Regulation of *Staphylococcus aureus* Lactate Dehydrogenase

W. GARRARD AND J. LASCELLES

Department of Bacteriology, University of California, Los Angeles, California 90024

Received for publication 4 October 1967

The effect of growth conditions on the specific activity of nicotinamide adenine dinucleotide (NAD)-linked lactate dehydrogenase (LDH) in extracts of *Staphylococcus aureus* strain SG 511A was examined. Kinetic and electrophoretic experiments, with extracts prepared from aerobically and anaerobically grown cells, provided evidence for only one physiologically significant enzyme. The aerobic level of NAD-linked LDH of *S. aureus* remained constant and was independent of the carbon source. In contrast, the level of LDH produced in an anaerobic environment was variable and was dependent on the carbon source. Growth anaerobically on pyruvate, as the sole fermentable carbon source, resulted in a maximal level of LDH activity, a value about eightfold greater than the aerobic level. Anaerobic growth either on pyruvate plus glucose or on glucose alone, however, resulted in approximately a threefold decrease in this maximum. Experiments with a heme-requiring auxotroph derived from *S. aureus* demonstrated that the aerobic level of LDH activity was dependent on a functional respiratory chain.

The end products of glucose catabolism by *Staphylococcus aureus* vary depending upon the growth conditions. Lactate is the major end product of anaerobic growth, whereas acetate and carbon dioxide are the predominant end products produced aerobically (4). Collins and Lascelles (3) found that the production of lactate from glucose under anaerobic growth conditions was paralleled by a higher level of lactate dehydrogenase (LDH) activity. The nicotinamide adenine dinucleotide (NAD)-linked LDH activity was approximately eightfold higher under anaerobic growth conditions than the activity found in aerobically grown cells.

Kaplan et al. (9) have shown that two principal forms of NAD-linked LDH occur in mammalian tissues. The “muscle” type predominates in organs which are particularly suited for anaerobic metabolism, whereas the “heart” type is found in tissues with a high aerobic metabolism (1, 10, 16). Goodfriend et al. (5) demonstrated that, under conditions of low oxygen tension, mammalian tissue cultures produced enhanced levels of “muscle” LDH.

In view of the above observations, a study of the NAD-linked LDH activity of *S. aureus* was undertaken. We found that only one primary enzyme species is produced under different growth conditions, and that the specific activity of the enzyme varies considerably with the conditions of growth.

**MATERIALS AND METHODS**

**Organisms and culture media.** The *S. aureus* strains (SG 511A and SG 511Var) used in this study have been described elsewhere (4). Strain SG 511Var was a heme-requiring auxotroph derived from the parent strain SG 511A. The maintenance of stock cultures and the composition of the basal medium used have been reported previously (4). The basal medium contained a mixture of amino acids, vitamins, and salts. This medium was supplemented after autoclaving, as indicated in the text. Uracil is required for anaerobic growth of *S. aureus* (14). All anaerobic cultures were therefore supplemented with uracil, and, in some experiments, for comparative purposes, aerobic cultures were similarly supplemented.

**Growth and harvesting of organisms.** Cultures were grown aerobically in 2.5-liter flasks, containing from 0.5 to 1.0 liter of medium, on a reciprocal shaker at 80 cycles per min. Cells were grown anaerobically in screw top flasks which were filled completely with medium. All cultures were incubated at 37 C. Turbidity was measured with a Klett-Summerson colorimeter, and cultures were harvested in the late exponential phase of growth by centrifugation at 2,500 × *g* for 10 min at 4 C. Cells were washed once with 0.05 M potassium phosphate buffer (pH 6.5) and stored at −20 C.

**Preparation of cell-free extracts.** Cells were suspended in approximately 20 volumes of 0.05 M potassium phosphate buffer (pH 6.5) and disrupted by three passages through a precooled French pressure cell (American Instrument Co., Inc., Silver Spring, Md.) at approximately 18,000 psi. Cell debris was removed by centrifugation at 27,000 × *g* for 15 min at 4 C.
Extracts prepared in this manner generally contained approximately 0.3 mg of protein per ml.

