Lectin Type Fold as a Scaffold for Massive Sequence Variation

Tech ID: 19502 / UC Case 2005-108-0

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OTHER INFORMATION

CATEGORIZED AS

- **Medical**
  - Disease: Autoimmune and Inflammation
  - Other
- **Research Tools**
  - Antibodies

RELATED CASES

2005-108-0

BACKGROUND

There is a great need in biotechnology and pharmaceutical development for manipulable high affinity binding proteins. At present, the best available technologies supporting this purpose are based on immunoglobulins. However, immunoglobulins are difficult and costly to produce and unstable in a reducing environment. Although they can provide high affinity molecular interactions with a diverse set of target molecules, their utility is limited by the difficulty with which their complex scaffold is amenable to manipulation. This limitation results in a number of disadvantages, including the need to produce the molecules in mammalian cells or a suitably engineered system, the need for complicated molecular cloning to diversify the binding site, and the confounding structural considerations that need to be made when modifying any of the six individual complementarity determining regions.

TECHNOLOGY DESCRIPTION

This invention provides a new class of high affinity binding proteins, based on a modified C-type lectin (CLec)-fold like structure, with a broad range of binding specificities (~10^{20} unique variations). Variation at select amino acid positions within the variable binding site of the CLec-fold results in a scaffold with a protein binding interface highly amenable to directed manipulation. This technology is based upon the natural ability of the Bordetella bacteriophage to produce extensive amino acid sequence variation in its receptor-binding protein, the major tropism determinant. As these scaffolds are native to bacteriophage, they can be effectively produced in common bacterial strains. The stability of these scaffolds is superior to that of immunoglobulins and other existing antibody alternatives. The variable positions in this invention may be readily modified using standard oligonucleotide based cloning methodologies to produce a library of binding proteins with unique binding specificities and affinities. The library may be screened to identify one or more scaffolds which bind a ligand of interest.

ADVANTAGES

- ▶ Simplified scaffold affords the creation of a population of binding proteins with highly diverse binding specificities and affinities through the modification of a single polypeptide chain.
- ▶ Optimized profile of variable residues within the CLec-fold scaffold, which are manipulable without influence on tertiary structure.
- ▶ Variable residues provide ~10^{20} possible combinations of amino-acid side chains in the binding site, allowing the generation of high affinity binding reagents for a variety of molecules, including nucleic acids, polysaccharides, lipids, small molecules, or a combination thereof.
- ▶ Can be readily displayed using phage display or other common methodologies to screen populations of scaffolds for structures that interact with a particular ligand.
- ▶ These scaffolds are soluble in E. coli and can be produced quickly and at far less expense than immunoglobulins.
- ▶ The structure of these scaffolds is tolerant to a reducing environment (relative to immunoglobulins, CTL_4-like sandwich domains, V-like domains, and tendamistatin-based structures).
- ▶ The binding site is distant from both the N- and C-termini, allowing for the structure of these scaffolds to remain intact when attached to an immobilization surface (such as a bead) or otherwise modified at one end (such as pegylation).
- ▶ Selected scaffolds can be readily modified with a moiety for detection including a detectable label, a toxin, or an activatable pro-drug.

APPLICATIONS

This invention could be used to target a variety of ligands; including, but not limited to, a viral antigen, a bacterial antigen, a fungal antigen, an enzyme, an enzyme inhibitor, a cell surface molecule of any composition, a reporter molecule, a serum protein, or a receptor.
Library of scaffolds or binding proteins can be expressed as a phage display, ribosome display, polysome display, cell surface display, array, or microarray format, and scaffolds with your specific affinity of interest can be produced:

- ▶ A labeled scaffold can be used to identify its high affinity binding partner in samples from, cells, serum, tissue, body fluid, etc; using microscopy, flow, or a variety of other techniques available in the existing art.
- ▶ A labeled scaffold can be generated for the detection of a diagnostic molecule in serum, tissue, body fluid, or another assayable material (such as the detection of hCG in urine samples as an indicator of pregnancy).
- ▶ A labeled scaffold could be used to detect pathogenic microorganisms, or a diseased or infected cell.
- ▶ A scaffold selected to interact with a marker for a diseased or infected cell could be used for the targeted delivery of therapeutics.
- ▶ Selected scaffold molecules could be attached to a toxin or an apoptosis signal to target a cell surface marker such as a marker of a cancer cell.

Scaffolds can be selected which bind antibodies from a particular species of animal for use as a secondary reagent in assays using antibodies as the primary detection agent.
The composition and methods of this invention could be used to build a kit, consisting of a particular scaffold molecule or molecules, or for the expression and production of a library of these scaffolds.

STATE OF DEVELOPMENT

This technology is offered exclusively or nonexclusively for U.S. and/or certain foreign countries. A commercial sponsor for potential future research is sought. See patent application 20070275367.

RELATED MATERIALS

The inventor is Partho Ghosh, UCSD Professor, Chemistry and Biochemistry. Technology-relevant papers are listed below.

The C-type lectin fold as an evolutionary solution for massive sequence variation, McMahon et al., Nat Struct Mol Biol. 2005 Oct;12(10):830-1.
Selective ligand recognition by a diversity-generating retroelement variable protein, Miller et al., PLoS Biology 2008 Jun 3;6(6):e131.

PATENT STATUS

Country	Type	Number	Dated	Case
United States Of America	Issued Patent	7,749,694	07/06/2010	2005-108

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