Directed Evolution of the Periplasmic Chaperone Spy

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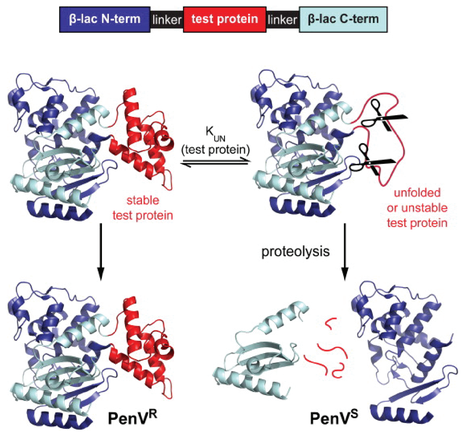
Abstract

Protein directed evolution combines genetic mutation with artificial selection. Chaperones are a large family of proteins that facilitate protein folding (refolding) or prevent protein aggregation. Chaperone's promiscuous property, interacting with a numerous clients, implies their enormous potential of enhancement. We linked the antibiotic resistance to the substrate folding with a biosensor to produce its more efficient variants.



Foit *et al., Molecular Cell biology, 2009*

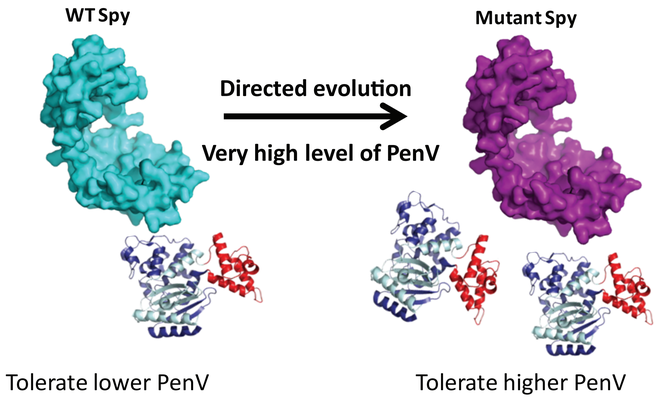
Significance

1. Explore the important interaction sites between Spy and its substrates.
2. "Super Chaperones": Super bacteria for protein production in industry, treatment for protein misfolding or aggregation diseases, etc.
3. Understand the natural evolution of Spy.

Introduction

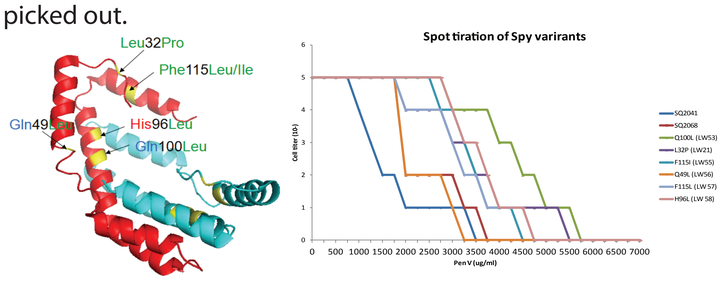
Spy (Spheroplast Protein Y) was rediscovered as a novel periplasmic chaperone (dimer) of Escherichia coli utilizing an elegant in vivo biosensor developed in our lab. This selection system linked antibiotic resistance with test protein folding by fusing the poorly folded test protein immunity protein 7 mutant (Im7-L53AI54A) into the middle of β-lactomase to form a sandwich structure. Our lab did a genome-wide random mutagenesis to screen for mutants with increased penicillin resistance which implies the stabilization of natively unfolded test protein and found Spy expression was dramatically up-regulated in nearly all the selected mutants. A series of in vitro chaperone assays finally unveiled Spy as a novel ATP-independent molecular chaperone in periplasmic envelope.

Directed evolution is to engineer proteins by actively applying iterative rounds of random mutations and selecting artificially to optimize functional proteins instead of passively accepting naturally selected mutants, which is named as "directed evolution".

