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Antibody engineering and phage display technology

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Abstract

Here we provide our pipeline for antibody discovery process. Starting from *de novo* isolation from custom made phage display libraries to expression and downstream applications; it allows us to obtain recombinant antibodies with desired properties to vast set of antigens. We also developed the CYB5-fusion system for periplasmic expression of multimeric proteins with the possibility of process monitoring. The combination of redox dependent absorbance spectrum of red-colored cytochrome b_5 with its high value molar extinction coefficient allows us to monitor antibody fusion proteins localization, redox state and quantify them in reliable way in turbid solutions. Moreover, it was revealed that due to outstanding stability and solubility, cytochrome b_5 significantly enhances expression level of antibody fragments in *Escherichia coli* periplasm.

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Introduction

Antibodies are an essential biotechnological tool being a part of immunodiagnostics, therapeutics and life science research instruments. Due to the advances of molecular biology methods, it became possible to manipulate structure and properties of antigen-binding domains of antibodies (1). This led to the creation of a variety of small-sized, single-domain, bi-specific, conjugated antibodies, alternative antibody scaffolds for antigen recognition. Nevertheless, development of universal recognition modules for clinical diagnostics and therapeutic usage that possesses low immunogenicity, high stability and could be easily conjugated with nanoparticles is still a big challenge for academia and pharma.

Materials and Methods

In order to isolate specific antibodies to vast set of targets with affinities and specificities that rival or exceed those of natural antibodies, we utilized the technology of "synthetic" phage display libraries. We have analyzed *in silico* more than 2500 Protein Data Bank structures of antibody-antigen complexes and diversified CDRs (complementaritydetermining regions) amino acids involved in antigen recognition to mimic natural immune system diversity. Generated Fab and single-domain phage display libraries had a diversity of 10^{10} various antibody variants. Such an approach makes it possible to raise antibodies with desired properties in carefully controlled conditions to virtually any antigens including toxic, non-immunogenic and self-antigens (2).

In order to monitor recombinant antibodies expression in *Escherichia coli (E. coli)* system in real time and to control their folded state we developed a novel antibody cytochrome b_5 fusion system and proposed its implementation for antibody discovery (3). The proposed monitoring module of fusion protein – cytochrome b_5 is hemeprotein, which has an excellent solubility, characteristic absorbance spectrum with the high value of molar extinction coefficient (ε_{413}=117.0 mM^{-1} cm^{-1}) and stable intense red color.

Results

As the proof-of-concept of suitability of synthetic antibody libraries a number of specific antibodies with moderate affinities to various cytokines and pharma targets (human growth hormone, erythropoietin, thyroperoxidase, cytochrome P450 11B2) were isolated and characterized. Development of highly specific antibodies against membrane mitochondrial proteins CYP11B2 (aldosterone synthase) and CYP11B1 (steroid 11β-monooxygenase) is necessary for investigation of molecular basis of enzyme specificity and for various immunostaining applications. We succeeded in generating specific binders to CYP11B2 (aldosterone synthase) that do not bind to CYP11B1 – proteins with 93% sequence identity.

Discussion

We found that red-colored cytochrome b_5 protein fused to antibody fragments enhance the recombinant antibodies expression level more than three-fold, simplifies their purification process and subsequent applications. Since the maximum of absorbance in the Soret region depends on oxidation state of cytochrome b_5, it became possible to evaluate relative redox potential of the fusion protein environment by measuring 413/423 nm absorbance ratio. Furthermore, we can definitely determine protein localization during expression. In *E. coli* cytoplasm cytochrome b_5 is expressed in reduced state with characteristic maximum in the Soret region at 424 nm. In the oxidative environment of *E. coli* periplasm, cytochrome b_5 has absorbance maximum at 413 nm. Thus, it became possible to estimate the relative distribution of periplasmic and cytosolic localization of fusion protein, by measurement of difference spectrums and using molar extinction coefficient $\varepsilon_{424\text{-}413}$.

Cytochrome b_5 fusion system could be used as a versatile tool for characterization of antibody-conjugated nanoparticles, as cytochrome b_5 fusion protein absorbance spectrum provides information whether the protein properly folded or not and make it possible to quantify it in reliable way in turbid solutions.

As the result, a complete pipeline for antibodies discovery and design was created. Starting from hybridoma cloning or *de novo* isolation from phage display libraries to expression and downstream applications, it allows us to obtain recognition modules with desired properties.

References

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