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Lasalocid-Catalyzed Proton Conductance in *Streptococcus bovis* as Affected by Extracellular Potassium†

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The effect of extracellular potassium on lasalocid-catalyzed proton conductance in *Streptococcus bovis* 24 was measured by using the fluorescent probe 9-aminoacridine. Increasing external potassium concentration resulted in decreased proton flux into *S. bovis* cells exposed to the ionophore. These results suggest that lasalocid catalyzes K^+/H^+ exchange diffusion in *S. bovis* cells.

Carboxylic ionophores catalyze exchange diffusion reactions that translocate cations or protons in opposite directions across biological membranes (9). For instance, lasalocid transports many divalent and monovalent cations (including protons) and displays a particularly high affinity for potassium (10). Ionophores frequently are added to beef cattle diets because they increase the efficiency of feed utilization by growing ruminants (1). Although monensin is the ionophore most widely used in this capacity, lasalocid has also been approved by the Food and Drug Administration. *Streptococcus bovis* is a ruminal bacterium which proliferates when large amounts of starch are fed, and its lactic acid production can exceed the buffering capacity of ruminal fluid (6). Lactic acidosis is associated with acute indigestion in affected animals. This report demonstrates that lasalocid causes proton flux into *S. bovis* cells at concentrations of potassium up to 140 mM but that lasalocid-catalyzed proton conductance decreases as external potassium concentration increases.

S. bovis 24 was isolated from the rumen of a steer fed alfalfa hay. The isolate was characterized as described by Diebel and Seely (5). Cells were grown under anaerobic conditions (6) at 39°C on medium 10 (3) supplemented with 10% (vol/vol) clarified ruminal fluid and containing 0.75% (wt/vol) glucose. Cells in the exponential growth phase (optical density at 600 nm, 0.5 to 0.8) were harvested under N_2 by centrifuging 10 ml of culture at 5,800 × *g* for 25 min in a Corex tube (Corning Glass Works, Corning, N.Y.) sealed with a flanged serum style stopper no. 03-225; Fisher Scientific Co., Pittsburgh, Pa.). Bacterial pellets were washed and suspended in assay buffers containing energy substrate.

Buffer consisted of 10 mM 2-(*N*-morpholino)ethanesulfonic acid (MES; pH 6.0), bis(2-hydroxyethyl) Tris (bis-Tris; pH 6.5), 3-(*N*-morpholino)-2-hydroxypropanesulfonic acid (MOPSO; pH 6.9), or 3-(*N*-morpholino)propanesulfonic acid (MOPS; pH 7.2) (each of these buffers was used to establish a given pH as indicated); 5, 70, or 140 mM potassium chloride; 135, 70, or 0 mM choline chloride; and 0.2% (wt/vol) glucose. The sum of potassium chloride and choline chloride concentrations in each buffer totaled 140 mM. Lasalocid, 9-aminoacridine (9AA), MES, bis-Tris, MOPSO, and MOPS were from Sigma Chemical Co., St. Louis, Mo. All other buffer components were products of Fisher Scientific.

Cell suspensions were transferred immediately and anaerobically to a glass cuvette and sealed under N_2 with a rubber septum stopper, and the assay for proton conductance was initiated. A standard curve for bacterial cell dry matter per milliliter was determined over the range of optical densities from 0.2 to 1.5, allowing correction for cell dry matter concentration.

The fluorescent dye 9AA was used to measure lasalocid-catalyzed proton conductance in *S. bovis*. The fluorescence of 9AA was measured with a filter fluorometer (Turner Model 111; Sequoia-Turner, Mountain View, Calif.). The excitation filter was a Turner 110-812 transmission filter with a peak wavelength of 405 nm. Emission was measured through a Turner 110-817 bandpass filter (>485 nm) and a Turner 110-825 transmission filter with a peak wavelength of 495 nm.

Each assay was started when an aqueous suspension of 9AA was injected into the cell suspension to a final concentration of 2.5 μM. The assay tube was inverted several times to ensure mixing. After a stable fluorescence base line was achieved, 2 μl of an ethanol solution containing lasalocid was injected into the assay tube to a concentration of 5 μg of lasalocid ml^{-1}. The tube was inverted rapidly to mix the ionophore with the cell suspension and returned to the fluorometer in less than 5 s.

The experiment was analyzed as a completely randomized design with a 3 × 4 factorial arrangement of treatments (three potassium concentrations and four pH levels) by analysis of variance (14). Each treatment combination was replicated three times.

The addition of lasalocid to cell suspensions of *S. bovis* 24 previously equilibrated with 9AA resulted in decreased fluorescence (Fig. 1), indicating flux of protons into the cells (11). However, progressively less ($P < 0.001$) lasalocid-catalyzed proton conductance occurred at higher external potassium concentrations (Fig. 2). Neither a pH effect ($P > 0.2$) nor a K-pH interaction ($P > 0.15$) was detected.