Lactate dehydrogenase assay. Extracts prepared as above were assayed immediately for LDH activity. The complete assay mixture contained the following components in a final volume of 1 ml: potassium phosphate buffer (pH 6.5), 50 μmoles; sodium pyruvate, 2.5 μmoles; reduced nicotinamide adenine dinucleotide (NADH₂), 0.136 μmole; and from 1 to 10 μg of protein, depending on the activity of the extract. After measurement of the endogenous NADH₂ oxidase activity, reactions were initiated by addition of pyruvate. The decrease in optical density at 340 μm, resulting from the oxidation of NADH₂, was followed with a Zeiss M4 QIII spectrophotometer (Carl Zeiss, Inc., New York, N.Y.) equipped with a Varicord model 43 recorder. Assays were conducted at 24 °C. Specific activity is expressed as micromoles of NADH₂ oxidized per minute per milligram of protein.

Preliminary experiments established that, under the above conditions, the rate of NADH₂ oxidation was linear with protein concentration and time. The possible side reaction catalyzed by 2,3-butylene glycol dehydrogenase could not be detected at the protein concentration employed in the lactate dehydrogenase assay.

Polyacrylamide gel electrophoresis. To obtain concentrated cell-free extracts for electrophoresis, cells were suspended in approximately 2 volumes of 0.05 M potassium phosphate buffer (pH 6.5) and passed through a French pressure cell as described above. The resulting extracts were then subjected to sonic oscillation for three 30-sec intervals by use of a Branson Model LS 75 Sonifier at a setting of 4. Cell debris was removed by centrifugation as described above. Prior to electrophoresis, 1.0-ml portions of cell-free extracts were passed through a column (1.5 × 9.0 cm) of G-25 Sephadex, which was equilibrated with and eluted by 0.05 M potassium phosphate buffer (pH 6.5). Electrophoresis was conducted at 4 °C according to the method of Hjortén et al. (6). Gels were developed for LDH activity by use of a mixture of DL-lactate, according to the conditions of Schrauwegen (15), with the exception that sodium cyanide was omitted from the reaction mixture.

Protein determinations. Protein determinations were performed according to the method of Lowry et al. (12).

RESULTS

Properties of lactate dehydrogenase produced by S. aureus under different growth conditions. Preliminary experiments were concerned with determining the number of primary enzyme species of NAD-linked LDH produced by S. aureus under different conditions of growth. To detect possible differences in kinetic properties, the effect of pyruvate concentration on enzymatic activity was examined by use of extracts prepared from cells grown under a variety of conditions. Figure 1 shows the pyruvate saturation curve for an extract prepared from aerobically grown cells. Similar profiles were obtained when extracts from anaerobically grown cells were examined. Although the specific activities of LDH varied nearly 10-fold depending on the growth conditions, no significant differences in the kinetic properties of these preparations could be detected (Table 1).

Differences in the molecular properties of LDH produced under different growth conditions were examined with various extracts by polyacrylamide gel electrophoresis. This method, however, revealed that the organism formed only one species of enzyme under all conditions tested. Furthermore, when extracts prepared from aerobically and anaerobically grown cells were mixed prior to electrophoresis, only one soluble lactate-dependent band was observed (Fig. 2). Another band of activity was also noted (Fig. 2), but the intensity of development of this band was independent of DL-lactate. This band of activity could not be removed from extracts by gel filtration, and the nature of the reaction is unknown.

