Thermal Lysis of Bacterial Membranes and Its Prevention by Polyamines

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SUMMARY

Protoplasts of *Sarcina lutea* and *Streptococcus faecalis* underwent thermal lysis when heated to 60° and above. [¹⁴C]Glycine was released from the internal pool of *Strep. faecalis* at 50°. Spermine, spermidine, cadaverine and Mg²⁺ partially protected protoplasts against thermal lysis.

It is generally agreed that the ability of thermophiles to grow at high temperatures is due, at least in part, to their ability to synthesize heat-stable macromolecules. Brock (1967) suggested that the thermostability of the cell membrane may be an additional important factor permitting thermophiles to grow at high temperatures and this hypothesis is supported by several recent reports (Abram, 1965; Golovacheva, 1967; Bodman & Welker, 1969; Brock, 1969). Although much work has been done with spheroplasts and protoplasts of mesophilic bacteria, there have been no direct investigations of their heat stability (McQuillen, 1960).

We report thermal lysis of the protoplasts of *Sarcina lutea* and *Streptococcus faecalis* and their stabilization to heat by polyamines, agents which have been shown to stabilize bacterial protoplasts to osmotic lysis (Mager, 1959; Tabor, 1962; Harold, 1964).

When protoplasts of *Streptococcus faecalis* were held at 0°, 37° or 50° for 60 min. no lysis occurred. Incubation at 60° caused slow lysis and incubation at 70° caused complete lysis of the suspension in 5 to 10 min. (Fig. 1). Microscopic observations showed that the changes in extinction were due to lysis. Similar results were obtained with the protoplasts of *Sarcina lutea*.

Since the decrease in extinction of a protoplast suspension indicates gross lysis, a more sensitive technique was used to investigate slight membrane damage. In the presence of chloramphenicol, *Streptococcus faecalis* incorporates [¹⁴C]glycine into the free amino acid pool only and this compound is retained when the organisms are incubated in buffer at 0° or 37° (Brock & Moo-Penn, 1962). Whole bacteria containing [¹⁴C]glycine in their pools can thus be used to study the effect of temperature on the stability of the membrane. Radioactive glycine was released from the internal pool at a significant rate at 50°, and at temperatures of 60° or over, loss of the radioactive material was essentially complete in 3 min. (Fig. 2). When suspensions of *S. faecalis* labelled in DNA by incubation with [¹⁴C]thymidine with excess uracil were subjected to the same temperatures for up to 30 min. essentially no radioactivity was released.

Addition of polyamines and metal ions to protoplasts of *Sarcina lutea* and *Streptococcus faecalis* gave some protection against heating for 60 min. at 60°. Spermine (5 mM) and

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spermidine (20 mM) gave essentially complete stabilization, while magnesium, cadaverine, and streptomycin (all 20 mM) were partly effective. Other cations (Ca^{2+} , lysine, ornithine and putrescine) were only weakly active or ineffective. The order of effectiveness of these compounds is similar to that on osmotic fragility (Harold, 1964) and binding to nucleic acid (Brock & Wooley, 1963), the effective compounds being those which have the highest cation charge density. Lower concentrations of polyamines gave partial protection against thermal lysis, and in the presence of 50 mM-NaCl or phosphate the polyamines were no longer effective in preventing thermal lysis.

These results show that temperature causes destruction of the protoplast membrane of mesophilic bacteria and that this destruction can be prevented by polyamines.

Fig. 1

Fig. 2

Fig. 1. Effect of temperature on protoplasts of *Streptococcus faecalis* (ATCC 9790) in isotonic medium. The protoplasts, made as described by Harold (1964), were stabilized in 1 M-sucrose. The suspensions were incubated at various temperatures for the time periods given, and after cooling the extinction was measured at 640 nm.

Fig. 2. Effect of various temperatures on the release of [^{14}C]glycine from the pool of suspensions of *Streptococcus faecalis*. [^{14}C]Glycine was allowed to accumulate in the free amino acid pool of *S. faecalis* (Brock & Moo-Penn, 1962). After washing in $\text{PO}_4\text{-NaCl}$ buffer the suspensions were incubated at the various temperatures described and filtered through membrane filters which were counted in a Nuclear Chicago gas-flow counter.

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