Toward the Structure of the C-terminal Domain of EcoR124I Restriction Enzyme

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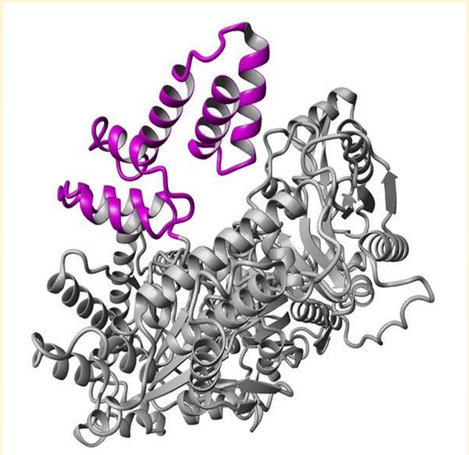
Background

The type I restriction-modification (R-M) system of plasmid EcoR124I is involved in distinguishing cellular and foreign DNA. Cellular DNA is protected by methylation within a specific recognition sequence, whereas foreign DNA, which lacks methylation, promotes DNA translocation through the stationary R-M enzyme followed by cleavage at distant nonspecific sites. The pentameric enzyme consists of three types of subunits: HsdS (specificity), HsdM (modification) and HsdR (translocation and restriction).



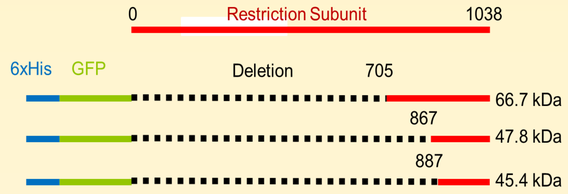
Proposed fifth C-terminal domain from the crystal structure of a mutant HsdR

Objectives

The published structure of the HsdR subunit of EcoR124I1 contains four functionally-integrated domains, but the last 150 C-terminal residues are unresolved. To facilitate its expression and crystallization, the C-terminal part of HsdR was appended after GFP and a hexahistidine tag.

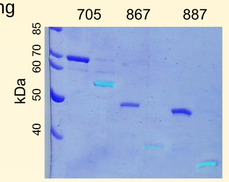
Methods

Plasmid Construction



Protein Verification

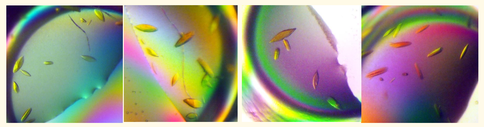
SDS-PAGE
DNA Sequencing



Protein Purification

Histag-affinity and anion exchange chromatography

Crystallization



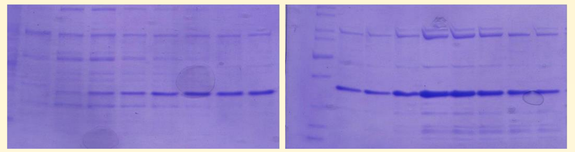
20% PEG 3350	20% PEG 3350	16% PEG 3350	16% PEG 3350
• 0.2M KH_2PO_4	• 0.2M KH_2PO_4	• 0.2M KH_2PO_4	• 0.2M KI

X-Ray Diffraction

Goal: less than 2.8 Angstroms

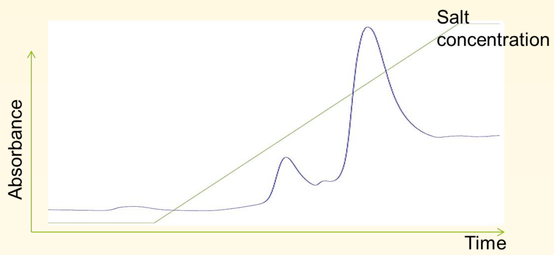
Results

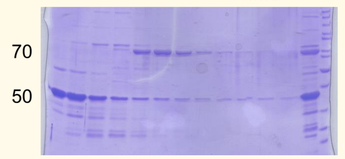
Purification by Affinity Chromatography



Samples were purified by a Ni^{+2}-NTA affinity column. The fusion protein was eluted from the column at 60-80 mM imidazole. Fluorescent, clean fractions were combined for anion exchange chromatography.

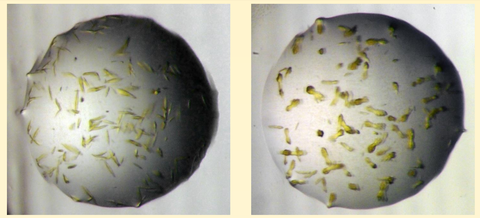
Purification by Anion Exchange Chromatography

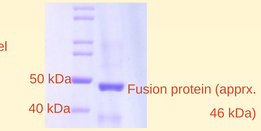




Samples were further purified by a DEAE-sepharose column. The fusion protein was eluted by a Tris buffer with a salt gradient (~300 mM NaCl).

Results





A 96-well crystallization robot was used to screen each protein for optimal conditions. Protein samples after both purification techniques were concentrated to 12 mg/mL and screened with Morpheus and PEG/Ion screens with sitting drop method. Initial diffraction trials yielded 8 Å resolution, but was optimized to yield 2.24 Å.

Conclusion

E. coli has been used to express the C-terminal domain of EcoR124I's restriction subunit. Of the three constructs probed, the construct containing histag, pHluorine, and the restriction subunit (AA 887-1038) is readily crystallizable.

Future Directions

Optimization of purification and further screening of crystallization conditions is required to elucidate the secondary structure of the restriction subunit.

Acknowledgments

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Lapkouski M., Panjikar S., Janscak P., Kuta Smatanova I., Carey J., Ettrich R., Csefalvay E. (2009) Structure of the motor subunit of type I restriction-modification complex EcoR124I. Nat Struct Mol Biol. 16, 94-95.