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Antibody Fab display system that can perform open-sandwich ELISA

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ABSTRACT

Previously, immunological detection of small haptens or peptides was only possible in a competitive format, which needed competitor antigen either labeled by a reporter or attached to a carrier protein. Beside this, open-sandwich immunoassay (OS-IA) is a simple but powerful immunoassay that can noncompetitively determine monovalent antigen concentration by measuring the antigen-dependent increase in V_H/V_L interaction of an antibody. However, the procedure to obtain suitable assay reagents for OS-IA for a target antigen has not been straightforward because of the lack of easy-to-use antibody selection/manipulation methods. Here we devise a new Fab antibody phage display system that is useful for rapidly evaluating and selecting suitable antibody Fv fragments to OS-IA. The system is based on a phagemid vector in which two identical restriction sites were incorporated into both ends of a human constant region domain. After selection of the M13 phage displaying a Fab fragment, the vector can be easily converted to the vector that can simultaneously produce the V_H-displaying phage and the light chain in the culture supernatant, which can be directly used for OS-ELISA. The successful results of model selection as well as conversion to OS format show the potential in developing various OS-IA for clinically and environmentally important targets.

Specific detection as well as quantification of small molecules such as steroids and peptides is crucial for clinical diagnostics. For example, peptides with small molecular weight, whose precursor is a protein specifically expressed in certain disease cells, are useful marker candidates for diagnoses of diseases. On the other hand, environmental pollution by small toxic chemicals has been a global social problem, especially in developing countries. However, the current detection methods for these small substances mainly rely on instrumental analyses such as LC/MS and GC/MS/MS, which require costly equipment and long measurement time despite generally low sensitivity.

As a powerful but inexpensive alternative to these conventional techniques, recently immunoassays (IA)[1] are becoming more and more popular. However, while most proteins can be quantified with sandwich IA using two antibodies with high sensitivity [1], for smaller antigens with a molecular weight of less than 1000, sandwich immunoassays are no more available due to a limited binding surface for the two antibodies in such small substances. In such case, the common approach is a competitive assay, in which either labeled antigen or labeled antibody is used as a competitor to monitor the amount of antigen in the sample. However, generally the assay needs careful optimization with preexperiments, and the detectable concentrations are almost always higher than the theoretical expectations [2].

Open-sandwich immunoassay (OS-IA) is a novel immunoassay approach that employs the antigen dependency of the interaction between the separated heavy chain (V_H) and light chain (V_L) of an antibody variable region (Fig. 1A). Without antigen, the two fragments are prone to dissociate, while in the presence of antigen, they associate owing to increased interaction by the bridging antigen. This phenomenon could be successfully applied to measure the concentration of many small molecules in various samples, without competition and in a short time [3–7]. Even if the antibody showed suboptimal properties for OS-IA, namely stronger V_H/V_L interaction in the absence of antigen, protein engineering at the V_H/V_L interface could successfully convert the Fv to a suitable one [8].

However, the procedure to obtain suitable assay reagent for OS-IA for a target antigen has not been straightforward because of the lack of an easy-to-use antibody selection/manipulation method.

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[1] *Abbreviations used:* BGP, bone gla protein (osteocalcin); BGP-C7, the C-terminal 7 amino acids of human BGP; BSA, bovine serum albumin; cfu, colony forming unit; ELISA, enzyme-linked immunosorbent assay; Fab, antibody-binding fragment; Fv, antibody variable region; V_H, antibody H chain variable region; V_L, antibody L chain variable region; HEL, hen egg lysozyme; IA, immunoassay; OS, open sandwich; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; SDS–PAGE, polyacrylamide gel electrophoresis in sodium dodecyl sulfate; SOE, splice overlap extension; TMBZ, 3,3′,5,5′-tetramethylbenzidine.

