Periodate Inhibition of Transformation and Competence Development in *Haemophilus influenzae*

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**ABSTRACT**

RANHAND, JON M. (University of Cincinnati, Cincinnati, Ohio), AND HERMAN C. LICHTSTEIN. Periodate inhibition of transformation and competence development in *Haemophilus influenzae*. J. Bacteriol. 92:956–959. 1966.—Periodate treatment of competent cells reduced the frequency of transformation to streptomycin resistance about 90% while reducing cell viability about 30% or less. Moreover, when periodate was added to cells early in the competence-development phase, these, too, were unable to develop maximal competence. Periodate inhibition was dependent on time and concentration as well as on the composition of the suspending menstruum. Periodate had no effect on transforming deoxyribonucleic acid (DNA), nor did it prevent transformation when added to competent cells which had already reacted with DNA. Furthermore, the progeny from cells inactivated 90% could be made fully competent, showing that the inhibition was not genetic. It was concluded that the periodate-sensitive substrate may involve the DNA binding site(s).

The bacterial cell wall has been implicated as a primary structure for competence development (2, 9, 10). Sneath and Lederberg (7) have shown that sodium periodate treatment of male *Escherichia coli* K-12 can prevent the conjugation process, which also involves a cell wall component (Lancaster, Goldschmidt, and Wyss, Bacteriol. Proc., p. 30, 1964). It was of interest, therefore, to see whether periodate could prevent transformation in competent *Haemophilus influenzae*. Recently, Polsinelli and Barlati (in preparation) observed periodate inhibition of transformation in *Bacillus subtilis*.

**Materials and Methods**

Organism and deoxyribonucleic acid (DNA). The Rd strain of *H. influenzae* and the method of DNA purification have been described in detail by Goodgal and Herriott (3).

Chemicals. The sources of chemicals were as follows: sodium metaperiodate (Fisher Scientific Co., Union, N. J.); vitamin-free, acid-hydrolyzed casein, 8-azaguanine, and Tween 80 (Nutritional Biochemical Corp., Cleveland, Ohio); inosine (Calbiochem); Trypticase (BBL); Brain Heart Infusion (BHI) broth and agar (Difco); streptomycin sulfate (Parke, Davis & Co., Detroit, Mich.); lactic acid and various salts (Mallinckrodt Chemical Works, New York, N. Y.).

Potassium lactate solution. Potassium lactate was prepared by neutralizing the free acid with KOH.

Phosphate-buffered saline. Phosphate-buffered saline (PBS) contained 0.01 m phosphate buffer, pH 7.1, in 0.85% NaCl.

DNA solution. The DNA solution contained 21 µg/ml of streptomycin-resistant DNA plus 100 µg/ml of 8-azaguanine in PBS. The 8-azaguanine was included to retard further competence development.

Modified saline solution. The modified saline solution was essentially that of Cabrera-Juarez and Herriott (1). It consisted of the following: 0.85% NaCl; 0.02 m potassium phosphate buffer, pH 7.0; 5 × 10⁻⁴ m CaCl₂; 5 × 10⁻⁴ m MgSO₄; and 0.01% Tween 80.

Competence development. Competence was obtained by first growing *H. influenzae* in a Trypticase medium (Tc) described by Ranhand and Herriott (6) containing inosine and lactate, both at 333 µg/ml, and, in addition, 0.3% glycerol (v/v) and 0.02 m potassium phosphate buffer, pH 6.7. The cells were then transferred to a competence-development medium (CDM), pH 7.1, which was essentially that of Spencer and Herriott (8) except that it contained 0.05% hydrolyzed casein. This addition allowed maximal competence to develop in 60 to 80 min as compared with 180 to 200 min (Fig. 1).

Phase 1 (growth phase). A 2.5-ml amount of frozen stock cells (2 × 10⁹ to 3 × 10⁹ per milliliter) in 3.7% BHI broth containing 15% glycerol was thawed rapidly at 37°C, washed once with 2.5 ml of PBS, and resuspended in 3.0 ml of PBS. These were diluted 1:30 in Tc in a 300-ml side-arm flask (18 to 19 mm in diameter), and were shaken gently (100 strokes per minute with a 2.54-cm displacement) on a reciprocating shaker at 37°C. Growth was estimated as an increase in optical density (OD) at 650 µm with a
Fig. 1. Kinetics of competence development by Haemophilus influenzae in phase 2. Cells grown in Tc were diluted in the competence medium. At zero-time and 15-min intervals thereafter, the DNA solution was added for 30 min, at which time the cells were diluted and plated. For details, see Materials and Methods.

Coleman Jr. spectrophotometer. When the culture reached an OD of 0.080 (4 × 10⁶ cells per milliliter), 28 ml was washed once with 4.0 ml of PBS and resuspended in 2.1 ml of CDM minus the hydrolyzed casein. (When larger quantities of cells were grown, i.e., 50 ml, the ratio, milliliters of cells to milliliters of CDM, was maintained at 4:0.3.)

Phase 2 (competence-development phase). The concentrated cells were diluted 0.2:1.0 in CDM in 16-mm screw-cap tubes and shaken (as above) for the desired length of time, after which 0.05 ml of the DNA solution was added; shaking was continued for the usual 30 min to allow DNA uptake, and the cells were diluted in 0.37% BHI broth and plated in duplicate in 3.7% BHI agar. After 90 to 110 min of incubation at 37 C, the cells were overlaid with BHI agar containing streptomycin at 100 μg/ml. Colonies were counted after overnight growth at 37 C.

