Hydrogenase of *Clostridium acetobutylicum* ATCC 824

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ABSTRACT

C. acetobutylicum is an anaerobic bacterium that produces acetic and butyric acids, hydrogen gas, and carbon dioxide during the exponential phase of growth. When the culture pH is allowed to remain near 4.5, the metabolism switches to the production of the neutral compounds (solvents) - acetone, n-butanol, and ethanol. The two metabolic phases are known as the acidogenic and solventogenic phases. The enzyme hydrogenase plays an important role in this bacterium because it converts excess reducing power into hydrogen gas to maintain a balance in the oxidation-reduction state in the cell. During solventogenesis, additional reducing power is used in the production of n-butanol and ethanol, which leaves excess reducing power to be vented as hydrogen gas. There are conflicting reports about the level of hydrogenase in acidogenic and solventogenic cells. There is also evidence that hydrogenase may consume too much reducing power during solventogenensis that it actually decreases the cell's capacity to produce solvents. The purpose of this study was to examine the level of hydrogenase in acidogenic and solventogenic cells and to search for clues that may indicate the presence of multiple forms of hydrogenase in *C. acetobutylicum*. Both the hydrogen-oxidation (uptake) and the hydrogen-production (evolution) activities were measured in this study. The level of hydrogenase was found higher in acidogenic cells than in solventogenic cells, but there was no difference in the molecular weight of hydrogenase from these two types of cells. A significant increase in the ratio of the hydrogen-uptake over the hydrogen-evolution activity was observed in oxygen or heat-treated cell extracts and in hydrogenase partially purified on a DEAE-cellulose column. The results suggest the presence of more than one type of hydrogenase in this species or hydrogenase activities in the two directions may be differentially altered. These possibilities will be investigated in a future study.

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