Structural Changes of AdhE in C. thermocellum Improve Bacterial Ethanol Production by Containing Toxic Intermediates

Background

- *Clostridium thermocellum* is a key candidate for consolidated bioprocessing but does not yet produce ethanol at economical titers for industrial use. Efforts to enhance ethanol tolerance and yield in *C. thermocellum* often target the AdhE enzyme for mutagenesis. AdhE is a two-domain protein that first catalyzes acetyl-CoA to acetaldehyde in the aldehyde dehydrogenase (ALDH) domain, and then catalyze acetaldehyde to ethanol in the alcohol dehydrogenase (ADH) domain while forming large, spring-like ultrastructures called spirosomes.

Approach

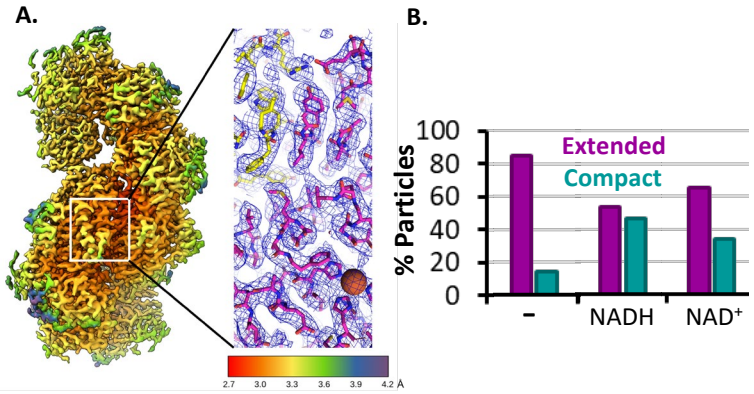
- Low-resolution staining TEM examined large changes in ultrastructure in response to reactants.
- High-resolution cryo-EM determined the structure of AdhE to begin assessing how mutants affect activity.
- Molecular dynamics simulations predicted how AdhE sequesters toxic aldehyde intermediates.

Results

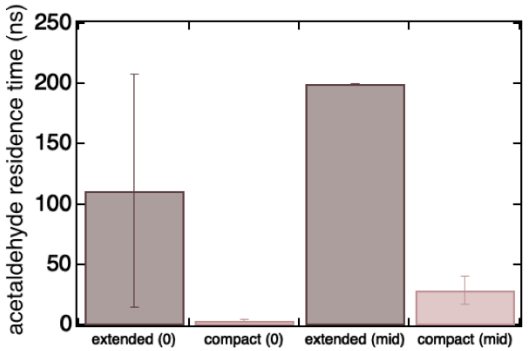
- AdhE spirosomes react to the presence of NAD^+ and NADH, contracting under NADH conditions and extending under NAD^+ conditions.
- Molecular dynamics simulations revealed a closed channel connecting the ALDH and ADH domains in the extended spirosome conformation, which allows the enzyme to transport toxic aldehydes between active sites without releasing them to the cytosol.

Significance

- This study presents the highest-resolution structure of an AdhE spirosome to date and provides valuable insights into how mutations might influence its catalytic activity and explain cofactor effects. It also offers the first evidence that compact spirosomes can form in Gram-positive organisms like *C. thermocellum*, opening up new avenues for bioengineering enzymes to improve ethanol production in bacterial systems.



A. Cryo-EM density of AdhE spirosome (left) colored by local resolution, with a zoom panel (right) showing the modeled amino acids of the interaction interface fit in the map.
B. Percentage of spirosomes in either extended or compact conformation in the presence of either NADH or NAD^+.



Measurement of the time a modeled aldehyde occupies the channel of the extended and compact spirosome starting at the ALDH active site (left two columns) and in the middle of the channel (right two columns).

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