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Affibodies

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Abstract

The introduction of recombinant DNA technology-based methodsfor the overproduction of either wild type or engineeredproteins in various host cells has had an enormous impact onthe availability of proteins for various therapeutic,diagnostic and research applications, which also has creatednew demands on efficient production and purification methods toprovide the desired material. Affinity chromatography hasproven to be an outstanding technique to obtain a single-stepincrease in purity and concentration. However, for affinitychromatography to be attractive for large-scale manufacture ofproteins, the affinity ligands have to meet industrial demandson stability and selectivity. The rather high cost and limitedstability of many affinity adsorbents available todayhaveundoubtedly contributed to the rather slow uptake in the use ofaffinity chromatography for protein manufacture, which callsfor new methodology for ligand development.

This thesis describes attempts to use combinatorial proteinengineering principles to construct large protein collectionsbased on the staphylococcal protein A (SPA) derivedα-helical three-helical-bundle Z domain, from which novelor optimized ligands can be identified using the powerfulin vitroselection technology "phage display".Combinatorial methods are attractive because they allowsystematic and rigorous screening (evaluation) of a largenumber of related compounds, in the search for target-specificmolecules.

Two combinatorial libraries of $4.5x10^7$members each were constructed, where 13 surfaceresidues on the Z domain simultaneously were randomized usingeither NN(G/T) or (C/A/G)NN degeneracy codons. Using the phagedisplay technology, binding proteins (denoted affibodies) withmicromolar affinities (K_D) were selected against a wide range of targets ofdifferent size and origin, including human insulin, humanapolipoprotein $A\text{-}1_M$,Thermus aquaticusDNA polymerase, and human coagulationfactor VIII. It was also shown that these affibodies couldconveniently be used as selective and robust ligands inauthentic affinity chromatography applications. For someinvestigated affibody ligands, a high stability against columnsanitation procedures involving repeated exposures to 0.5 MNaOH was demonstrated. Affibody ligands were shown to retaintheir binding function when further engineered into multimericconstructs or after fusion to other protein domains. Improvedligands were also obtained using affinity maturationstrategies, resulting in second generation binders having 15 to20 fold increased affinities, with $K_D$values in the range of 10-25 nM.

In conclusion, it was shown that libraries of the Z domainhave the potential to be used as general sources of affinityligands to a wide range of targets. Z domain variants withcompletely altered specificities but with affinities in parityto that between the parental wild type Z domain and its naturaltarget IgG have been selected. Affibody ligands should beinteresting alternatives

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to monoclonal antibodies in thepurification of proteins from complex backgrounds. Thepotential use of affibodies as new biotechnological tools alsoin other applications is discussed.

Key words:affibody, affinity chromatography, affinityligand, bacterial receptor, combinatorial library, phagedisplay, selection, staphylococcal protein A, Z domain

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