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Research paper

Engineering of recombinant antibody fragments to methamphetamine by anchored periplasmic expression

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Abstract

The detection of methamphetamine and other chemically related illicit drugs relies extensively on immunoassays. Here we report the cloning and affinity maturation of an anti-methamphetamine antibody which is being employed in the current commercial assays. An anti-methamphetamine scFv was cloned from hybridoma cells, expressed in bacteria and its affinity towards methamphetamine and *N*-ethylamphetamine (ethamphetamine) was determined by Surface Plasmon Resonance (SPR). The anti-methamphetamine scFv gene was subjected to random mutagenesis by error prone PCR and variants with improved affinity were isolated from the resulting library by a novel screening methodology termed Anchored Periplasmic Expression (APEx) [Harvey, B.R., Georgiou, G., Hayhurst, A., Jeong, K.J., Iverson, B.L., Rogers, G.K. (2004). Anchored periplasmic expression, a versatile technology for the isolation of high-affinity antibodies from Escherichia coli-expressed libraries. Proc. Natl. Acad. Sci. U. S. A. 101, 9193]. The isolated clones exhibited improved affinity to these illicit drugs, yet maintained low cross-reactivity to over-the-counter drugs. In addition, all clones displayed improved expression characteristics in *Escherichia coli*. The affinity improved scFv antibodies are thus likely to be useful in methamphetamine class immunodiagnostics.
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Keywords: Methamphetamine; Antibody; Affinity maturation; Anchored periplasmic expression (APEx)

1. Introduction

"Amphetamines" are a group of compounds that are powerful stimulants of the central nervous system. Derivatives of the key compounds, methamphetamine and ethamphetamine, include the drugs of abuse 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxy-*N*-ethylamphetamine (MDEA) respectively. Other closely related but legal derivatives of these compounds that can be found in widely used over-the-counter (OTC) and prescription medication

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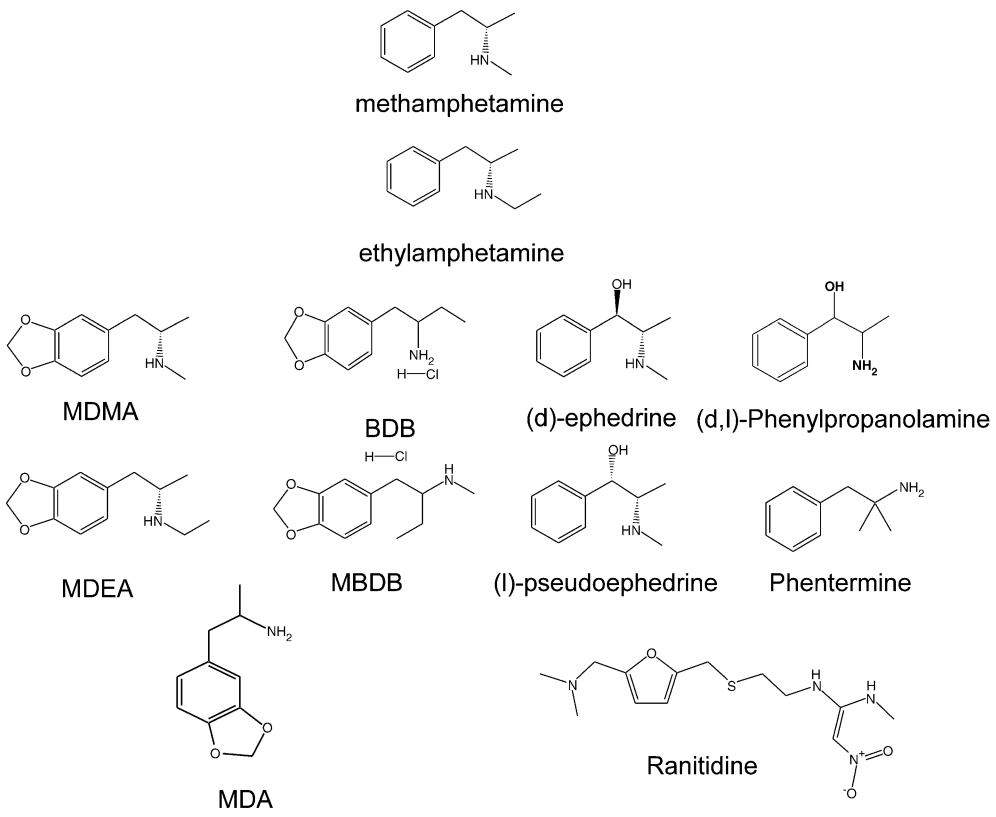

Fig. 1. Methamphetamine and its derivatives. Drugs of abuse methylenedioxymethamphetamine (MDMA), methylenedioxyethylamphetamine (MDEA), methylenedioxyamphetamine (MDA), benzodioxazolylbutanamine (BDB), and methylbenzodioxazolylbutanamine (MBDB) are on the left, and OTC drugs L-ephedrine, D-pseudoephedrine, ranitidine, (D,L)-phenylpropanolamine, and phentermine are pictured on the right.

include ephedrine and pseudoephedrine (Fig. 1) (Buchanan and Brown, 1988).

Immunoassays are routinely employed to detected methamphetamine and its derivatives in urine samples. Samples that test positive by immunoassay are subsequently analyzed further by gas chromatography–mass spectrometry (GC-MS) (Goldberger and Cone, 1994) to provide unambiguous determination of the absence or presence of drug of abuse for legal purposes. However, GC-MS is not practical for routine screening of large numbers of samples. Improving the utility of immunoassays for amphetamines could drastically reduce the number of false positives and therefore reduce the need for GC-MS analysis. This can be accomplished by employing antibodies that exhibit the following properties: (1) bind to methamphetamine with higher affinity, thus allowing more sensitive detection; (2) also exhibit higher affinity for other common derivatives of the drug; and (3) afford low cross-reactivity to over-the-counter (OTC) drugs that interfere with amphetamine detection. As illustrated by Fig. 1, this last point can be especially difficult when modifying antibody structure, as there is a high level of structural similarity between the OTC and illicit drugs.

In this study, we cloned the variable heavy and variable light domains of a methamphetamine-specific antibody, currently used in commercial assay kits, and expressed the corresponding recombinant antibody fragments in *Escherichia coli*. The recombinant scFv was subjected to random mutagenesis and clones exhibiting improved methamphetamine binding were isolated following a single round of screening by a new bacterial expression/flow cytometric technique, Anchored Periplasmic Expression or APEx, which was recently developed by our laboratory (Harvey et al., 2004). In APEx, antibody fragments are expressed as fusions to a 6 amino acid *N*-terminal sequence derived from the *E. coli* lipoprotein NlpA. Such NlpA-ScFv fusions are fatty acylated and become tethered to the periplasmic side of the cytoplasmic membrane of the bacterium. Upon chemical permeabilization of the outer membrane (Chen et al., 2001), fluorescent antigens can enter into the periplasmic space where they are recognized by the membrane-tethered antibodies. As a result, antibody-expressing cells

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