Periodate inactivation. Periodate was delivered in 0.05-ml volumes to 1.0 ml of cells in either CDM or the modified saline solution. The inactivation was allowed to occur for 10 min at 37 C in the dark, after which time the periodate was removed by centrifugation (4 min at room temperature); the pellet was then resuspended in 1.0 ml of fresh CDM. When periodate was added to cells at the beginning of phase 2 (low competence), the cells were treated in one of two ways after its removal: (i) the DNA solution was added immediately to estimate the zero-time inactivation, or (ii) the DNA solution was added after the cells had an additional 80 min in phase 2. In either case, after the DNA addition, the above protocol was followed. When periodate was added at 60 or 80 min (high competence) after its removal, the DNA solution was added for 30 min and the above protocol was followed. In all cases, controls were handled in an identical manner except for the addition of periodate.

RESULTS AND DISCUSSION

The data in Fig. 2 show the effect of periodate on transformation and cell viability when the inactivation was carried out in CDM (curves A and A') or in the modified saline medium (curves B and B'). The inactivation was complex in that it was not "one-hit"; there was also a marked decrease in the effective periodate concentration that depended on the suspending menstrum. For 90% inactivation in CDM, the periodate concentration had to be increased approximately sixfold, which may reflect its consumption by the medium per se, since Martin and Synge (4) and others (5) have shown that periodate will react with some amino acids contained in CDM. Figure 2 also shows that the effective concentration in both cases was limited to a narrow range, above which the cell viability decreased dramatically. These results also imply that it is some part of the cell that is being inactivated rather than a component of CDM, and that periodate, and not a byproduct from CDM, is causing the inactivation.

The data in Table 1 compare the effect of periodate on cells which were treated in phase 2 at zero-time (line 1, b to d; line 2, b to d) with those which were treated at 80 min (line 3, b to d). The values are the average from two independent experiments.

A comparison of lines 1b, 2b, and 3b shows that periodate inhibited transformation to the same extent (60 to 65%) regardless of the competent state of the culture (low versus high). The same effect was observed with higher periodate concentrations (1.8 × 10⁻⁴ M and 2.2 × 10⁻⁴ M). These results imply that the periodate-sensitive substrate is present at zero-time (when the transformability of the population is low), and that it does not increase during the additional 80 min of incubation in phase 2. If the periodate substrate had increased, then one might expect that the same level of periodate given at 80 min would result in a lesser inhibition owing to the presence of more substrate at that time.
Table 1. Effect of periodate on zero-time and 80-min cells treated in the phase 2 competence-development medium

<table>
<thead>
<tr>
<th>Expt condition</th>
<th>Periodate concn (X 10^-4 M)</th>
<th>Transformation per ml (X 10^-3)</th>
<th>Viable centers per ml (X 10^-3)</th>
<th>Per cent transformation</th>
<th>Per cent inhibition</th>
</tr>
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<tr>
<td>1 a</td>
<td>0.0</td>
<td>0.0027</td>
<td>1.6</td>
<td>0.0017</td>
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<tr>
<td>c</td>
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<td>0.00023</td>
<td>1.6</td>
<td>0.00014</td>
<td>92</td>
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<tr>
<td>d</td>
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<td>0.00028</td>
<td>1.3</td>
<td>0.00022</td>
<td>87</td>
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<tr>
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<td>0.69</td>
<td>65</td>
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<tr>
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<tr>
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<td>0.11</td>
<td>1.4</td>
<td>0.079</td>
<td>97</td>
</tr>
</tbody>
</table>

* (1) At zero-time, cells were treated first with periodate for 10 min, followed by the addition of the DNA solution for 30 min; they were then diluted and plated as described in Materials and Methods. (2) At zero-time, cells were treated first with periodate for 10 min. They were then allowed to develop competence for an additional 80 min, at which time the DNA solution was added for 30 min. The above protocol was then followed.

(3) At 80 min, the cells were treated with periodate for 10 min, followed by the addition of the DNA solution for 30 min. The above protocol was then followed.

Per cent inhibition = 100 – (per cent transformation of periodate-treated cultures x 100 divided by per cent transformation of untreated cultures).

These results also show that once the site(s) is destroyed it cannot be resynthesized during phase 2. However, the progeny from cells in which transformation was inactivated 90% could be transformed to novobiocin resistance to the extent of 3.6%, showing that the change was not genetic.

Other experiments showed that periodate had no inactivating effect on transforming DNA, nor did it prevent maximal transformation to streptomycin resistance when competent cells were first exposed to DNA for 30 min and then to periodate.

The most favorable interpretation of these data is that cells coming out of phase 1 contain a fixed number of sites which develop into DNA binding sites during the 60 to 80 min of treatment in phase 2, and that periodate can inactivate these sites to the same extent regardless of their state of development, i.e., their incapability or capability of binding with DNA. It is concluded, therefore, that periodate can recognize the DNA binding site(s) or prebinding site(s) before the cells can irreversibly bind DNA and express it phenotypically as a transformation.

The fact that the percentage of transformation does increase some 400- to 1,000-fold (shown by comparison of line 1b with 2b, 1c with 2c, and 1d with 2d) shows that the population of cells which did not react with periodate at zero-time can increase their competence as did the untreated cells (shown by comparison of line 1a with 2a and 3a). Higher periodate concentrations could not be used, because unwanted cell death would have resulted.

Since inosine and lactate have been shown to be essential in phase 1 for competence development
it is interesting to hypothesize that their metabolic fate either alone or together involves the periodate-sensitive substrate. Work is now in progress to describe more fully the fate of inosine and lactate.

ACKNOWLEDGMENTS

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LITERATURE CITED


