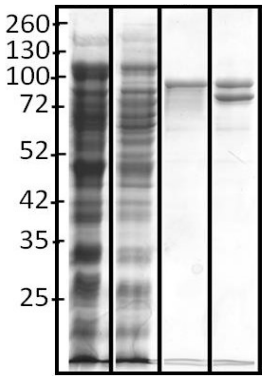
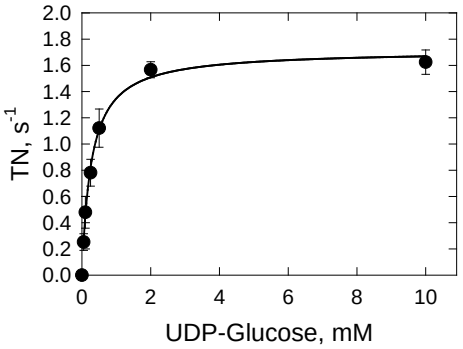
Chemical and Biochemical Characterization of Cellulose Synthesis

Purification of the AcsA-AcsB heterodimer from *G. hansenii*



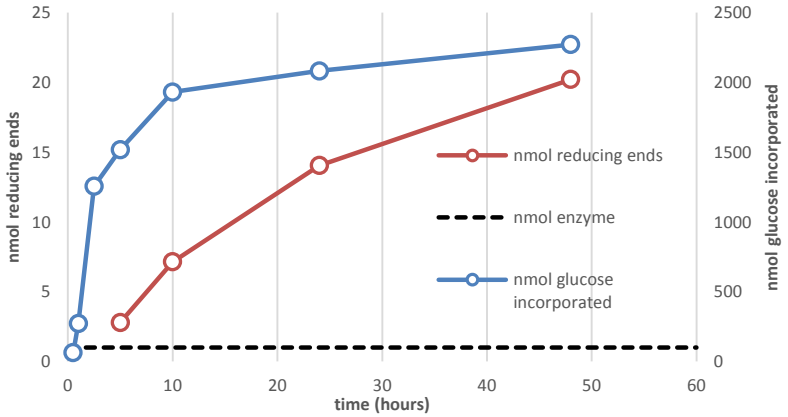
(L to R) Crude membrane, solubilized membrane, purified AcsA, purified AcsAB

Steady state kinetics of AcsA-AcsB



Enzyme	kcat	kcat/Km
G. hansenii	1.7 s^{-1}	1,600 M^{-1} s^{-1}
R. spaheroides	0.56 s^{-1}	1,120 M^{-1} s^{-1}

Cellulose synthase produces new reducing ends over time



Scientific Achievement

As a model for plant cellulose synthase, the CLSF has studied bacterial cellulose synthesis with *Rhodobacter sphaeroides* and with *Gluconacetobacter hansenii.* In contrast to *R. spaheroides*, the *G. hansenii* system synthesizes crystalline cellulose in a linear complex, mimicking the cellulose synthesis complex (CSC) in plants. With the purified enzymes from *G. hansenii*, we determined how the enzymes are post-translationally processed, forming the active heterodimer AcsA-AcsB. We also characterized the enzyme mechanism chemically and kinetically. Our chemical analysis of the product indicates cellulose is made without a primer.

Significance and Impact

With the purified cellulose synthase from *G. hansenii*, we determined the second order rate constant between the enzyme and its substrate to be 1.6 x 10^3 $M^{-1}s^{-1}$ and the kcat to be 1.7 s^{-1}. For the enzyme from *R. sphaeroides*, a similar kcat of 0.56 s^{-1} was observed. This compares with the estimated kcat of the plant enzyme of 5-16 s^{-1} based on the velocity of the CSC (Paredez et al. 2006).

The estimated degree of polymerization is consistent with new synthesis of cellulose chains during catalysis. The number of reducing ends produced by the purified enzyme is up to 20 greater than the number of active sites in the synthesis reaction mixture. This suggests that new chains of cellulose are being initiated during the time course and thus no special priming mechanism in required.

Research Details

- Both bacterial enzymes were affinity purified and quantified by molar extinction coefficient, permitting determination of molecular rate constants.
- The post translational modification of *Gluconacetobacter* enzyme was determined after purification.
- Steady-state kinetic parameters were determined by measurement of cellulose synthesized. Reducing ends were measured by bicinchoninic acid assay. Total cellulose was measured by phenol sulfuric acid.

McManus J, Deng Y, Nagachar N, Kao T, Tien M (2015) AcsA-AcsB: The Core of the Cellulose Synthase Complex from Gluconacetobacter hansenii ATCC23769. Enzyme and Microbial Technology 82: 58-65. DOI: 10.1016/j.enzmictec.2015.08.015

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