Schuldiner et al. (13) proposed a model of 9AA action which assumes (i) 9AA is distributed across a bimolecular lipid membrane in response to change in proton activity and (ii) intracellular 9AA does not produce detectable fluorescence. Partitioning of dye between the extracellular and intracellular compartments is assumed to be a function of the relationship between the pH of each compartment and the pK_a of 9AA (13). Unfortunately, 9AA can exhibit interactive membrane binding (4), and its behavior in vesicle or cell suspensions may deviate from that of an ideal amine. This complication has led some reviewers (2, 8) to conclude that

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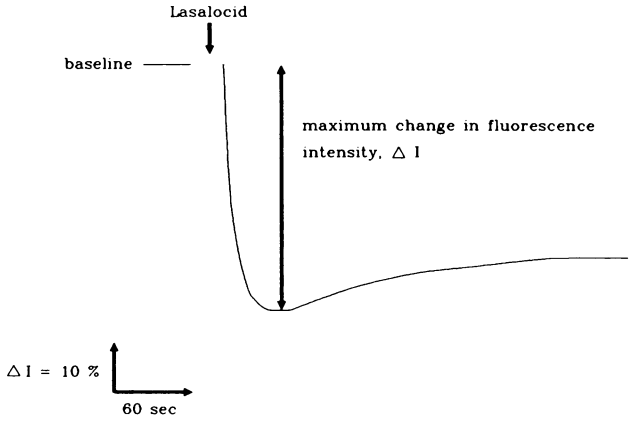

FIG. 1. Representative recording of 9AA fluorescence in cell suspensions (0.32 mg ml^{-1}) of *S. bovis* 24 after addition of lasalocid (5 μg ml^{-1}). The buffer consisted of 10 mM MOPS (pH 7.2), 5 mM potassium chloride, 135 mM choline chloride, and 0.2% (wt/vol) glucose. The cells were equilibrated in the presence of 9AA prior to the addition of lasalocid. Downward pen deflection denotes fluorescence quench accompanying proton flux into cells. ΔI is the percent change in fluorescence intensity (100% fluorescence was defined as full-voltage output of the fluorometer, 10 mV).

9AA is useful for qualitative purposes but can not be used successfully to quantitate transmembrane ΔpH. Casadio and Melandri (4), however, showed that the relative amount of bound to unbound 9AA in bacterial chromatophores was proportional and suggested that a correction factor can account for deviation of the behavior of 9AA from that of an ideal amine. Thus, 9AA would appear suitable for relative differentiation of the direction and extent of proton conductance, if not the absolute quantitation of ΔpH.

Russell (12) showed that *S. bovis* JB1 had a 70-fold concentration gradient of potassium from inside to outside the cell, resulting in a potassium concentration gradient which was much larger than that for sodium ions or protons. The potassium concentration decreased in cells upon exposure to monensin, and potassium efflux was coupled to the influx of protons into cells. Russell (12) speculated that increasing the potassium concentration external to *S. bovis* cells should result in decreased electrochemical potential in the transmembrane potassium gradient and, consequently, decreased monensin-catalyzed potassium/proton exchange diffusion.

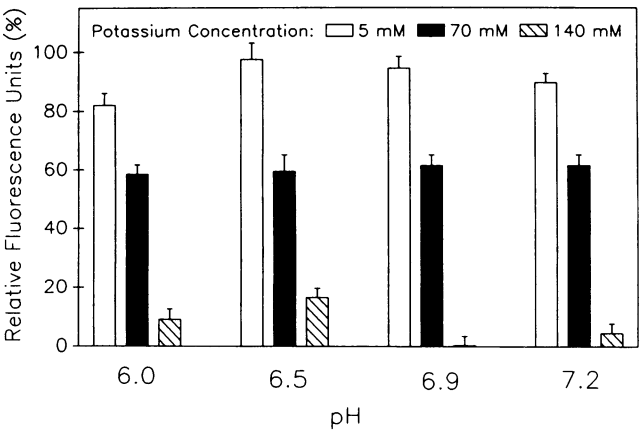

FIG. 2. Effect of pH and extracellular potassium concentration on maximum change (percent) of 9AA fluorescence caused by addition of lasalocid to cell suspensions of *S. bovis* 24. Preparation of washed cell suspensions was performed as described in the text. 9AA was added and allowed to equilibrate before lasalocid (5 μg ml^{-1}) was added. Maximum change in fluorescence is expressed in relative fluorescence units (100% fluorescence was defined as full-voltage output of the fluorometer, 10 mV) adjusted to a cell dry matter concentration of 0.5 mg ml^{-1}. Each value represents the mean of triplicate measurements.

The results reported here imply that lasalocid-catalyzed proton flux into *S. bovis* cells is also linked, by an exchange diffusion reaction, to potassium efflux from *S. bovis* cells. Suspending *S. bovis* 24 cells in buffers of various potassium concentrations altered the transmembrane potassium gradient because all cells grew under identical conditions and were presumably of identical intracellular composition (7). A smaller transmembrane potassium gradient, associated with high external potassium concentrations (12), resulted in decreased lasalocid-catalyzed proton conductance. Thus, potassium/proton exchange diffusion is implicated as a major event in *S. bovis* cells exposed to lasalocid.

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