Prevention of Autolysis in Suspensions of Neisseria gonorrhoeae by Mercuric Ions

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Received for publication 21 December 1977

Mercuric ions were the only metal ions which prevented autolysis of Neisseria gonorrhoeae, as measured both by stabilization of optical density and by prevention of release of [3H]diaminopimelic acid.

The fragility of Neisseria gonorrhoeae poses significant problems for biochemical and immunological studies because the half-life of the organism in buffer ranges from 23 to 35 min (4). Varying results have been obtained with methods for preventing autolysis by using ions (2-4), osmotic stabilizers (2, 3), manipulation of required nutrients (3, 6), and different suspension media (3-5, 7). This report is concerned with studies on stabilization of N. gonorrhoeae with metal ions, including the more toxic heavy metals, because viability is not an essential requirement for immunological study of surface components (1).

N. gonorrhoeae strains Black, Mel, and 2686 were obtained from T. Buchanan. Organisms were grown on defined liquid medium (5) in stir culture. Cells were sedimented, washed once, and suspended in tris(hydroxymethyl)aminomethane (Tris)-maleate buffer (0.05 M each; pH 7.0) to an optical density of 0.5 to 0.6 (1.2 × 10⁸ to 1.5 × 10⁹ colony-forming units per ml; optical density was measured at 620 nm with a 1-cm light path). Autolysis was measured by changes in turbidity after suspension in buffer with the required additive. The optical density at 620 nm was measured at zero time and at 30-min intervals thereafter with incubation at 37°C. Results were plotted as the percent change from the zero time reading.

Autolysis was also measured by determination of the leakage of [3H]diaminopimelic acid (DAP) from the cell (4). Early-log-phase cells were sedimented, suspended in fresh defined medium containing 5 µCi of [3H]DAP per ml and 0.01 M lysine, and grown for two generations. Cells were sedimented, washed in warm defined medium, suspended in fresh warm medium containing 0.01 M DAP and 0.01 M lysine, and incubated for 10 min. Cells were washed in buffer and suspended in buffer plus the metal ions. At zero time and at 30-min intervals thereafter, a sample of the cell suspension was centrifuged at 8,000 × g for 5 min at 25°C, and 1.0 ml of the supernatant was added to 10 ml of Bray scintillation fluid and assayed for radioactivity. The counts per minute per milliliter was plotted as the percent change from the zero time determination.

To ascertain that the radioactivity appearing in the supernatant was derived from DAP and/or from lysine, supernatant was collected and precipitated with cold 5% trichloroacetic acid; the precipitate was hydrolyzed in 4 N HCl for 14 h at 105°C and spotted on 3MM chromatography paper. The chromatogram was developed in ethanol-water-ammonia (18:1:1, vol/vol). One set of chromatographs was sprayed with ninhydrin, and the others were cut into 1-cm strips and assayed for radioactivity.

Tris-maleate buffer was used for the study because most of the ions remained soluble in this buffer [exceptions were BaCl₂, AlK₃(SO₄)₂, and PbC₂H₃O₂, whereas other buffers, such as HEPES (N-2-hydroxyethyl piperazine-N'-2-ethanesulfonic acid) and TES (N-tris(hydroxymethyl)methyl-2-aminomethane-sulfonic acid), formed precipitates with many ions. A variety of metal ions were tested, and the upper limits of concentrations used were 10 to 100 mM. Metal ions which bind sulfhydryl groups were tested first. Mercury stabilized the turbidity at concentrations of 1.0 mM and greater (Fig. 1). Silver ions gave good stability from 0.1 mM. Turbidity was also stabilized by 100 mM MnCl₂, 5 mM CuSO₄, 10 mM CaCl₂, 100 mM MgCl₂, and 100 mM ZnCl₂. A variety of ions failed to give stability; these were SrCl₂, FeCl₃, Fe(NO₃)₃, CsCl, TIC₃H₂O₃, K₂Fe(CN)₆, Na₂WO₄, NaF, KBr, Na₂AsO₃, NaCl, KCl, and I₂ in KI. Stabilization by Hg²⁺ was also observed with strain 2686, colony type 3. Colony types 1 and 2 also appeared to be stabilized, but strong autoagglutination made the results difficult to assess.

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When the release of $^{3}$H]DAP was tested, only the addition of Hg$^{2+}$ permitted stabilization (Fig. 2); ions such as Cu$^{2+}$ and Zn$^{2+}$, although they stabilized turbidity, failed to prevent label release. Chromatographic analysis of the supernatants of autolyzing cells indicated 3,530 cpm at the $R_f$ of DAP and 2,050 cpm at the $R_f$ of lysine, suggesting that the procedure was measuring the release of cell wall components. Phase-contrast microscopic examination after 2 h of incubation showed few whole cells and large amounts of cell debris in untreated samples, large deformed cells and cell debris in those samples showing stable turbidity but label leakage, and typical diplococci with little debris in samples treated with Hg$^{2+}$. The addition of various salts to autolysis inhibitors changed turbidity-stabilizing properties; NaCl lowered the ability of ions such as Zn$^{2+}$ and even Hg$^{2+}$ to stabilize turbidity, and NaNO$_3$ appeared to increase stability, although the addition of NO$_3^-$ did not suppress label release.

The mechanism of action of heavy metals as toxic agents is apparently mercaptide formation with sulfhydryl groups of enzymes. The efficiency of action of the various metals in this study was Hg$^{2+} >$ Ag$^{+} >$ Cu$^{2+} >$ Zn$^{2+}$, an order which correlates exactly with the negative logarithm of the solubility products of these metal sulfides and with their relative toxicity for bacteria. The most stringent means of determining cell stability appears to be measurement of the prevention of release of labeled incorporated compounds from the cell. Optical methods fail to differentiate between the cessation of autolysis and the stabilization of osmotically sensitive forms produced by continuing autolysis.

During the preparation of this manuscript, Wegener et al. (8) described the relationship between autolysis in HEPES buffer and the hydrolysis of the peptidoglycan of N. gonorrhoeae. This study indicated that stabilization of autolysis as measured turbidimetrically does not necessarily mean that the peptidoglycan is not continuing to hydrolyze. They also found that mercuric ions both stabilized cell autolysis and prevented peptidoglycan hydrolysis, findings which are substantiated by the studies with Tris-maleate buffer reported herein. Mercuric ions are unique in this respect, as we did not find other effective ions in the present study.

This study was supported by Public Health Service training grant AI 00125 from the National Institute of Allergy and Infectious Diseases.

**LITERATURE CITED**