The similarities in kinetic and molecular properties of LDH produced by S. aureus, grown either aerobically or anaerobically, thus provide firm evidence for only one major enzyme species. Further experiments were directed
Table 1. Effect of growth conditions on kinetic properties of lactate dehydrogenase of Staphylococcus aureus

<table>
<thead>
<tr>
<th>Additions to basal medium</th>
<th>Growth condition</th>
<th>$K_m$ (10$^{-4}$ M$^{-1}$)</th>
<th>Pyruvate inhibition ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>Aerobic</td>
<td>5.72</td>
<td>1.24</td>
</tr>
<tr>
<td>Glucose</td>
<td>Aerobic</td>
<td>5.42</td>
<td>1.21</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>Anaerobic</td>
<td>5.68</td>
<td>1.15</td>
</tr>
<tr>
<td>Glucose + pyruvate</td>
<td>Anaerobic</td>
<td>6.03</td>
<td>1.17</td>
</tr>
</tbody>
</table>

* The optimal pH, determined by assaying in 0.05 M potassium phosphate buffer (pH 5.0 to 7.5) and in 0.05 M tris(hydroxymethyl)aminomethane chloride buffer (pH 8.0 to 9.0) was 6.5. The optimal pyruvate concentration, determined as described in Fig. 1, was 2.5 × 10$^{-4}$ M.

b Additions: 0.05 M glucose and 0.02 M pyruvate. Anaerobic cultures were also supplemented with 10$^{-4}$ M uracil.

c Specific activity at 7.5 × 10$^{-4}$ M pyruvate/specific activity at 10$^{-2}$ M pyruvate.

Fig. 2. Polyacrylamide gel pattern of lactate dehydrogenase of Staphylococcus aureus. Gel numbers: (1) and (2) 248 μg protein of an extract prepared from an aerobic culture grown in unsupplemented medium; (3 and 4) 96 μg protein of an extract prepared from an anaerobic culture grown in basal medium supplemented with 0.02 M pyruvate and 10$^{-4}$ M uracil; and (5) a mixture containing 124 μg protein of the above aerobically grown cell extract and 48 μg protein of the above anaerobically grown cell extract. Gel numbers 1 and 3 were developed in the absence of DL-lactate.

toward examining the levels of LDH activity under a variety of growth conditions.

Effect of growth conditions on the specific activity of lactate dehydrogenase in extracts of S. aureus. The activity of LDH produced by S. aureus grown aerobically, with a variety of carbon sources, did not vary significantly. The presence of a fermentable carbon source did not enhance the aerobic level of LDH (Table 2).

Under anaerobic conditions, the LDH activity of cells grown with pyruvate as the sole fermentable carbon source was approximately eight times higher than the aerobic level (Table 3). Anaerobic growth, either on glucose alone or on glucose plus pyruvate, resulted in only about a threefold increase in LDH activities compared to the aerobic levels (Table 3).

Effects of oxygen and a functional cytochrome system on the specific activity of lactate dehydrogenase in extracts of S. aureus. The effect of aerobiosis on the level of LDH activity was examined with a mutant strain of S. aureus. This mutant strain required added heme for the development of respiration by a cytochrome system (7). When grown without heme, this organism produced similar enhanced levels of LDH both aerobically and anaerobically (Table 4). A decrease in the specific activity of LDH aerobically occurred only upon addition of heme to the growth medium. Such supplementation, however, did not significantly affect the anaerobic level of enzyme activity (Table 4).

Discussion

The results of this study provide evidence for only one physiologically significant species of NAD-linked LDH for S. aureus. Examination of the aerobic and anaerobic levels of this enzyme showed that LDH could assume several levels during anaerobic growth, depending on

Table 2. Effect of aerobic growth conditions on the specific activity of lactate dehydrogenase in extracts of Staphylococcus aureus

<table>
<thead>
<tr>
<th>Addition to basal medium</th>
<th>Generation time (min)</th>
<th>Specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>90</td>
<td>4.2</td>
</tr>
<tr>
<td>Uracil</td>
<td>90</td>
<td>4.0</td>
</tr>
<tr>
<td>Glucose</td>
<td>50</td>
<td>3.9</td>
</tr>
<tr>
<td>Glycerol</td>
<td>50</td>
<td>3.4</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>50</td>
<td>4.3</td>
</tr>
<tr>
<td>DL-Lactate</td>
<td>50</td>
<td>3.9</td>
</tr>
<tr>
<td>Fumarate</td>
<td>90</td>
<td>4.0</td>
</tr>
</tbody>
</table>

a Additions: 0.05 M glucose; 0.02 M fumarate, glycerol, DL-lactate, or pyruvate; or 10$^{-4}$ M uracil.

