CHIMERIC TRANSDUCERS TO MIMIC OXYGEN SENSING IN BACTERIA

Heme-based sensors are important mediators of cellular responses to metabolic and environmental stimuli such as NO, CO and O_2. They are multidomain proteins that tend to function in homo- and hetero-multimeric states. Changes in intracellular gas concentrations are sensed by a heme moiety and result in either aerotaxis or some sort of gene regulation. Presently, there are six known types of heme sensors. The HemATs are the only heme sensors known to provoke aerotaxis, whereas the remaining heme sensors function in gene regulation. The globin-coupled sensor (GCS), HemAT-*Bs*, an aerotactic receptor from *Bacillus subtilis*, consists of an N-terminal globin domain connected to a C-terminal signaling domain. HemAT-*Bs* senses oxygen via a chromophore linked to residue Histidine 123. The goal of this project was to investigate how oxygen is sensed by the globin domain. In order to do this, we created a chimeric receptor and tested its function in *Escherichia coli*. We also mutated Histidine 123 to Alanine and observed its effects on 1) heme and oxygen binding and 2) the ability to trigger an aerotactic response in *E. coli*. We discovered that the chimeric receptor did sense oxygen while the *E.coli* cells without the expressed protein failed to show any reaction. Therefore the protein caused an aerotactic response in *E.coli* when it effectively bound to heme and oxygen. However, the mutated histidine 123 prompted a slight aerotactic response in *E.coli*. These results indicate that non-specific heme-binding may cause an aerotactic response or HemAT-*Bs* has multiple functions to trigger aerotactic responses.

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INTRODUCTION

Organisms have evolved to perform optimally within their own systems. However, if it were possible to swap systems with another organism, would the system still function or fail? I sought the answer to this question by using a chimeric protein to mimic the function of HemAT found in the bacterium *Bacillus subtilis*. HemAT-*Bs*, the heme-based aerotactic transducer from *Bacillus*, is a globin responsible for oxygen sensing. Previous studies at the University of Hawaii discovered HemAT's in *Halobacterium salinarum*, from the Kingdom Archaea and *Bacillus subtilis*, a bacteria commonly found in soil, triggered aerotactic reactions and demonstrated spectral properties comparable to those of myoglobin.[1]

MATERIALS AND METHODS

The DNA, Bs-Tsr 193 with cloning sites EcoRI and BamHI, was mutated using 2 µL of primers YHFV H123A F and YHFV H123R, 1 µL of DNA, 5 µL of 10× Pfu buffer, 3 µL of DMSO 5 µL of dNTP, and 31 µL of sterile water. PCR protocol started with a single 2 minute hot start at 94°C. After one minute, 1 µL of *Pfu* polymerase was added, followed by twelve cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 68°C for 12 minutes. After the mutagenesis PCR was completed, 1 µL of Dpn I was added to the PCR product to digest the original templates. The PCR product was purified using a Qiagen PCR purification kit. Plasmids were then transformed into competent TOP10 *E.coli* cells and incubated overnight. The Qiagen Prep Kit was used for plasmid isolation. The plasmid was transformed into both BL 21 *E.coli* cells for protein purification (BioCad Sprint) and spectrum (Varian) delta 5 *E.coli* cells for motion analysis (MotionAnalysis). Each capillary for motion analysis consisted of a delta 5 *E.coli* cells, LB, and caged oxygen. The capillaries were left

