REGULATION OF CELLULOLYTIC ENZYMES AND METABOLIC PATHWAYS IN *CLOSTRIDIUM THERMOCELLUM*

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Introduction. *Clostridium thermocellum*, an anaerobic, thermophilic, ethanogenic, and cellulolytic anaerobe, produces a complex extracellular cellulolytic organelle called the cellulosome, which contains various depolymerizing enzymes that effectively degrade complex cellulosic substrates (1, 2). During cellulose fermentation, the bacterium evolves hydrogen at a high rate, in addition to producing ethanol. Analysis of its genome sequence reveals the existence of at least four putative hydrogenase genes central to hydrogen metabolism. Furthermore, at least 20 genes are potentially related to hydrogen metabolism. The bacterium is thus remarkably versatile in employing various enzymes for hydrogen metabolism, indicating the significance of this biological process. Yet little is known concerning the pathway for hydrogen production and the regulatory mechanism and network that control these hydrogen genes as well as cellulolytic process and other metabolic pathways.
The objective of this study is to understand the H_2 metabolic pathway in this cellulose-degrading bacterium and its regulatory network at the molecular and systems levels. We hypothesize that hydrogen metabolism is coupled to cellulolysis and fermentative pathways in this bacterium.

Methods. We used one of the *hyd* promoter regions to fish out potential DNA-binding proteins or transcription factors using affinity chromatography. The bound proteins were identified by LC/MS/MS. The DNA-binding activity of the protein was confirmed by using EMSA (electrophoretic mobility shift assay). An *in vitro* transcription assay was used to determine the effect of the recombinant transcription factor on gene expression. In this assay, the DNA template contained the promoter region and part of the *hyd* structure gene and crude cell lysate was used as the source of RNA polymerase. The resulting mRNA was reverse-transcribed and quantified by qPCR using gene-specific primers.

Results. A protein homologous to Rex was found to bind to the *hyd* promoter region as determined by EMSA. The binding site was a 16-bp palindromic sequence interrupted by 4 bp in the center of the sequence. The DNA-binding activity was inhibited by NADH, but not NAD^+ (Fig.1). In the *in vitro* transcription assay, the recombinant Rex-like protein repressed *hyd* gene in a dose dependent manner (Fig. 2), indicating that Rex is a repressor. The repression was reversed by NADH, but not NAD^+.

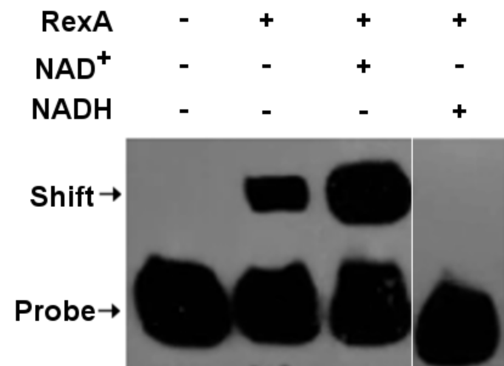

Fig.1 Effect of NAD^+/NADH on Rex binding to *hyd* promoter region as revealed by EMSA.

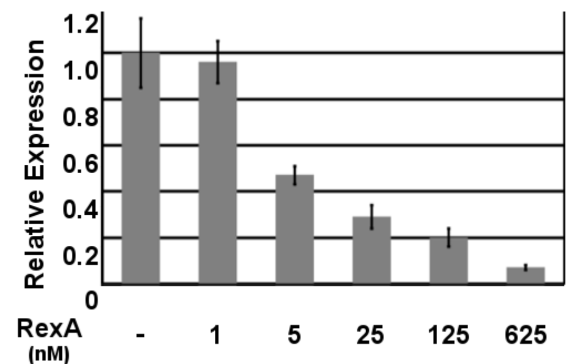

Fig.2 RexA-mediated repression of *hyd* expression in the *in vitro* transcription assay

Conclusions. Binding of the Rex-like protein to the *hyd* promoter region is NADH-dependent. The Rex binding represses *hyd* gene. Such repression is reversed when NADH level is high. Thus hydrogen production is a mechanism by which the cell balances its NAD^+/NADH ratio.

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