OSMOTIC STABILITY OF BACTERIAL PROTOPLASTS RELATED TO MOLECULAR SIZE OF STABILIZING SOLUTES*

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The solute requirements for osmotic stabilization of bacterial protoplasts have puzzled many investigators. Weibull (1956) concluded from his work with Bacillus megaterium protoplasts that "the stabilization phenomenon can hardly be explained in exclusively osmotic terms." Membrane permeability appears to play a major role in the phenomenon (Mitchell and Moyle, 1956a, 1956b), and solutes that can readily penetrate the protoplast membrane, e.g., glycerol, do not serve as stabilizers. Other solutes that enter the cell by respiration-coupled mechanisms, e.g., sucrose and aminoisobutyrate, serve as stabilizers for nonrespiring protoplasts but not for respiring ones (Weibull, 1955, 1956; Marquis and Gerhardt, 1964). Further, solutes vary widely in regard to their effective osmolalities (Weibull, 1955), and noncolligative properties appear to be important for stabilization. This report presents evidence, derived from experiments with mono-, di-, tri- and tetra-saccharides, that molecular size is an important factor in the stabilizing capacity of sugars for nonrespiring protoplasts. Sugars with larger molecular radii were found to be more effective stabilizers, and this finding was interpreted as an indication that the protoplast membrane may be perforated with small, aqueous channels.

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METHODS

Organism. B. megaterium strain KM was grown in aerated 2% Oxoid peptone broth. Cells were harvested by centrifugation in the cold at the phase of declining growth rate.

Experimental procedures. Protoplasts were prepared at room temperature by suspending cells in sucrose solutions containing 0.1 mg muramidase per ml and 2 mM phosphate buffer, pH 7. The protoplast suspensions were then chilled in an ice bath and diluted forty-fold with appropriate chilled solutions in order to lower the osmolality of the suspending medium. Protoplast suspensions were not centrifuged because centrifugation, even in the cold, altered the apparent internal osmolality of the protoplasts.

Protoplast concentrations were determined with a Petroff-Hausser counting chamber, and at least 100 protoplasts were counted per sample. Chilled protoplasts were found to be stable in ribose solutions for about three hours and in solutions of glucose and larger sugars for more than two days.

Protoplast diameters were measured with an ocular micrometer and also by use of photomicrographic enlargements. More than 50 protoplasts were measured to arrive at a single value for the average diameter.

RESULTS

Osmotic responses of protoplasts. As shown in Fig. 1, the bursting response of protoplasts to lowered external sucrose molality was markedly influenced by the osmolality of the original medium in which they were formed. For example, half the protoplasts formed in 2 molal sucrose solution burst when the external osmolality was reduced to 1.0; whereas a reduction to ca. 0.44 molal sucrose was necessary to cause 50% bursting of protoplasts prepared in 0.5 molal sucrose solution. These differences in protoplast stability presumably reflected differences in internal osmolality (Mitchell and Moyle, 1956b), especially since the yield of protoplasts per cell here was unaffected by the osmolality of the suspending medium. Protoplasts prepared in 2 molal sucrose solution were used in all subsequent experiments.

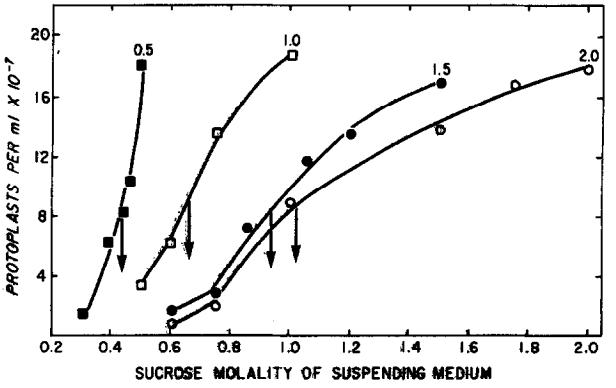

Figure 1. Osmotic stability of protoplasts related to the sucrose molality of the medium in which they were formed. Initial suspending media contained: ■ 0.5 molal, □ 1.0 molal, ● 1.5 molal or ○ 2.0 molal sucrose prior to dilution. Arrows indicate the extracellular sucrose molalities at which 50% protoplast bursting occurred.

Protoplasts responded to lowered external osmolality by swelling as well as by bursting (Fig. 2). The two responses occurred conjointly, and the fraction of protoplasts bursting in a suspension was found to be proportional to the size of the remaining protoplasts. The results (Figs. 1 and 2) indicate also that apparent internal osmolality is a continuous variable in protoplast populations.

