

The Lysis of *Fibrobacter Succinogenes*

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Introduction

In ruminant animals, microbial protein is the primary source of protein for the animal, and microbial protein turnover is a wasteful process that decreases amino acid availability. Protozoal predation has often been cited as the prime cause of bacterial protein turnover in the rumen, but high rates of bacterial turnover have been noted in defaunated sheep. Bacteria need autolytic enzymes to expand their cell wall and grow, but the cells have a very high turgor pressure. If the action of autolytic enzymes is not carefully orchestrated, the cells can lyse. Because *Fibrobacter succinogenes* is a ruminal bacterium that lyses easily, it makes a good model for studying rumen bacterial turnover.

Materials and Methods

Fibrobacter succinogenes S85 was grown anaerobically in a minimal medium that contained cellobiose. Cultures were grown (39 °C) in 80 ml serum bottles, 18 x 150 mm tubes or a continuous culture device. Growth and lysis were monitored by the changes in optical density as well as changes in cell protein. Protein was determined by the Lowry method. RNA and DNA were determined using orcinol and diphenylamine methods, respectively. Cell N was calculated from protein, RNA and DNA with the N content of protein and nucleic acids being 16 and 12%, respectively. The membrane potential ($\Delta\Psi$) was calculated from the uptake of [³H] TPP⁺ using the Nernst equation ($62 \text{ mV} \times \log [\text{in}]/[\text{out}]$), and the non-specific binding of [³H] TPP⁺ was estimated from cells which had been treated with nigericin plus valinomycin (10 mM each) or toluene (1% of a 1:9 [v/v] toluene to ethanol). ATP was assayed with a luminometer. Cellobiose and cellular polysaccharide were assayed by an anthrone procedure. Soluble protein was measured by the Bradford dye method. Ammonia was assayed by a calorimetric method. Free amino acids were

measured by high pressure liquid chromatography. Peptides were hydrolyzed with HCl prior to derivatization and amino acid analysis.

Results

Growing cultures of *F. succinogenes* assimilated more ammonia than could be accounted by cellular protein, RNA or DNA, and released large amounts of non-ammonia nitrogen. The difference between net and true growth was most dramatic at low dilution rates, but mathematical derivations indicated that the lysis rate was a growth rate-independent function. The lysis rate was 7-fold greater than the true maintenance rate (0.07 h⁻¹ versus 0.01 h⁻¹). Because slow-growing cells had as much protonmotive force and ATP as fast-growing cells, lysis was not a starvation response per se. Stationary cells had a lysis rate that was 10-fold less than growing cells. Rapidly-growing cells were not susceptible to the proteinase inhibitor, PMSF, but PMSF increased the lysis rate of the cultures when they reached stationary phase. This latter result indicated that autolysins of stationary cells were being inactivated by a serine proteinase. When growing cells were treated with the glycolytic inhibitor, iodoacetate, the proteinase-dependent transition to stationary phase was circumvented, and the rate of lysis could be increased by as much as 50-fold.

Discussion

Lysis is usually considered to be a property of stationary phase bacteria that have depleted their nutrients, but *F. succinogenes* had a much faster rate of lysis when it was growing. In the surface stress model of bacterial growth, peptidoglycan is first deposited at the inner surface, and the older, outer layers are then cut by autolytic enzymes. This continual process of synthesis and degradation allows the stress to be gradually transferred to more recently synthesized portions

of the peptidoglycan. Since each new layer of the peptidoglycan is slightly longer than the preceding one, the wall is continually expanded.

Some workers hypothesized that the autolysins might be regulated by protonmotive force. In this model of autolytic regulation, protonmotive force decreases pH near the cell membrane, and acidic pH prevents the autolysins from completely degrading the peptidoglycan. Uncouplers that decreased protonmotive force promoted the lysis of *F. succinogenes*, but lysis and protonmotive force could not always be correlated. Growing cells with a high protonmotive force lysed, and stationary-phase cells that had a low protonmotive force did not lyse very rapidly.

Stationary cells of *F. succinogenes* had a mechanism of preventing lysis, and the experiments that included PMSF indicated that a proteinase was involved in autolysis. Because PMSF had no effect on exponentially growing *F.*

succinogenes cultures but promoted lysis when the cells reached stationary phase, it appeared that a serine proteinase was inactivating the autolysins when growth was no longer possible. One might view the lysis of growing *F. succinogenes* cells as a detrimental phenomenon that would decrease its niche in the rumen, but this assumption does not address the aspect of maximum growth rate and cell division. If autolytic activity is too low, growth rate would be sacrificed.

If the autolysis and growth of *F. succinogenes* are indeed highly integrated processes, lysis may be a very difficult phenomenon to alter. Lysis is, however, a dynamic process that must compete with the fluid dilution rate. Mineral salts have no direct effect on growth or lysis per se, but they increase the ruminal fluid dilution rate. When fluid dilution rate is increased, fluid phase bacteria have less time to lyse and turn over.