Hydrogenase of *Clostridium acetobutylicum* ATCC 824

by

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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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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>i</td>
</tr>
<tr>
<td>ACKNOWLEDGMENT</td>
<td>ii</td>
</tr>
<tr>
<td>TABLE OF CONTENTS</td>
<td>iv</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>vi</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>vi</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>LITERATURE REVIEW</td>
<td>3</td>
</tr>
<tr>
<td>Historical background</td>
<td>3</td>
</tr>
<tr>
<td>Structural and functional properties of hydrogenases</td>
<td>3</td>
</tr>
<tr>
<td>The physiological roles of hydrogenase</td>
<td>5</td>
</tr>
<tr>
<td>The importance of solvent production and the role of hydrogenase in solvent-producing clostridia</td>
<td>6</td>
</tr>
<tr>
<td>Hydrogen production and hydrogenase level in acidogenic and solventogenic cells</td>
<td>9</td>
</tr>
<tr>
<td>Modulation of electron flow in solvent-producing clostridia</td>
<td>11</td>
</tr>
<tr>
<td>Molecular characterization of hydrogenase genes of clostridia</td>
<td>11</td>
</tr>
<tr>
<td>Purpose of study</td>
<td>12</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>14</td>
</tr>
<tr>
<td>Materials</td>
<td>14</td>
</tr>
<tr>
<td>Cultures of ( C. acetobutylicum ) ATCC 824</td>
<td>14</td>
</tr>
<tr>
<td>Preparation of cell-extracts</td>
<td>14</td>
</tr>
<tr>
<td>Column chromatography</td>
<td>15</td>
</tr>
<tr>
<td>Protein determination</td>
<td>15</td>
</tr>
<tr>
<td>Assays for hydrogenase</td>
<td>15</td>
</tr>
<tr>
<td>Assay for acetoacetate decarboxylase</td>
<td>16</td>
</tr>
<tr>
<td>Assay for phosphoglucoisomerase</td>
<td>16</td>
</tr>
<tr>
<td>Polyacrylamide gel electrophoresis and activity staining</td>
<td>16</td>
</tr>
<tr>
<td>Determination of molecular weights</td>
<td>16</td>
</tr>
<tr>
<td>Determination of oxygen sensitivity</td>
<td>16</td>
</tr>
<tr>
<td>Determination of thermostability</td>
<td>17</td>
</tr>
<tr>
<td>RESULTS AND DISCUSSION</td>
<td>18</td>
</tr>
<tr>
<td>Preparation of solventogenic and acidogenic cell of ( C. acetobutylicum ) ATCC 824</td>
<td>18</td>
</tr>
<tr>
<td>Activities of hydrogenase in extracts of solventogenic and acidogenic cells</td>
<td>18</td>
</tr>
<tr>
<td>Molecular weight of hydrogenase</td>
<td>20</td>
</tr>
<tr>
<td>Oxygen sensitivity of hydrogenase in solventogenic and acidogenic cell-extracts</td>
<td>21</td>
</tr>
<tr>
<td>Thermostability of hydrogenase in solventogenic and acidogenic cell-extracts</td>
<td>22</td>
</tr>
<tr>
<td>Isolation of hydrogenase by ion-exchange chromatography</td>
<td>22</td>
</tr>
<tr>
<td>Isolation of hydrogenase by gel filtration</td>
<td>23</td>
</tr>
<tr>
<td>Resolution of hydrogenase by polyacrylamide gel electrophoresis</td>
<td>24</td>
</tr>
<tr>
<td>Section</td>
<td>Page</td>
</tr>
<tr>
<td>-------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>Concluding remarks and future work</td>
<td>25</td>
</tr>
<tr>
<td>LITERATURE CITED</td>
<td>26</td>
</tr>
<tr>
<td>VITA</td>
<td>31</td>
</tr>
</tbody>
</table>
LIST OF TABLES

Table 1. Enzyme activities in solventogenic cells of \textit{C. acetobutylicum} ATCC 824 

Table 2. Enzyme activities in acidogenic cells of \textit{C. acetobutylicum} ATCC 824

LIST OF FIGURES

Figure 1. Glucose fermentation of \textit{Ruminococcus albus}  
Figure 2. Hydrogenases in \textit{Alcaligenes eutrophus} H16  
Figure 3. Metabolic pathway of acid and solvent production in clostridia  
Figure 4. An example of growth and solvent production of \textit{C. acetobutylicum} ATCC 824 in 10 L of the clostridial medium at pH 4.5  
Figure 5. Molecular weight of hydrogenase as determined by gel filtration on a Sephacryl S-300 column  
Figure 6. Elution of hydrogenase activities from a DE-52 column  
Figure 7. Elution of hydrogenase activities from a Sephacryl S-300 column  
Figure 8. Elution of hydrogenase activities from a Sephacryl S-300 column