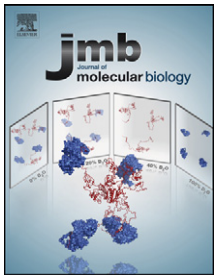

Shifting Fitness and Epistatic Landscapes Reflect Trade-offs along an Evolutionary Pathway

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Abstract

Nature repurposes proteins via evolutionary processes. Such adaptation can come at the expense of the original protein's function, which is a trade-off of adaptation. We sought to examine other potential adaptive trade-offs. We measured the effect on ampicillin resistance of ~12,500 unique single amino acid mutants of the *TEM-1*, *TEM-17*, *TEM-19*, and *TEM*-15 β-lactamase alleles, which constitute an adaptive path in the evolution of cefotaxime resistance. These protein fitness landscapes were compared and used to calculate epistatic interactions between these mutations and the two mutations in the pathway (E104K and G238S). This series of protein fitness landscapes provides a systematic, quantitative description of pairwise/tertiary intragenic epistasis involving adaptive mutations. We find that the frequency of mutations exhibiting epistasis increases along the evolutionary pathway. Adaptation moves the protein to a region in the fitness landscape characterized by decreased mutational robustness and increased ruggedness, as measured by fitness effects of mutations and epistatic interactions for TEM-1's original function. This movement to such a "fitness territory" has evolutionary consequences and is an important adaptive trade-off and cost of adaptation. Our systematic study provides detailed insight into the relationships between mutation, protein structure, protein stability, and epistasis and quantitatively depicts the different costs inherent in the evolution of new functions.

Introduction

Proteins often evolve to serve new roles. Such repurposing can come at the expense of the original function, which is one type of adaptive trade-off. For instance, different beta-lactamases (β-lactamases) provide different levels of resistance to beta-lactam (β-lactam) antibiotics [1]. Selective pressure for resistance to one class of β-lactam may decrease resistance to a second class. Because selective pressures can change over time, the selective history and evolutionary pathways of protein function may be complex [2,3]. When selective pressure is removed for an original function, alleles compromised in this function may become fixed in a population. Although such an event offers new evolutionary pathways for evolution to follow [4–6], it will close off other pathways available to the original allele. If the new "fitness territory" surrounding the adaptive allele is disadvantageous compared to that surrounding the original allele (i.e., beneficial mutations do not result in equivalent high fitness values, and other mutations tend to have more negative effect and result in epistatic effects producing rugged landscapes), this territorial disadvantage would be another type of adaptive trade-off. Quantitative and mechanistic understanding of all types of adaptive trade-offs is important for understanding evolutionary dynamics and outcomes. However, we lack an extensive, systematic study of changes in the fitness landscape surrounding a protein along an adaptive pathway and the extent and types of trade-offs that resulted. To better understand the trade-offs inherent in adaptation, we used protein fitness landscapes to extensively quantify the effects of adaptation on the prevalence of epistasis and how the fitness territory after adaptation differs from that before the accumulation of adaptive mutations.

The advent of deep sequencing has provided the ability for extensive studies of the effect of mutation on function and fitness for a single gene or protein [7,8]. Protein fitness landscapes provide a description of the effects of mutation on protein function or

the phenotype they provide. Most studies of protein fitness landscapes have focused on the effects of single mutations in a set genetic background, characterizing only the first possible evolutionary steps from a given allele. However, the coupled effects of mutations (i.e., epistasis) give rise to rugged landscapes, making the effect of multiple mutations difficult to predict from the effects of individual mutations [9–14]. Intragenic epistasis is believed to be enriched during adaptive evolution [14–17], but the evidence for this enrichment mostly comes from epistatic interactions between adaptive mutations or through homolog comparisons rather than a systematic study of epistasis throughout the protein along an adaptive pathway. The few large-scale studies of protein epistatic landscapes [18–23] were not designed to globally address epistasis in the context of adaptive mutations and have been limited to pairwise epistasis. With one exception [22], these studies have not examined mutations throughout an entire protein in a physiological setting. Other studies [24] relied on statistical inference of epistasis, which is subject to bias [25]. To best capture the relationships between adaptation, epistasis, and trade-offs, physiological fitness landscapes of full-length genes involving a series of alleles along an evolutionary pathway must be analyzed and compared. Here, using the *TEM-1* β-lactamase gene, we examine how protein fitness landscapes change with respect to the original function as adaptive mutations for a new function accumulate. We also investigate how the prevalence and types of epistasis change along an evolutionary pathway.