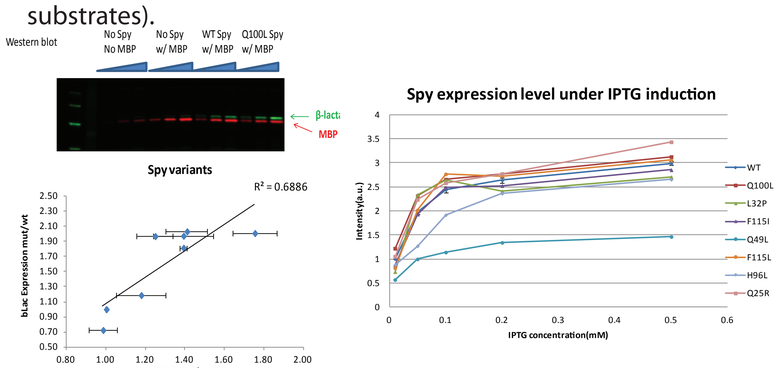


Methods and Results

A random mutagenesis was conducted on the mature sequence of Spy and mutants with enhance antibiotic resistance were picked out as promising candidates, and confirmed with spot titration experiment. Several outstanding mutated sites like Q100L(48 occurrences), F115L/I, Q25R , L32P, Q49L and H96L were picked out.



Selected mutants were remade with site directed mutagenesis. We did spot titration for MICs(minimal inhibition concentration) to Penicillin V as an index to evaluate their *in vivo* activity (folding substrates).



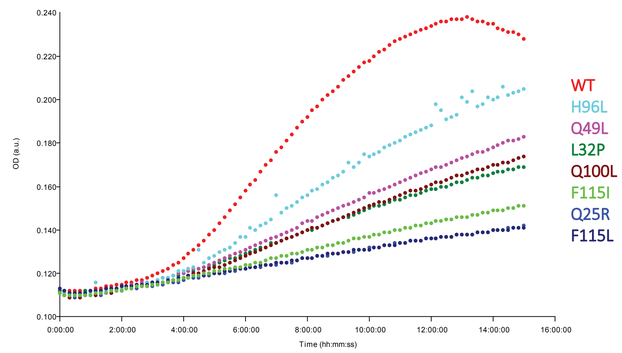
Quantitative western blot shew that there was no significant increase in the expression of the Spy mutants but higher level of beta-lactamase which implies their raised chaperone activity.

Properties of the Spy mutants

Spy variants	MIC_{norm}	Activity (MDH agg. Prev)	Activity (α-LA agg. Prev)	k_{on} ($\times 10^5$ mol^{-1} s^{-1})	k_{off} (s^{-1})	KD (μM)	Tm (°C)	ΔHm (kcal mol^{-1})	ΔCp (kcal K^{-1} mol^{-1})	ΔG_{NU}(25 °C) (kcal mol^{-1})
wt	1.00	1.00	1.00	3.98	0.46	1.15	48.1	66.6	0.64	4.24
Q25R	1.44	1.08	0.99	2.29	0.20	0.87	46.5	73.7	0.98	4.2
L32P	1.92	1.20	1.24	1.51	0.03	0.20	31.7	52.1	0.71	0.99
Q49L	1.60	0.81	0.84	2.30	0.18	0.76	51.8	59.9	0.68	4.19
H96L	1.62	1.13	1.05	2.68	0.27	1.00	50.1	56.2	0.71	3.66
Q100L	2.19	1.18	1.50	1.19	0.03	0.23	53.5	28.9	0.23	2.24
F115L	1.52	0.95	1.08	2.73	0.25	0.90	41.6	56.6	0.76	2.6
F115I	1.65	0.84	1.18	2.82	0.33	1.17	42.1	54.3	0.98	2.43

We measured the binding affinity (KD) of Im7L53AI54A with Spy using Bio-layer interferometry (BLI). The KD of variants are lower than the WT indicating that there was a tighter binding due to the much lower dissociation rates (Koff). We also made the melting curves of Spy variants and found that those mutants were more unstable or flexible than WT.

In vitro chaperone assay



We conducted a series of in vitro chaperone assay to investigate those variants' chaperone activity in preventing the aggregation of misfolding substrates (alpha-lactalbumin, MDH and aldolase). It's interesting that although these mutants were selected out as better folding of Im7L53AI54A, they still shew higher chaperone activity on other substrates.

Conclusion

We used a fused biosensor to select 7 promising Spy variants that have higher antibiotic resistance, which were found to have increased protein level of beta-lactamase. The protein level of Spy was not significantly raised implying that these enhancement was due to improved chaperone activity. In vitro assay demonstated that these variants bound the substrate tighter and inhibited the substrate aggregation more efficiently. In sum, we utilized directed evolution strategy to obtain better Spy variants.

References

Quan, S., P. Koldewey, et al. (2011). "Genetic selection designed to stabilize proteins uncovers a chaperone called Spy." Nature Structural & Molecular Biology 18(3): 262-269.
Foit, L., G. J. Morgan, et al. (2009). "Optimizing protein stability in vivo." Mol Cell 36(5): 861-871.