Directed Evolution of Antibody Molecules in Phage-Displayed Combinatorial Libraries

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Advances in methods for conformational prediction, structural analysis and site-directed mutagenesis of proteins have contributed to the understanding of their structure and function. However, with the exception of a few successes, the generation of practical functional proteins solely by rational design remains a difficult challenge. The aim of our study is to investigate molecular design relying on evolutionary processes, called as “directed evolution”, to generate a novel class of biofunctional molecules. This evolutionary approach consists of three steps; 1) constructions of protein libraries based on structural information, 2) expressions of the libraries on phage particles, and 3) selections with investigator-imposed selective pressures. In this work, I would like to introduce our strategy for directed evolution of catalytic antibodies in phage-displayed antibody (Fab) libraries.

Enzymes have evolved their ability to use binding energies for catalysis by increasing the affinity for the transition state of a reaction and decreasing the affinity for the ground state. To evolve abzymes toward higher catalytic activity, we have reconstructed an enzyme-evolutionary process in vitro. Thus, a phage-displayed combinatorial library from a hydrolytic abzyme, 6D9, generated by the conventional in vivo method with immunization of the transition-state analog (TSA), was screened against a newly devised TSA to optimize the differential affinity for the transition state relative to the ground state. This method was able to provide the evolved mutants which were 6-20 fold higher active than the parent antibody 6D9.