TEM-1 is highly optimized to provide penicillin resistance to bacteria but has nearly no ability to confer cefotaxime resistance. *TEM-17* (E104K), *TEM-19* (G238S), and *TEM*-15 (E104K/G238S) are clinically isolated alleles of *TEM-1* with the indicated mutations [26]. These mutations confer increased cefotaxime resistance and exhibit positive epistasis. E104K and G238S individually confer four- and eightfold increases in cefotaxime resistance, respectively, but when combined, they confer a 128-fold increase [27]. Improved resistance results from active site changes that synergistically increase catalytic activity on cefotaxime, but this adaptation comes at the expense of penicillinase activity and thermodynamic stability [28]. In particular, the G238S mutation causes the largest increase in cefotaximase activity, the largest decrease in penicillinase activity, and the largest decrease in stability ($\Delta\Delta G = -1.94$ kcal/mol) [28]. Whether E104K is slightly destabilizing [28] or slightly stabilizing [29] is uncertain, but there is agreement that the combination of G238S and E104K is approximately additive in terms of their effect on stability [28,29].

Since TEM-1 is highly specialized for penicillin hydrolysis, these adaptive mutations for cefotaxime resistance expose the allele to risk for loss in the capacity to provide resistance to penicillins such as ampicillin (Amp). For example, the G238S mutation reduces the minimum inhibitory concentration (MIC) for Amp by fourfold and reduces the k_{cat}/K_m for Amp hydrolysis by 25-fold [30]. Here, we quantify how the protein fitness landscape for Amp resistance changes along the evolutionary pathway from TEM-1 to TEM-15. Since either mutation can occur first in the evolutionary pathway to TEM-15 [31], we characterized the fitness landscapes along both possible evolutionary trajectories.

Fitness conferred by antibiotic-resistant alleles can be measured through growth competition experiments in the presence of the antibiotic; however, the fitness values depend greatly on the concentration of antibiotic used, and the method cannot distinguish fitness differences among alleles conferring antibiotic resistance far above or far below the level of resistance required for growth [32]. We skirt these limitations by measuring the effect of mutations on TEM-1's ability to confer Amp resistance using a synthetic-biology-based method that quantifies the protein's underlying fitness landscape and thus its intrinsic evolutionary potential with respect to its primary cellular function— to confer antibiotic resistance [33]. This method combines high-throughput, site-directed mutagenesis [34], a band-pass genetic circuit to partition alleles based on fitness [35], and deep sequencing to assign fitness values [33] (Supplementary Fig. S1). Although the resulting protein fitness landscape is the major determinant of an organismal fitness landscape for growth of the bacteria in the presence of the antibiotic [22], the two types of landscapes are not equivalent. However, unlike most previous large-scale studies of protein epistatic landscapes, our landscape is determined in a physiological setting and includes a mutation's effect on protein-specific activity, protein cellular abundance, and potentially other factors arising from the native cellular context. Although synonymous mutations can have small fitness effects in *TEM-1* [33], here we average the effect of synonymous mutations and measure protein fitnesses.

Results and Discussion

Fitness measurements

Our protein fitness measurements quantify the ability of the protein to provide the bacteria with resistance to the β-lactam antibiotic, much like a MIC does. Although our fitness measurements linearly correlate with the MIC [35], our measurements are technically not a MIC. We use a synthetic gene circuit that makes the bacteria behave like a band-pass filter for β-lactam hydrolysis activity. We designed this gene circuit such that sublethal levels of β-lactam