrochemical (ElectroEMAT) signal transduction provides an effective means for dilute chemical detection. Our EMAT system relies on a β-gal-ligand conjugate whose activity is dependent on the binding of the natural receptor to the covalently attached ligand. Receptor association with enzyme-bound ligand causes complete enzymatic activity repression, due presumably to steric hindrance at the enzyme's active site. Enzyme activity is de-repressed in the presence of free ligands, which compete with the enzyme-coupled ligand for receptor binding. Activity is measured through enzyme-catalyzed hydrolysis of the substrate *p*-aminophenyl β-D-galactopyranoside, which liberates the electrooxidizable product, *p*-aminophenol.

ElectroEMAT was used to monitor estrogen and endocrine disrupting chemical (EDC) binding to the human estrogen receptor (hER) with an estradiol-β-gal conjugate, as well as benzodiazepine drug affinity to the central benzodiazepine receptor (CBR) with a gidazepam-β-gal conjugate. EDCs interfere with normal operation of the endocrine system, often through mimicry of hormones. Using ElectroEMAT, we investigated the following estradiol-mimicking EDCs: nonylphenol, genistein, bisphenol A, and diethyl phthalate. Benzodiazepines enhance the inhibitory effect of the neurotransmitter, GABA, through binding to the $GABA_A$ receptor complex and are known to act as anti-convulsants, anxiolytics, myorelaxants, and hypnotics. We estimated CBR binding affinities to the following benzodiazepines using ElectroEMAT: gidazepam, lorazepam, and diazepam. This is the first reported homogeneous electrochemical receptor assay for detection of benzodiazepines. Binding affinities were derived from empirical data using a competitive binding assay model.

Our EDC findings indicate that nonylphenol and genistein ($K_a \sim 10^8$ M^{-1}) have a greater affinity for hER than bisphenol A (10^6 M^{-1}) and diethyl phthalate (10^4 M^{-1}). CBR binding affinity was determined, in decreasing affinity, to be: lorazepam (10^8 M^{-1}), diazepam (10^7 M^{-1}), and gidazepam (10^7 M^{-1}). These results are consistent with published results and illustrate the effectiveness of an ElectroEMAT system for the simple detection of hormone mimicking compounds and for the screening of receptor-binding drugs. We currently are working on the construction of a reusable biosensing probe for convenient application of ElectroEMAT. This β-gal-based EMAT approach could be extended readily to other ligand/receptor pairs or to enzyme/inhibitor systems to enable high-throughput screening for ligand- and inhibitor-mimicking drugs.