Selection by reaction: a general strategy to develop novel enzymes and catalysts and for functional cloning

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Controlling and catalyzing reaction like enzymes is always the central topic of chemistry. In 1986, Lerner RA's group [1] and Schultz PG's group [2] proposed the catalytic antibody strategy (abzyme), which allow chemist to develop novel artificial enzymes to certain reactions, which was a breakthrough in this field. Catalytic antibody is antibody induced by synthetic transitional state mimics, it may stabilize the real transitional state therefore catalyze the reaction. However, catalytic antibody strategy has its intrinsic limitations, which greatly hindered its industrial application:

1) Only use antibody as scaffold, limited structure diversity and therefore limited functions.

2) Hard to utilize coenzyme and subunit.

3) The good transitional state mimics are not easy to design and synthesize, and even a good transitional state mimics cannot exactly mimic the real transitional state.

4) Need to know the clear reaction mechanism while many reactions' mechanisms are not clear yet and there are other ways for catalysis besides stabilizing transitional state.

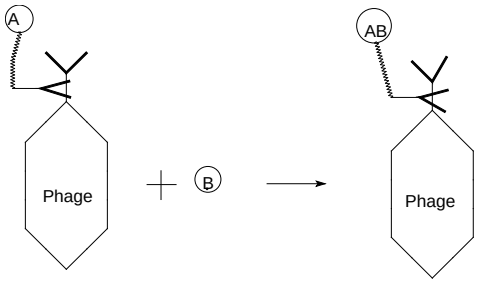
5) Most importantly, most antibodies induced by transitional state mimics have only very good binding but no catalytic activity at all.

Till now only one catalytic antibody has been used in small industrial scale (and in fact even this one is developed using reactive hapten [3], an ingenious improvement on catalytic antibody by Janda KD instead of using transitional state mimics).

In 1997, the author invented a new strategy: Selection by Reaction. It overcome the limitations of catalytic antibodies and could work for most reactions theoretically. The detail is: first use a phage displayed library such as a phage displayed antibody library

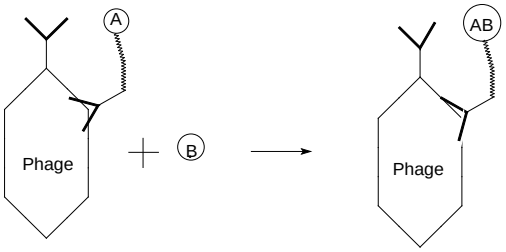
(not limited to antibody library, therefore have greater scaffold diversity), then couple the substrate with the phage, if there is a phage expressed certain antibody that can catalyze the substrate to give reaction product, under suitable condition the substrate on this phage would become product and we can pick this phage out by affinity column for the product. A directed evolution strategy could be applied until the catalytic activity is optimized. The cross catalysis between phages could be reduced by adjusting concentration, viscosity or immobilization. The following figure gives a more detailed illustration, which use antibody specific to the surface protein on phage or the constant region of antibody expressed to immobilize the substrate instead of covalently coupling using chemicals, which may lack specificity. For a reaction A+B→ AB,

1)



Substrate-PEG-helper antibody-constant region of catalytic antibody - phage

2)



Substrate-PEG-helper antibody-other protein on the phage

Fig1, 2 catalytic antibodies on phage catalyze substrate A which is immobilized on phage by the helper antibody to give reaction product AB

This method eliminate the need for design and synthesizing transitional state mimics and need not to know the mechanism of the reaction, and could utilize other catalyst scaffolds and other display system such as ribosome display library. The author had proposed this

strategy to both inventors of catalytic antibody and received the reply from Dr. K. D. Janda. Several months later without acknowledging the author's original proposal, Schultz PG' group published a paper using a know enzyme as model proved the validity of this strategy and suggested it could be used for protein functional cloning in proteomic study [4]. This method overcomes the limitation of catalytic antibody and is showing [5, 6] great potential in life science and chemistry.

This strategy is also not limited in searching novel protein enzymes, for example, it could also be used in developing small molecule catalysis on polymer support:

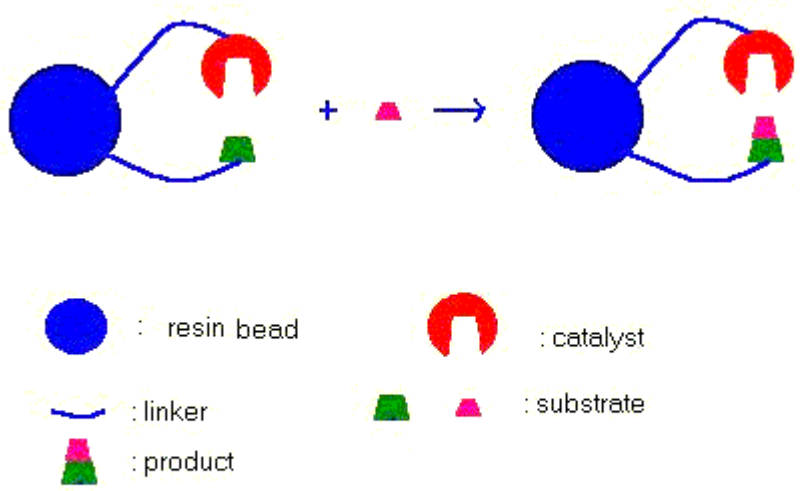


Fig.3 Catalyst on the resin bead converts the substrate nearby into product for selection, a one bead one compound library is used, antibody selective to product or isotope, dyes coupled to the free substrate could be used to pick out the special bead having catalytic activity .

1) Tramontano A, Janda KD, Lerner RA. Proc Natl Acad Sci U S A 1986 September;83(18):6736-40; Science 1986 Dec 19;234(4783):1566-70

2) Pollack SJ, Jacobs JW, Schultz PG; Science 1986 December 19; 234 (4783):1570-3

3) Janda KD, Lo LC, Lo CH, Sim MM, Wang R, Wong CH, Lerner RA. Science 1997 February 14;275(5302):945-8

4) Henrik Pedersen , Holder S, Sutherlin DP, Schwitter U, King DS, Schultz PG. Proc. Natl. Acad. Sci. USA. 1998 September 1; 95 (18): 10523–10528

5) Liu, D.R.; Schultz, P.G. Angew. Chem. Int. Engl. Ed., 38:36-54, 1999.

6) Xia G, Chen L, Sera T, Fa M, Schultz PG, Romesberg FE. Proc Natl Acad Sci U S A 2002 May 14;99(10):6597-602