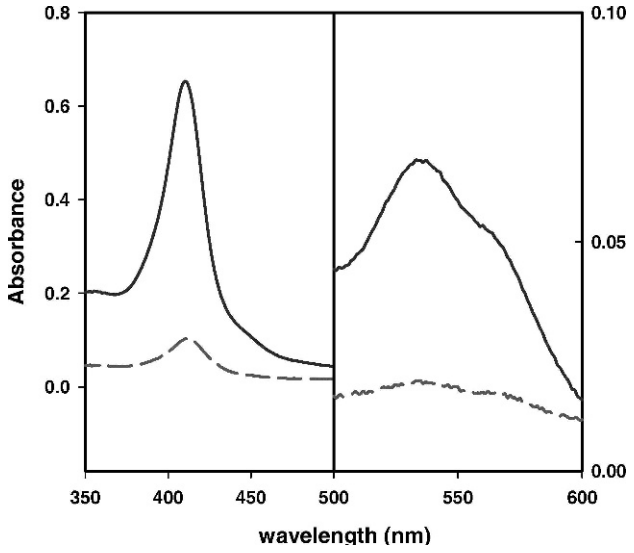

Fig 1. Spectrum of Bs-Tsr 193 (solid line) and mutant (dotted)

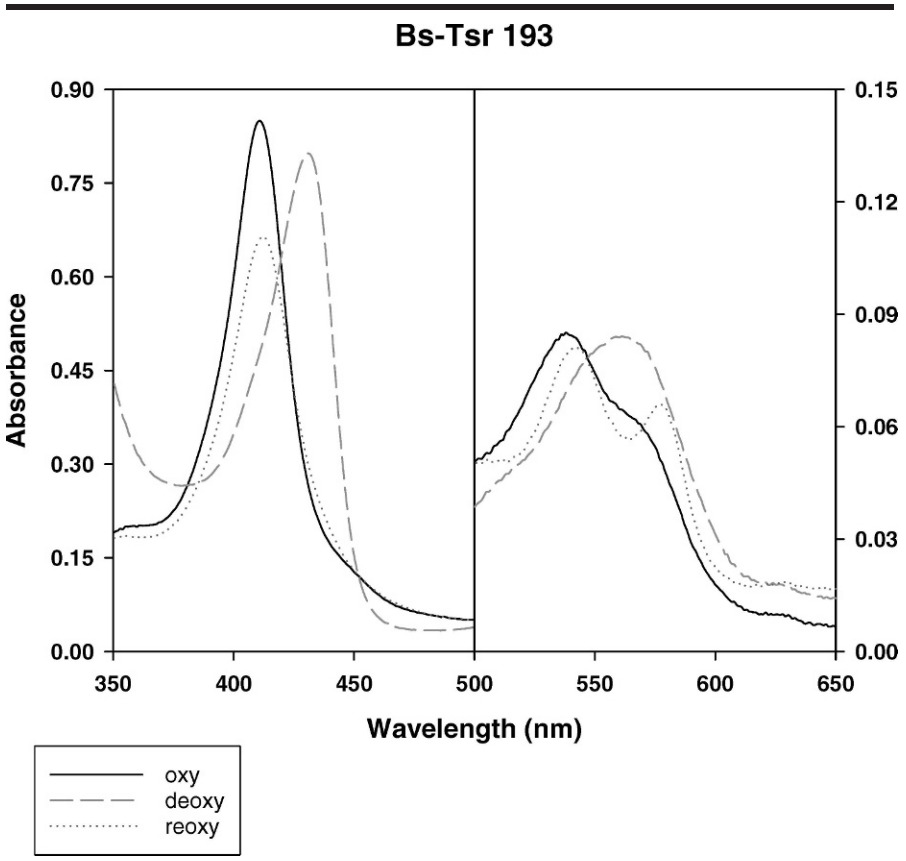

Fig 2. Spectrum of Bs-Tsr 193

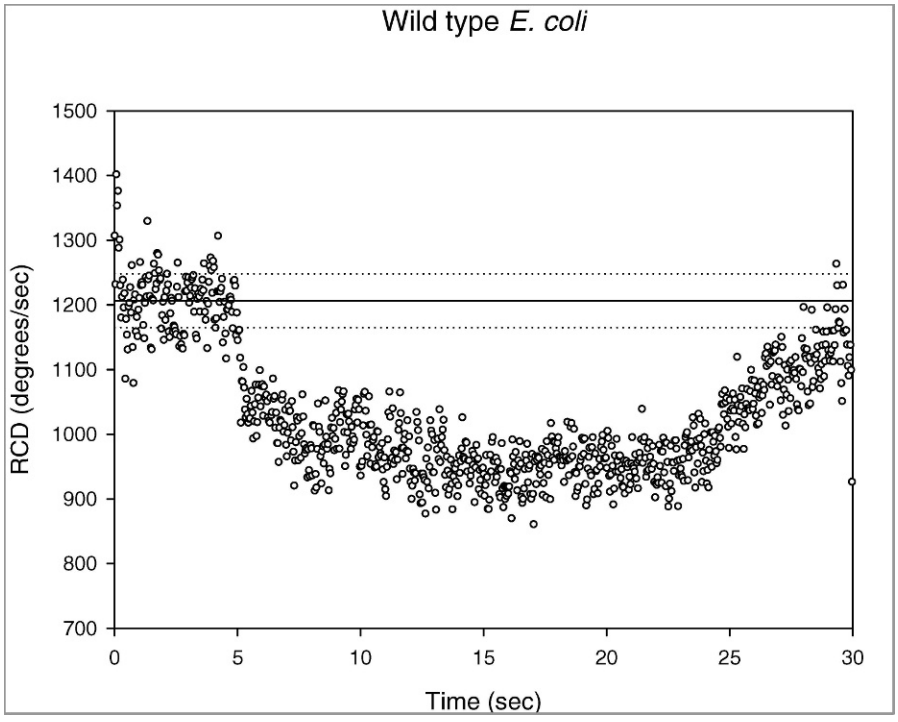

Fig 3. Wild type *E.coli* with all aerotactic transducer

for thirty minutes before motion analysis occurred to create a semi anaerobic state.

RESULTS

To see whether the protein could successfully bind heme and oxygen, spectra were measured for both the chimera and its mutant (Figure 1). The chimera indicated it was capable of heme and oxygen binding by its soret peak at 410 nm and alpha and beta peaks at 580 and 540 nm, respectively. The mutated histidine 123 also showed a slight absorbance. Bs-Tsr 193 was able to bind, release, and rebind heme (Figure 2). The four samples for motion analysis were as follows: two controls, the wild type *E.coli* (Figure 3) and delta five *E.coli* cells (Figure 4), the chimera Bs-Tsr 193 (Figure 5), and the mutated Histidine 123 (Figure 6). The two controls showed obvious responses. The wild type *E.coli* with all aerotactic transducers functional showed a positive aerotactic response while the same delta five *E.coli* cells with their aerotactic transducers knocked out showed no response. HemAT-*Bs* was successful in creating an aerotactic response in Bs-Tsr 193, but instead of a positive response, oxygen became a repellent. Unexpectedly, the mutated Bs-Tsr 193 also created an aerotactic response.

DISCUSSION

The relationship between the proximal histidine, heme and oxygen is essential, for example, if the proximal histidine is unable to bind to heme then the protein will be unable to bind oxygen. Absorbance spectrums use light to see whether a protein can bind heme and oxygen. The spectrum for the wild type chimera showed the expected soret band at 410 nm and alpha and beta bands at 580 and 540 nm while the mutated chimera showed a slight soret band at 410 nm. The soret peak at 410 nm is representative of heme and

