HARNESSING THE POWER OF EVOLUTION TO CREATE NANOSCALE BIOSENSORS

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There is a need by the Army for rapid, accurate and sensitive methods for the detection of synthetic organic compounds (eg., organophosphates, explosives residues) in cluttered environments. These methods should lend themselves to miniaturization ("on-chip") and are expected to meet the multiple functional sensor needs of a modern soldier. Such systems need to be robust, versatile and survive hostile environments. For example, a small patch on a soldier is envisioned to be capable of monitoring the chemical or biological environment, and automatically delivering a suitable antidote directly into the blood stream on detecting a hostile environment.

Rapid detection of synthetic organic compounds has depended to a large extent on the ability of biosensors that specifically and selectively recognize a target molecule, even in a complex environment. Biosensors are based on receptors that are made up of polymers of either nucleic acid or amino acid residues. For example, numerous antibodies are available that bind specifically to their target. However, the key problem in biosensor development is signal transduction. Receptors are normally engineered on a case-by-case basis to provide a signal upon target binding. Ideally, what is needed for biosensor development is a receptor family each member of which has different target specificity, and which produces the same type of signal upon target binding. To achieve this goal we have developed a novel process for the creation of such a receptor family, based on the *in vitro* evolution and selection (IVES) process of a naturally occurring polypeptide-receptor, the estrogen receptor. The technology being developed has the potential to be used for a wide variety of situations in which a rapid, accurate and sensitive method for detection of compounds in complex mixtures is needed.

The estrogen receptor contains a ligand-binding domain that is responsible for the specific and high affinity interaction with various ligands. To create novel receptors based on the estrogen receptor random mutations were introduced into the regions contained in the ligand-binding domain that are known to be important in ligand binding. These random mutations were introduced using the polymerase chain reaction with doped oligonucleotides. Receptors that bind the organic compound of interest are identified by using a yeast-based one-hybrid screen. This screen depends on the ability of the estrogen receptor to enhance gene expression in response to ligand binding. Thus, only yeast that contain a mutant receptor that binds the organic compound of interest will grow in the yeast-based one-hybrid screen. The mutant receptor cDNAs can easily be isolated from the yeast and characterized. We have shown proof-of-principle for this approach by creating a novel receptor that specifically interacts with a pesticide, called methoxychlor. The estrogen receptor, which was used to create the methoxychlor receptor, does not detectably interact with methoxychlor. Our methoxychlor receptor is highly specific, as it does not interact with DDT, the parent compound of methoxychlor, nor with the organohalide, dieldrin. We are currently in the process of identifying receptors for the explosive compound, TNT and for the organophosphate, Demeton-S, a close structural analogue to VX.

In parallel with the identification of novel receptors is the development of fluorescence-based signal transduction mechanisms that will provide quantitative detection of the receptors interaction with the organic compound of interest. We are developing two signal transduction systems. In the first system the ability of the mutant receptors, which are obtained from the IVES process, to activate transcription in response to the targeted organic compound has been exploited to develop a yeast-based biosensor. Yeast have been engineered to produce green fluorescent protein (GFP) in response to the presence of the organic compound of interest. The presence of the organic compound is detected by a receptor, which then enhances the expression of GFP. To demonstrate the feasibility of this approach we have constructed a yeast-based methoxychlor biosensor that is able to detect methoxychlor at levels >2 ppm in a complex mixture.

The other signal transduction system provides rapid and quantitative detection of the organic compound of interest. It depends on the conformational change that occurs in the ligand-binding domain of the receptors upon binding to the organic compound. As proof-of-principle of this approach we have produced a methoxychlor biosensor that rapidly generates a fluorescence signal in response to methoxychlor. The ligand-binding domain of the methoxychlor receptor obtained from the IVES process was re-engineered to create a methoxychlor biosensor. Cyan fluorescent protein (CFP) was covalently attached to the N-terminus of the ligand-binding domain of the methoxychlor receptor and yellow fluorescent protein (YFP) was attached to the C-terminus of the domain. CFP and YFP possess intrinsic fluorescence that requires no cofactors or *in vitro* chemical manipulations

to induce light emission. The emission spectrum of CFP overlaps substantially with the excitation spectrum of YFP. At distances less than 100 A, fluorescence resonance energy transfer (FRET) occurs in which the emission of CFP excites YFP and result in stimulated emission of YFP. FRET between a pair of fluorophores varies with the sixth power of their separation, and is therefore a direct and sensitive readout of ligand binding. The methoxychlor biosensor generates a FRET response to methoxychlor and can currently detect methoxychlor levels > 10 ppm in a complex mixture. We are currently optimizing the signal-to-noise levels obtained with the biosensor to improve sensitivity.

In summary, we have used the IVES process to create a novel polypeptide-based receptor that detects the pesticide methoxychlor, and used this receptor to create nanoscale biosensors. The mutant libraries that have been created are a resource that can be reused indefinitely to find specific polypeptide-based receptors for a number of organic compounds of interest. In principle, given the complexity of polypeptide structures and the appropriate selection pressure, it is likely that a polypeptide-based receptor could be created that would be specific for any organic compound of interest. The receptors isolated from the IVES process can be easily, cheaply and rapidly modified to defeat countermeasures. The signal generating systems that have been developed will be applicable to any of the receptors developed through the IVES process, which will eliminate the need to develop new signal generating systems for each organic compound of interest. Yeast-based biosensors would be useful as cost-effective sentinels in which response time was not of paramount importance. The FRET-based biosensors have the potential to be multiplexed to create a sensor chip that could detect hundreds of different organics simultaneously and would be useful in a battle situation where rapid responses are essential.