Table 3. Effect of anaerobic growth conditions on the specific activity of lactate dehydrogenase in extracts of Staphylococcus aureus

<table>
<thead>
<tr>
<th>Addition to basal medium</th>
<th>Specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic</td>
<td>Anaerobic</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>4.3</td>
</tr>
<tr>
<td>Glucose</td>
<td>3.7</td>
</tr>
<tr>
<td>Glucose + pyruvate</td>
<td>3.8</td>
</tr>
</tbody>
</table>

a Additions as described in Table 1. Cultures were also supplemented with 10$^{-4}$ M uracil.
TABLE 4. Effects of oxygen and a functional cytochrome system on the specific activity of lactate dehydrogenase in extracts of a heme-requiring auxotroph of Staphylococcus aureus

<table>
<thead>
<tr>
<th>Heme addition* (10⁻⁴ M)</th>
<th>Specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aerobic</td>
</tr>
<tr>
<td>None</td>
<td>24.0</td>
</tr>
<tr>
<td>2</td>
<td>17.0</td>
</tr>
<tr>
<td>5</td>
<td>8.1</td>
</tr>
</tbody>
</table>

* Organisms were grown in basal medium supplemented with 0.05 m glucose, 0.02 m pyruvate, 10⁻⁴ M uracil, and heme at the concentrations indicated.

the carbon source. In contrast, the aerobic level of the enzyme remained constant, irrespective of the carbon source.

It is of particular interest to note the high anaerobic level of the enzyme under conditions where pyruvate is the sole fermentable carbon source. If the turnover number and molecular weight of the bacterial enzyme are similar to mammalian "muscle" LDH (13), then the LDH of S. aureus would comprise approximately 6% of the soluble cell protein. Furthermore, the aerobic level of the enzyme is exceedingly high, even with amino acids as the sole carbon source, when LDH would appear to have no physiological function. This high aerobic level is comparable with the LDH activity in several strains of streptococci grown anaerobically with glucose as the carbon source (17).

The results of the experiments with the heme-requiring mutant showed that control of LDH activity was not governed by oxygen per se but required, in addition to oxygen, the presence of a functional cytochrome system. Regulation of LDH and alcohol dehydrogenase levels in a quinone-deficient strain of Escherichia coli has also been shown to be dependent upon a functional respiratory chain (8). In contrast, oxygen per se appears to play a fundamental role in determining the activities of S. aureus nitrate reductase (2), E. coli fumarate reductase (8), and mammalian "muscle" LDH (5).

The observed changes in the specific activity of LDH from S. aureus could be attributed to alterations in the activity of preformed enzyme, or to changes at the level of enzyme synthesis, or to both. Speculation on the processes involved in the regulation of LDH activity by S. aureus should be compatible with the following observations: (i) LDH levels are enhanced aerobically in the absence of a functional respiratory chain, and (ii) LDH levels are enhanced in anaerobic cultures containing pyruvate as the sole fermentable carbon source. The former observation can be explained by the hypothesis that the pool size of some electron carrier regulates enzyme formation. This hypothesis, however, does not account for the effect of pyruvate. Perhaps the stress imposed on the organism for use of pyruvate anaerobically, as an energy source, or for gluconeogenesis are of primary importance in the further increase of LDH activity. Our present knowledge of the metabolism of S. aureus, however, does not include the nature of these reactions.

ACKNOWLEDGMENTS

This investigation was supported by grant 1 R01 AM-11,148-01 from the National Institutes of Health and grant 2268 from the Academic Senate of the University of California.

LITERATURE CITED