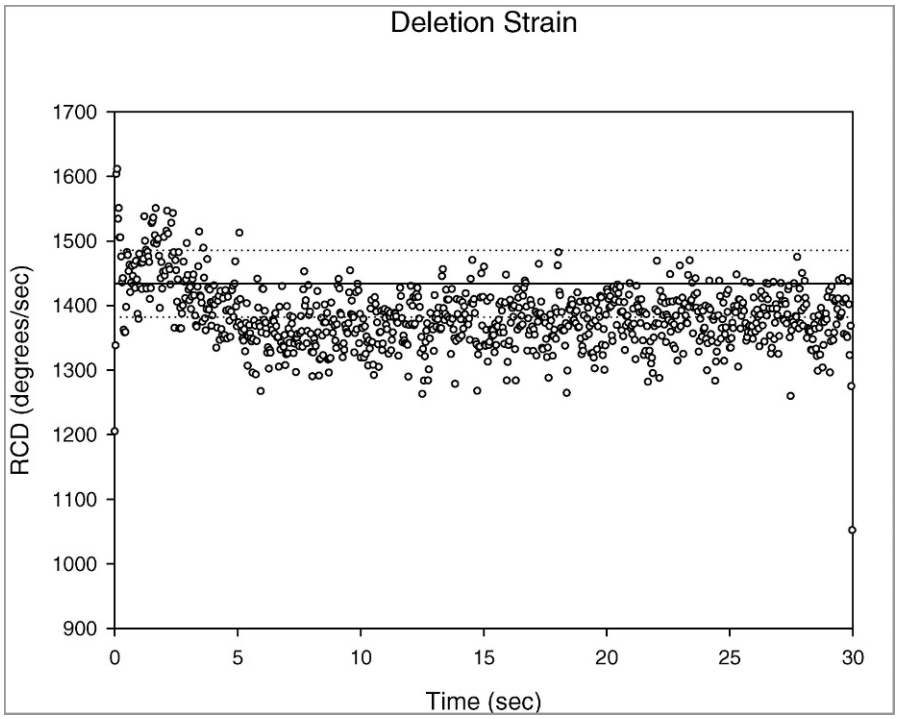

Fig 4. *E.coli* with all five aerotactic transducers deleted

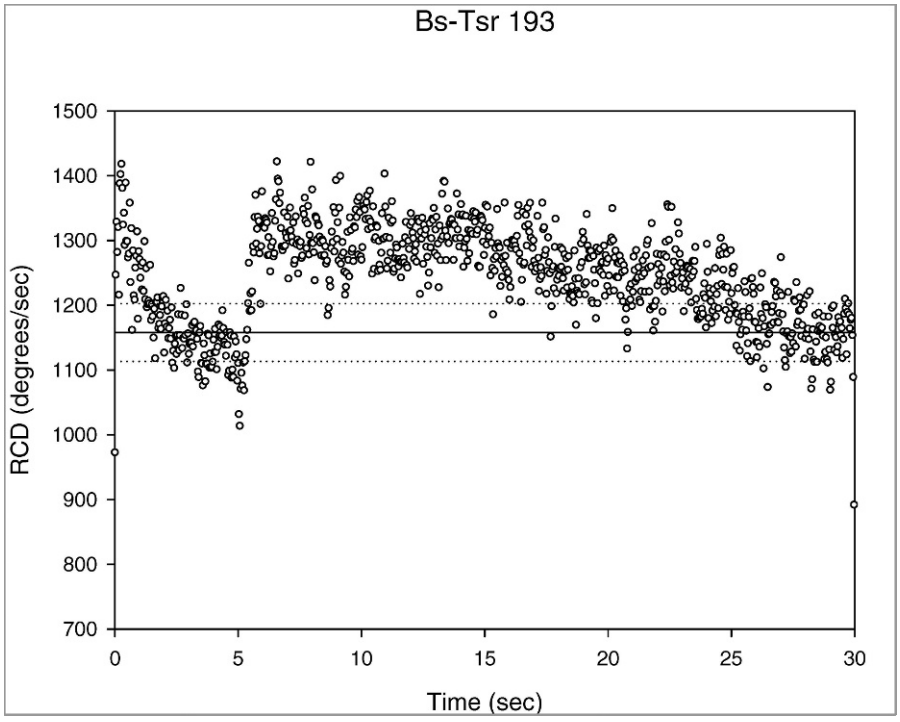

Fig 5. Non mutated Bs-Tsr 193

oxygen binding. The result for the wild type chimera was expected, to allow the protein to be to bind heme and oxygen. However, the result for the mutated chimera was unexpected. The point mutation occurred to the proximal histidine 123 codon in order to prohibit heme and oxygen binding; yet in the spectrum, there is a slight peak at 410 nm meaning the mutated protein is capable of heme and oxygen binding. An explanation for this phenomenon is non-specific heme binding supported by slight soret peak (Figure 1). The other part of my project was to see the physiological response of the chimeric protein in *E.coli* cells by conducting a motion analysis experiment. The wild type *E.coli* (Figure 3) with all its natural aerotactic transducers responded to oxygen normally, for oxygen