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Rex-Family Repressor/NADH Complex

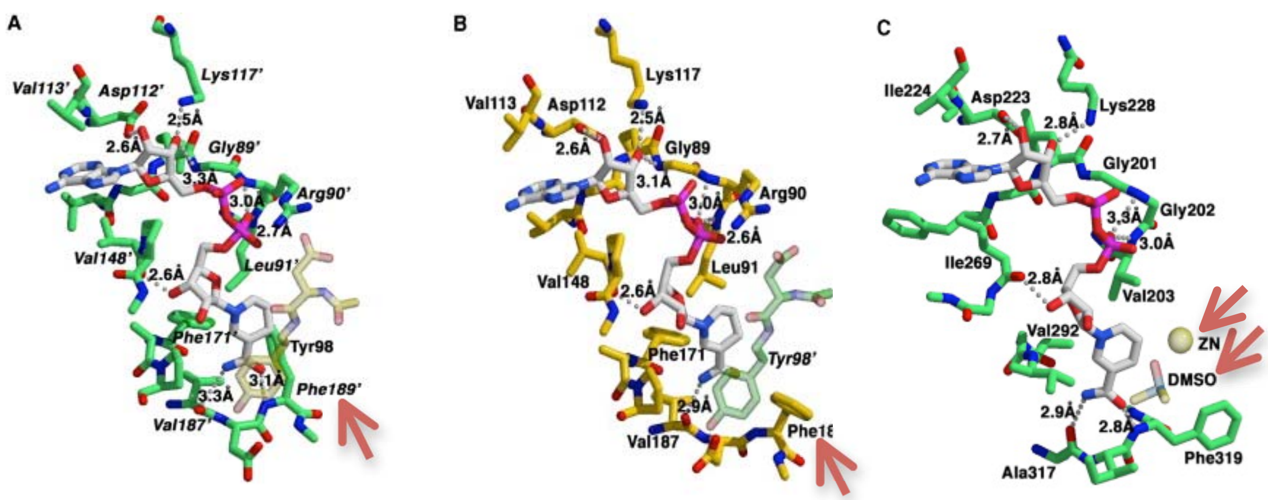
Part A

The biological sensing protein that we selected is the Rex-family repressor/NADH complex. We chose this sensor because it is a calcium ion sensing protein, which has some very obvious, and also very important links, to the human biological system. Gaining an understanding of this protein will only aid in gaining a further appreciation for how the human body works. This specific sensor deals with the respiratory system,[1] which has always been a system of personal interest. How can something so seemingly basic, such as breathing be so complex? Can this sensor help us understand our respiratory system better, and therefore enable us to develop ways to enhance our respiratory system, allowing us to perform better at things from simple, everyday tasks like walking across campus, to excelling under high-stress, intensely aerobic conditions? Recently, a new redox-sensing repressor was discovered in *Streptomyces coelicolor*, also known as Rex. Rex regulates components of the respiratory system by repressing the transcription of certain respiratory chain components in response to intracellular NADH/NAD^+ ratios.[2] Rex is one member of a "conserved bacterial family" that is widespread among the human pathogens anthrax, pneumonia, and lockjaw. While both NADH and NAD^+ can bind to Rex, only NADH inhibits DNA binding, so when oxygen levels are limiting, the lowered respiratory rate is believed to increase NADH levels. This increase in NADH allows NADH to replace NAD^+ that may be bound to Rex, therefore further inhibiting DNA binding at these sites. This reduced binding affinity removes the Rex repressor from the DNA target site

(ROP), inducing genes to ensure more efficient oxygen use by the cells. Excess NADH is then recycled.[1]

Part B

The redox-sensing repressor Rex regulates transcription of respiratory genes in response to the intra cellular NADH/NAD^+ redox poise. The X-ray structure of *Thermus aquaticus* Rex (T-Rex) bound to the effector NADH has been determined at 2.9 Å resolution. There are actually two active sites for this biological sensing protein. The first is under the NADH binding domain and the winged helix (WH) domain. Each NADH molecule is bound in an extended conformation with the distinguishing feature that the nicotinamide binding site is located at the T-Rex dimer interface rather than the traditional position between NAD(H) and substrate binding domains of enzyme active sites. Although most of the NADH contacts are similar within distinct subunits, a preferential hydrogen bond is formed between the carboxamide oxygen and the backbone of the Phe189′ side chain (Figure 1: A).[1]



