PROTEINS TARGETING ORTHOLOGS

FIELD OF THE INVENTION

[0001] In a particular aspect, the present invention is relevant to proteins and to their generation by protein evolution.

BACKGROUND

[0002] Protein engineering via site-directed mutagenesis and, more recently, molecular evolution has been successfully employed to improve enzymatic properties in industrial applications and therapeutic properties in antibodies. Characteristics such as thermostability, pH optimum, enantioselectivity, specificity and binding affinity have all been altered to better adapt proteins and antibodies for specific purposes.

[0003] Since its inception, many different methods for molecular evolution have been described and applied to improve characteristics of the target protein. For example, sets of single point mutants can be generated and screened for upmutants. Beneficial single amino acid substitutions can then be recombined and screened to further optimize the desired characteristics in the target molecule.

[0004] However, the successful evolution of a target molecule starting with single point mutations requires that the (sometimes) subtle changes in performance can be accurately measured to identify the upmutants. In cases where a sensitive assay does not exist, single point mutations cannot be successfully screened. Simultaneous mutations of several sites can be done, however the number of combinations created, increases very quickly and reaches the limits of cloning efficiency and screening capability.

SUMMARY ~~OF THE INVENTION~~

[0005] The present ~~invention~~disclosure relates to methods of identifying cross-species active mutant polypeptides formed from, or based upon, a template polypeptide. The template polypeptide shows an activity on a target from a first species. The method comprises the steps of generating a library of mutant polypeptides from the template polypeptide; selecting mutant polypeptides that show the activity on an ortholog of the target from a second, different species; and screening the selected mutant polypeptides for a cross-species mutant polypeptide that also shows the activity on the target from the first species.

[0005a] In one aspect, there is provided a method of identifying a cross-species mutant polypeptide from a template polypeptide of a species, wherein the template polypeptide shows an activity on a first target from the species, the method comprising the steps of:

i. generating a library of mutant polypeptides from the template protein;

ii. selecting mutant polypeptides that show the activity on an ortholog of the first target in another species; and

iii. screening the selected mutant polypeptides for a cross-species mutant polypeptide that shows the activity on the first target.

[0005b] In another aspect, there is provided a method of identifying a cross-species mutant peptide from a template peptide from a species, wherein the template peptide shows an activity in a first system from the species, the method comprising the steps of: generating a library of mutant peptides from the template peptide; selecting mutant peptides that show the activity in an orthologous system from another species; and screening the selected mutant peptides for one or more cross-species mutant peptides that shows the activity in the first system.

[0006] The present ~~invention~~disclosure relates to comprehensive methods of identifying and mapping mutant polypeptides formed from, or based upon, a template polypeptide. Typically, the polypeptide will comprise n amino acid residues, wherein the method comprises (a) generating n

separate sets of polypeptides, each set comprising member polypeptides having X number of different predetermined amino acid residues at a single predetermined position of the polypeptide; wherein each set of polypeptides differs in the single predetermined position as confirmed by sequencing or other technique; assaying each set for at least one and preferably two predetermined properties, characteristics or activities; (b) for each member identifying any change in said property, characteristic or activity relative to the template polypeptide; and (c) creating a functional map reflecting such changes. The number of different member polypeptides generated is equivalent to n x X

[0007] In the alternative, the method comprises generating a single population comprising the sets of mutated polypeptides. In this embodiment, the entire population is sequenced, tested for expression and screened for a function, the individual members identified, and, preferably, the functional map generated.

[0008] Typically, where each naturally occurring amino acid is used, X will be 19 (representing the 20 naturally occurring amino acid residues and excluding the particular residue present in a given position of the template polypeptide). However, any subset of amino acids may be used throughout, and each set of polypeptides may be substituted with all or a subset of the total X used for the entire population.

[0009] Any mutational or synthetic means may be used to generate the set of mutants. In one embodiment, the generation of polypeptides comprises (i) subjecting a codon-containing polynucleotide encoding for the template polypeptide to polymerase-based amplification using a 63-fold degenerate oligonucleotide for each codon to be mutagenized, wherein each of the 63-fold degenerate oligonucleotides is comprised of a first homologous sequence and a degenerate N,N,N triplet sequence, so as to generate a set of progeny polynucleotides; and (ii) subjecting the set of progeny polynucleotides to clonal amplification such that polypeptides encoded by the progeny polynucleotides are cloned, sequenced, expressed and screened.

[0010] In one embodiment, the entire polypeptide is subjected to comprehensive mutagenesis. In another embodiment, one or more regions are selected for comprehensive mutagenesis. In such case, n represents a subset or region of the template polypeptide. For example, where the polypeptide is an antibody, the entire antibody or one or more complementarity determining regions (CDRs) of the antibody are subjected to comprehensive mutagenesis.

[0011] The ~~invention~~disclosure thus includes methods of mapping a set of mutant antibodies formed from a template antibody having at least one, and preferably six, complementarity determining regions (CDRs), the CDRs together comprising n amino acid residues, the method comprising (a) generating n separate sets of antibodies, each set comprising member antibodies having X number

of different predetermined amino acid residues at a single predetermined position of the CDR; wherein each set of antibodies differs in the single predetermined position; and the number of different member antibodies generated is equivalent to n x X; (b) confirming by sequencing or other method that each member antibody has been made; (c) expressing each member antibody; (d) assaying each set for at least one predetermined property, characteristic or activity; (e) for each member identifying any change in a property, characteristic or activity relative to the template polypeptide; and (f) creating a structural positional map of such changes. For antibodies, the predetermined property, characteristic or property may be binding affinity and/or immunogenicity. As set forth above, in the alternative a single population comprising all sets of mutated antibodies may be generated.

[0012] In addition, provided are methods of producing a set of mutant antibodies formed from a template antibody having at least one complementarity determining region (CDR), the CDR comprising n amino acid residues, the method comprising: (a) generating n separate sets of antibodies, each set comprising member antibodies having X number of different predetermined amino acid residues at a single predetermined position of the CDR; wherein each set of antibodies differs in the single predetermined position; and the number of different member antibodies generated is equivalent to n x X. In another embodiment, antibody comprises six CDRs, and together the CDRs comprise n amino acid residues.

[0013] One embodiment of the disclosure includes a functional positional map (EvoMap™) made by the methods described herein.

[0014] In an additional embodiment, certain residues particularly sensitive to change may be so indicated on the EvoMap™. Further optimization may be implemented by making additional mutational changes at positions outside of these sensitive positions.

[0015] In a specific embodiment, the mutations generated in the comprehensive evolution techniques of the disclosure are confirmed by sequencing, or some other method.

[0016] Methods of the present ~~invention~~disclosure are useful for generating polypeptides as drugs that are safe and effective in humans and also function in animal model systems, such as rat, mouse, rabbit, hamster, guinea pig, monkey, dog, cat, non-human primates and other non-humans, for evaluation.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] Figure 1 illustrates how comprehensive positional evolution (CPE™) is used to generate a molecule specific database (EvoMap™).

[0018] Figure 2 shows an example of a EvoMap™ and how additional optimization may be implemented by Synergy evolution.