is an attractant, and in response, the *E.coli* cells took on a smoother swimming pattern. In *B.subtilis*, oxygen also acts as an attractant. However, in the chimera the aerotactic response toward oxygen is negative. The same negative response is seen in the mutated chimera. Although the aerotactic response was unexpected, it was not implausible with the results from the spectra showing the likelihood of non-specific binding. The genetic differences between *B.subtilis* and *E.coli* may explain the negative response toward the oxygen. The motion analysis method was successful in proving that a protein from one bacterium could be hosted functionally in another cell; additional research is needed.

REFERENCES

1. Hou S, Larsen RW, Boudko D, Riley CW, Karatan E, Zimmer M, Ordal GW, Alam M. Myoglobin-like aerotaxis transducers in *Archaea* and *bacteria*. *Nature*. 2000;403(6769):540–544.

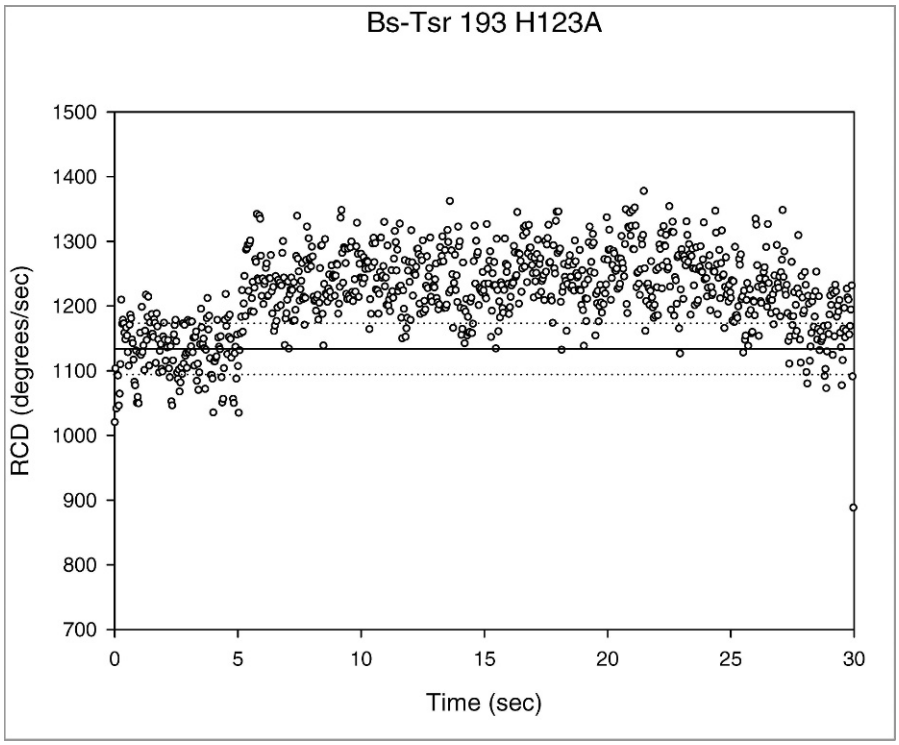

Fig 6. Mutated Bs-Tsr 193