A: Phe189 B: Phe189 folded back C: DMSO/Zn interactions

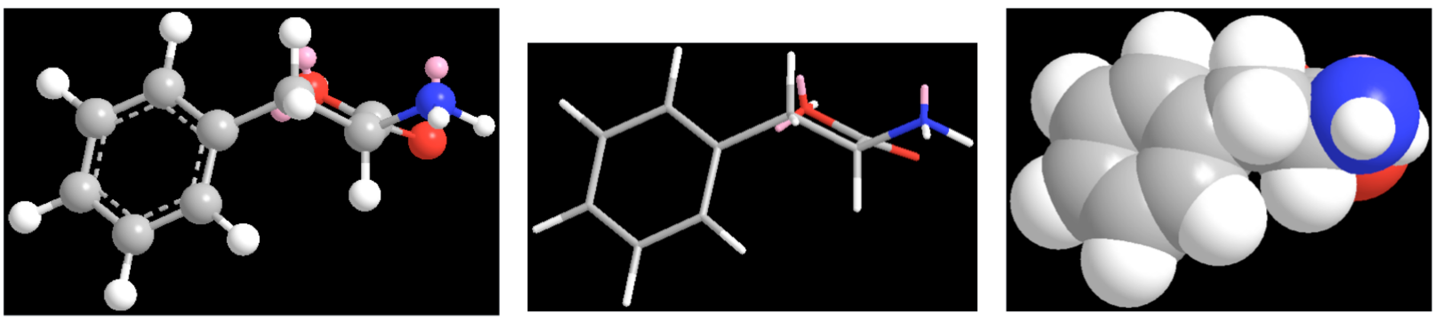
Figure 1. [1]

The DNA binding properties of T-Rex almost certainly stem from the presence of the N-terminal domain, because fusion with an N-terminal peptide inhibits DNA binding. The wing appears disordered in the three subunits in which Phe189 is folded back into the protein interior, but is readily visible in the three subunits in which *Phe189′* is inserted between the NADH molecules at the dimer interface (Figure 1: B).[1]

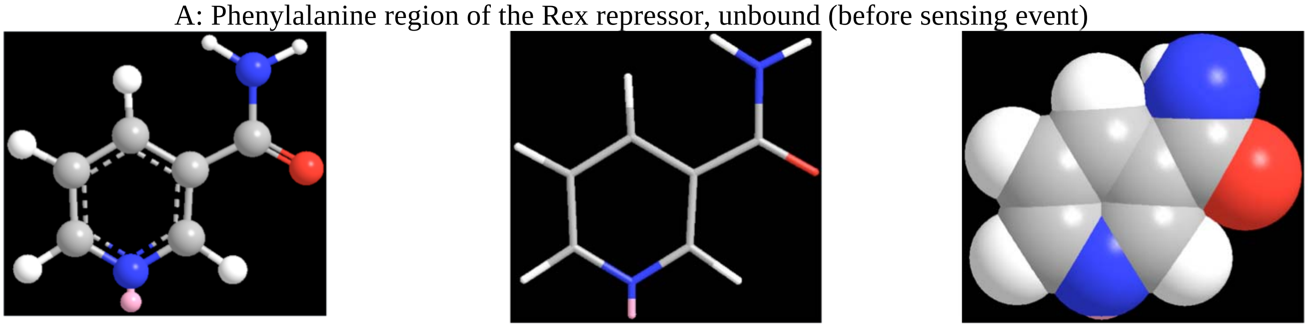
Although conformational changes are difficult to predict, comparison of the T-Rex structure with the classic substrate-induced domain closure of liver alcohol dehydrogenase (LADH) may indicate which residues play critical roles in NAD(H)-regulated DNA binding. When the NAD(H) binding domains of T-Rex and LADH are superimposed (1.9 Å), the LADH active site is located at the T-Rex dimerization interface, where the LADH substrate binding domain overlaps one of the T-Rex subunits. During substrate reduction by LADH, kinetic and structural analyses indicate that a bound Zn^{+2} ion plus histidine and serine side chains stabilize and facilitate protonation of an anionic intermediate (Figure 1: C).[1]