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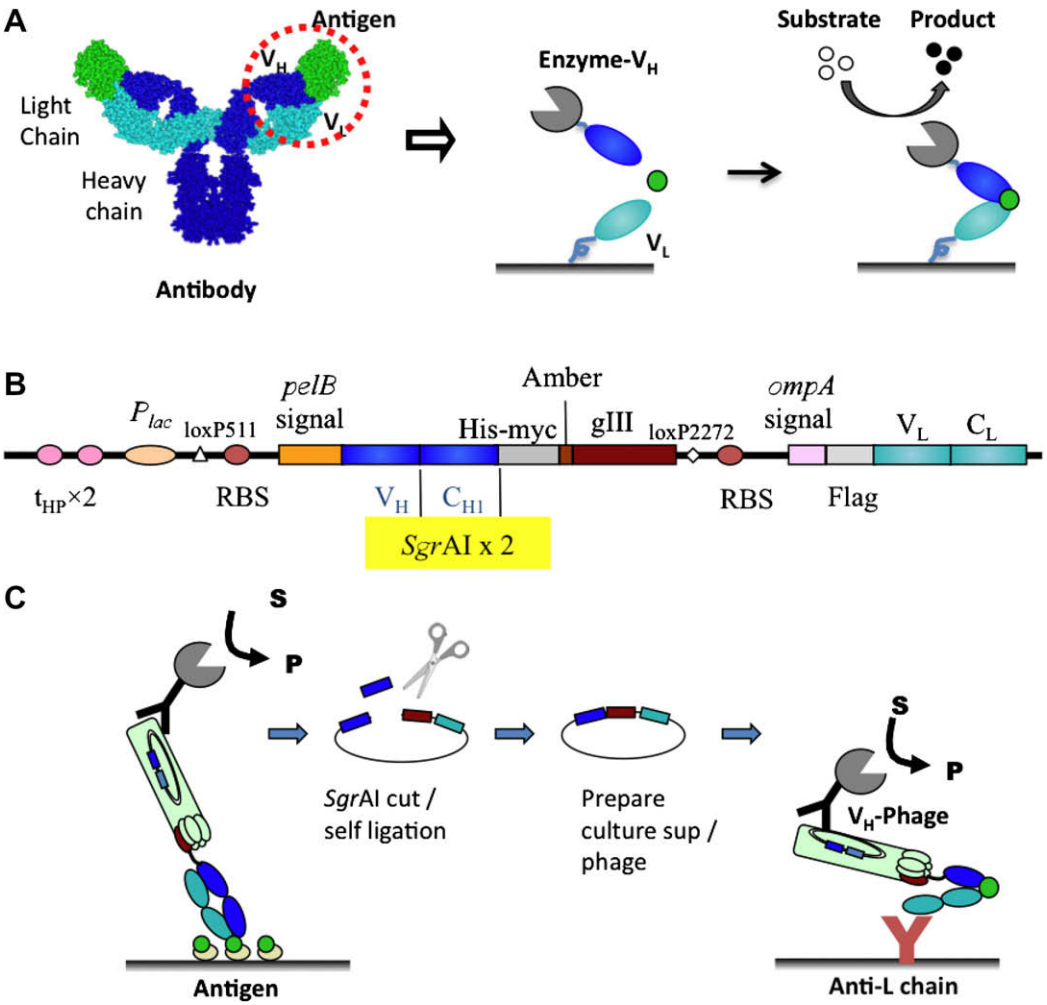

Fig. 1. (A) A schematic drawing of open-sandwich immunoassay. (B) Structure of the phagemid for Fab display (pDong1). (C) Schematic drawing of the conversion of Fab to OS formats.

Because whether an antibody is suitable for OS-IA or not is only decided after measuring the V_H/V_L interaction strength and its antigen dependency, a fast and convenient approach for evaluating the interaction is needed. For this purpose, phage display is a relatively new combination of molecular techniques for the selection and evaluation of protein–protein interactions [9]. The technology can be used for display of various antibody fragments, and is an efficient method for antibody display and selection. So far, a number of systems have been developed to display antibody and its fragments on phage surface, and to select high affinity binders from the libraries of various sources. Phage display vectors for single chain variable region (scFv), such as pIT2 and pCANTAB5E (GE Healthcare, Buckinghamshire, UK) and also for heterodimeric Fab display such as pComb3 [10] and pCES [11], have been used to select specific antigen-binding clones from the library constructed on them. However, all the current vectors except one are not intended for evaluating the interaction between the two displayed chains. The exception is the split Fv (spFv) system, which displays V_H and V_L fragments on the two N-termini of M13 phage coat proteins pIX and pVII, respectively [4]. In this system, amber codon was designed to locate between V_L gene and gene VII, and when an amber suppressor *Escherichia coli* is used for phage production, only V_H is displayed on the phage surface, and soluble V_L fragment is secreted to the culture supernatant. Thus it enables ELISA to investigate the V_H/V_L interaction by immobilizing the V_L on the surface of microplate wells either by an anti-tag antibody or protein L, and detecting the amount of immobilized phage after the reaction. However, while this system was successfully used to select and clone many Fvs that are suitable for OS-ELISA, some other Fvs did not show positive antigen binding, or the level of secreted V_L fragment was too low to perform OS-ELISA, possibly due to limited stability of the isolated V_L domain.

Compared with the scFv fragment that is known to have a high tendency to form multimers [12–14], the antibody Fab fragment is reported to stay monomeric, allowing selection for affinity in contrast to selection for avidity [15]. In this paper, we describe a novel antibody selection system based on phagemid vectors that are useful for monovalent display of Fab fragments on a phage surface. The system could be used for making Fab-displaying phage, selecting antibodies with specific antigen-binding affinity, and finally and most importantly, evaluating the interaction of V_H and V_L to perform OS-ELISA to see whether it is suitable for antigen quantitation.

Materials and methods

Materials

The plasmids pBR322(hIgγ1-10) and pBR322(hIgCκ) containing IgG1 C_{H1} and Cκ genes of human antibody, respectively, were provided by Health Science Research Resource Bank (Japan Health Sciences Foundation, Tokyo, Japan). The *E. coli* strains used in this