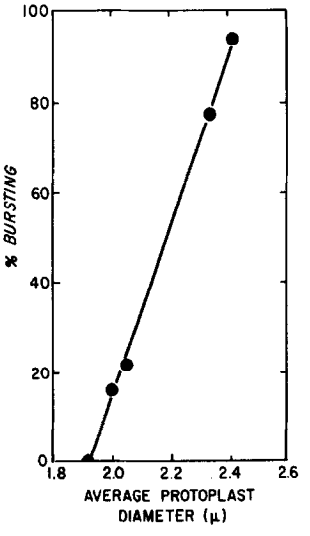

Figure 2. Relation between protoplast swelling and bursting in response to lowered osmolality of the suspending medium.

B. megaterium protoplasts have been found (Fitz-James, 1964) to lack mesosomes and to be bounded by an essentially unarticulated membrane. The capacity of protoplasts to swell suggests then that the limiting membrane is extensible, sufficiently so that in these experiments protoplast surface area increased on the average over 60%, from 11.4 μ^2 to 18.4 μ^2, when the volume increased from 3.6 μ^3 to 7.4 μ^3.

Stabilizing capacities of sugars. If protoplast swelling and bursting were due solely to osmotic pressure differences across a membrane completely impermeable to lipid-insoluble solutes, then protoplast stability should be predictable in terms of these differences, or for a given set of protoplasts, in terms of the external osmolality. However, the results presented in Fig. 3 indicate that the stabilizing capacities of sugar solutions depend not only on their osmolalities but also on molecular size of the dissolved sugars. (The sugars used were all considered to have osmotic coefficients of essentially one.) In essence, sugars with larger molecular radii were more effective stabilizers.

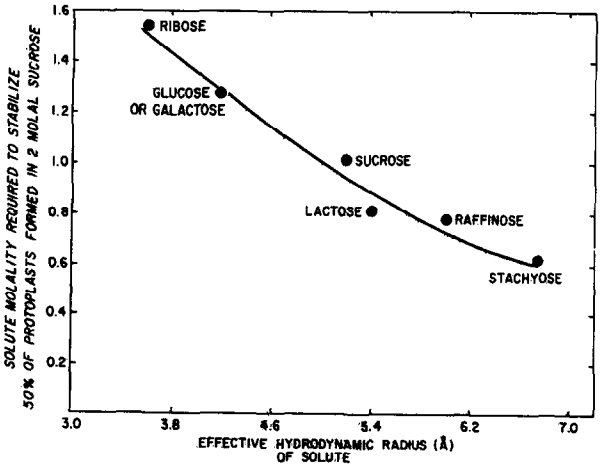

Figure 3. Protoplast stabilizing capacities of sugars as a function of hydrodynamic molecular radii. The molecular dimensions are those calculated by Schultz and Solomon (1961), with the exception of stachyose. Longsworth's (1953) formula was used to estimate the effective radius of stachyose from its diffusion coefficient. The position of the curve was shifted upward or downward when protoplasts with respectively higher or lower average apparent internal osmolalities were used; the shape of the curve remained unchanged.

DISCUSSION

One may interpret the above results by considering that membrane deformations underlying protoplast swelling and bursting are responses to osmotically induced stress on the membrane. Stretching of the extensible protoplast membrane could lead to the formation of small fissures or rents which expand in response to further stress. Thus with a particular stress (external osmolality of ca. 1.54 in the present experiments), the average protoplast would develop pores large enough for ribose molecules to enter. With more stress (external osmolality of ca. 1.28), the pores would become large enough for glucose molecules to enter, and so on.

Alternatively, one may interpret the results as an indication that the major factor in stabilization is the relative rate at which a stabilizing sugar can diffuse across the protoplast membrane through small, preformed, water-filled pores similar to those which Solomon (1960) has characterized in animal cells. Thus protoplasts would be stable in a particular sugar solution only if the initial external osmolality was sufficiently high so that diffusion of the sugar through membrane pores did not cause the internal osmolality to greatly exceed the external osmolality during the experimental period. This second hypothesis does not readily account for the finding that, once stabilized, chilled protoplasts remain intact and unswollen for long periods of time (days) in solutions of sugars larger than ribose.

The experimental results do seem to support the notion that aqueous channels penetrate the membrane of naked protoplasts, even though they may form only in response to stress. There is evidence that pores may be present in the membrane of wall-ensheathed protoplasts also. Scherrer and Gerhardt (1964) have described a sieving effect in the uptake of polyethylene glycols and dextrans by whole cells of B. megaterium. They found that the cell wall behaved as if it were a coarse sieve, while the underlying membrane behaved as if it were a finer sieve.

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