Part C

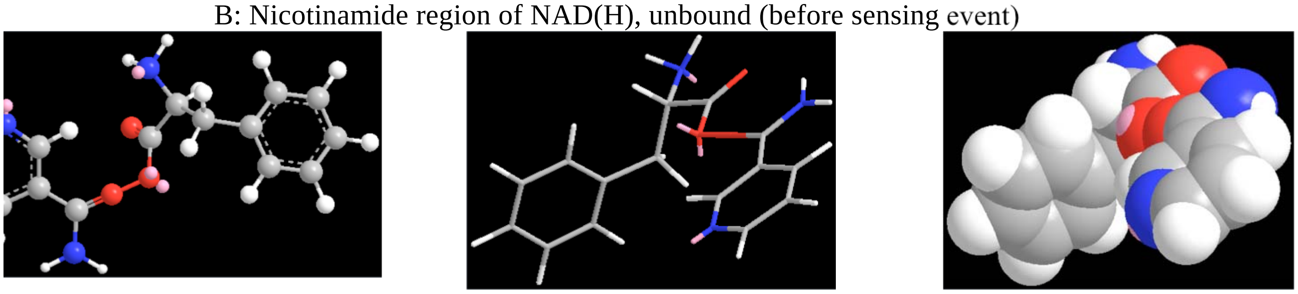
The sensing event for the Rex-family repressor/NADH takes place in the Rossmann fold, where NADH and/or NAD^+ will bind to Phe189` (phenylalanine). The T-Rex NAD(H) dinucleotide binding site can be divided into two distinct sets of interactions, one with the adenosine moiety at the loop regions of the C-terminal domain, and the other with the nicotinamide portion of the effector molecule bound to the opposing subunit[1]. We will focus on the interactions of the nicotinamide region of the $NADH/NAD^+$, with the phenylalanine region of the Rex repressor. Before the sensing event, the phenylalanine region of the Rex repressor is unbound and resembles a simple phenylalanine amino acid, and the $NADH/NAD^+$ present is also unbound (Figure 2: A and B). During the sensing event, Phe189` is inserted between the NADH molecules at the dimer interface, and a hydrogen bond between the carboxamide oxygen on the NAD(H) and the backbone of the Phe189` side chain, is formed, with an average length of 3.1 Å. This molecule of phenylalanine bound to NAD(H) is what will be seen after the sensing event (Figure 2: C). If it is NADH that binds at the Phe189` active site, DNA binding at the Winged Helix domain is inhibited, causing cells to use oxygen in a more efficient way. But if NAD^+ binds instead, DNA binding at the Winged Helix domain is not inhibited and oxygen use continues at a normal rate. It is the job of the Rex repressor to sense the $NADH/NAD^+$ ratio present in a specific cell and therefore mediate which enzyme will bind, ultimately controlling the respiration/oxygen intake of the cell.



A: Phenylalanine region of the Rex repressor, unbound (before sensing event)



B: Nicotinamide region of NAD(H), unbound (before sensing event)



C: Phenylalanine bound to nicotinamide (after sensing event), with a hydrogen bond between the carboxamide oxgygen of the nicotinamide and the backbone of the Phe, having an average bond length of 3.1 Å.

Figure 2.

References

[1] Sickmier EA, Brekasis D, Paranawithana S, Bonanno JB, Paget MS, Burley SK, Kielkopf CL.Sickmier. X-Ray Structure of a Rex-Family Repressor/NADH Complex Insights into the Mechanism of Redox Sensing. Protein Data Bank. **2005.** *13*, Issue 1. 43-54. http://www.ncbi.nlm.nih.gov/pubmed/15642260. Accessed on 3/18/2011.

[2] Breakasis and Paget. A novel sensor of NADH/NAD+ redox poise in *Streptomyces coelicolor*. EMBO J. **2003.** *22*. 4856–4865. http://www.nature.com/emboj/journal/v22/n18/full/7595364a.html. Accessed 3/18/2011.