Purification of HcpR in Porphyromonas gingivalis

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Abstract

Porphyromonas gingivalis is a gram-negative anaerobic oral bacterium that plays a critical role in periodontitis pathogenesis. The oral cavity salivary glands convert nitrites and nitrates from nitrite-rich foods into nitric oxide (NO), which helps control oral bacteria growth. However, P. gingivalis possesses a regulator called hcpR that enables it to resist the high levels of NO by managing the nitrosative stress response, virulence, and survival of the bacteria. HcpR controls the transcription of the hybrid cluster protein (hcp) and detects stimuli through its cofactor, hemin. This study aims to express and purify sufficient hcpR protein for crystallization to reveal its full crystal structure, gain insights into its sensing mechanism in adapting to nitrosative stress, and define the structure of hcpR's heme DNA complex for potential therapeutic strategies.

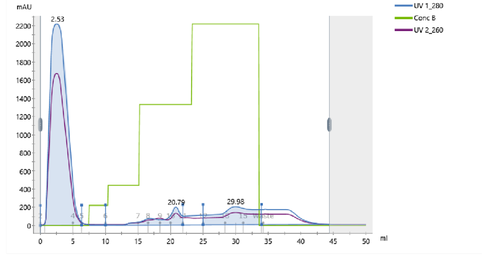
Background

- *Porphyromonas gingivalis* is mainly involved in periodontitis pathogenesis, an inflammatory disease that targets tissues supporing teeth, leading to tooth loss.
- *P. gingivalis* is also implicated in rheumatoid arthritis and amniotic fluid of women, leading to premature labor.
- *P. gingivalis* employs different mechanisms to circumvent nitrosative stress (excessive amounts of nitric oxide in our body), such as expressing hcpR, it's transcription factor
- HcpR is in the FNR-CRP family of regulators which utilize it's cofactors to sense stimuli and are responsible for the transcription of genes like hcp

Experiment

To accomplish this we cloned and expressed the hcpR gene into *E. coli* BL21 (DE3) cells overnight with antibiotics. The cells were induced using 1 mM IPTG. After disrupting the cells to release the protein and subsequent centrifugation, the supernatant was purified using a Ni-NTA column. Protein verification was performed using SDS-PAGE, and fractions with adequate protein were combined and further purified with a Superdex-75 column. The fractions expressing a 25kDa protein were pooled and treated with TEV Protease to remove the 6x His tag from hcpR. The sample was then passed through a Ni-NTA column to eliminate any uncut protein and the TEV Protease. SDS-PAGE was used to verify the purity of the protein.

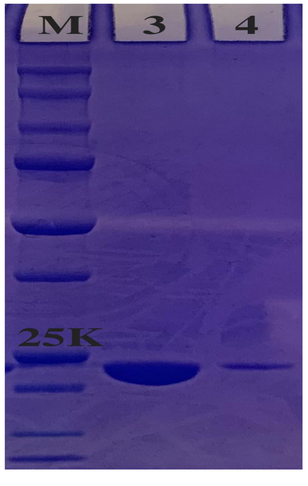
Results



Peak Table

Peak	Retention (ml)	Area (ml*mAU)	Area %	Ext coeff. (mg (3 ml cm !)-1	Fraction(s)	Volume (ml)	Conductivity (mS/cm)
Peak A	2.528	6862	77.96		2 - 4	6.338	19.40
Peak B	20.790	607.9	6.91		6 - 10	11.882	19.15
Peak C	29.977	1332	15.13		12 - 15	8.961	30.67

Elution Profile of second Ni-NTA column



M - Marker
3 - Fraction 3 (17 hours after TEV cut)
4 - Fraction 4 (17 hours after TEV cut)

Discussion/Future Direction

Overall, the goal of this experiment was to express and purify the hcpR protein in a pure form. Based on the results from the SDS-Page Gel, after the TEV Protease digestion, to remove the 6x His tag, and the second Ni-NTA column, fractions 3 and 4 expressed enough pure protein. To verify if a fraction has expressed enough pure protein, a solid band must be present at the 25kDa marker on the gel, given that is the molecular weight of hcpR. As shown on the gel under Results, both fractions 3 and 4 expressed a prominent line at 25kDa. Thus, we have expressed enough pure hcpR protein to later use in crystallization experiments. Once crystallization experiments are performed, this research could lead to a better understanding of how P. gingivalis resists NO and pave the way for potential therapeutic targets to combat periodontitis.

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