The mechanical properties of the *Microcystis* gas vesicle

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Gas vesicles isolated from the cyanobacterium *Microcystis* sp. retained their high stability and critical pressure on storage and could be held under a sustained pressure of 0.5 MPa without collapse. Application and release of pressures up to 0.5 MPa gave reversible changes in volume that indicated an elastic compressibility of 0.0087 MPa^{-1} , which was linear with pressure. The elastic bulk modulus of the *Microcystis* gas vesicle was 115 MPa. The average yield pressure of the gas vesicles, 4.3 MPa, was determined by infiltrating them with gas at high pressure and then releasing the pressure so that they exploded. From these two measurements the proteinaceous wall of the gas vesicle was calculated to have a Young's modulus of 3.8 GPa and a yield stress of 78 MPa. These values correspond closely to those of nylon, which has a similar secondary structure to that of the gas vesicle protein. The critical collapse pressure of the *Microcystis* gas vesicle, 0.8 MPa, is substantially higher than the theoretical buckling pressure, 0.2 MPa, for an unstiffened homogeneous cylinder of similar relative dimensions. The stiffening of the gas vesicle may be provided by its outer layer of GVPc, since the removal of this protein decreases the critical pressure to 0.23 MPa. The volumetric compressibility of the gas vesicle generates a small reversible change in turbidity (0.008 MPa^{-1}). Spectrophotometric measurements showed that a gas vesicle suspension containing $1 \mu\text{l ml}^{-1}$ of gas gives a pressure-sensitive optical density of 2.03 cm^{-1} at a wavelength of 500 nm.

Introduction

The gas vesicles of cyanobacteria and other aquatic prokaryotes are hollow structures that provide buoyancy. The wall of the gas vesicle is made of protein molecules stacked in ribs arranged in the form of a cylindrical tube closed at each end by a hollow cone. The structure is rigid and will withstand considerable pressures before collapsing to a flattened envelope (see Walsby, 1972, 1989, for reviews).

In nature, gas vesicles encounter hydrostatic pressures generated principally by the overlying water column and the osmotically generated cell turgor pressure. These pressures range from as little as 0.01 MPa (0.1 bar) inside halophilic bacteria inhabiting shallow brine pools, to more than 2 MPa (20 bar) in cyanobacteria of the deep oceans (Walsby, 1971, 1978). There has been natural selection for gas vesicles of different strength in bacteria that encounter different pressures; the strongest gas vesicles have the narrowest diameter and provide less buoyancy. Where high pressures are not encountered, weaker and wider gas vesicles are found (Walsby, 1972; Hayes & Walsby, 1986; Walsby & Bleything, 1988); they provide buoyancy with greater efficiency (Walsby, 1987).

In an attempt to understand what determines the pressure at which a gas vesicle collapses, the elastic properties of the gas vesicle have been investigated (Walsby, 1982). Measurements were made of the reversible, elastic compressibility of gas vesicles isolated from the cyanobacterium *Anabaena flos-aquae*. Application of a pressure $P = 0.1 \text{ MPa}$, considerably less than the critical collapse pressure, caused a relative volume reduction (dV/V) of only 0.0155. From this value, and measurements of the cylinder radius and wall thickness, an estimate of the average Young's modulus of the gas vesicle wall protein was obtained, $Y = 2.8 \text{ GPa}$. A general theory for buckling of thin-walled hollow structures (DeHart, 1969) indicates that a cylinder with the relative dimensions and properties of the *Anabaena* gas vesicle would collapse at a pressure of only 0.06 MPa, if it were of homogeneous construction. The observation that the critical collapse pressure (p_c) of the *Anabaena* gas vesicle was about 10-fold higher (0.6 MPa) suggested that the elements in the gas vesicle wall were arranged in such a way as to stiffen the structure and so postpone buckling. It was suggested that the arrangement of the gas vesicle protein into ribs might provide ring-stiffening support (Walsby, 1982) and that the stresses in the gas vesicle wall would be equalized in the hydrogen bonds

between the β -chains in the protein, which are orientated at a special angle to the principal axis of the cylinder (Walsby, 1987).

In a thin-walled cylindrical structure, like the gas vesicle, that collapses by instability failure (DeHart, 1969) a measure of its mechanical efficiency can be obtained by comparing the average critical stress (s_c) in the wall, as the structure approaches its critical pressure (p_c), with the yield stress (s_y) at which the wall material fails. In instability failure the cylinder goes out-of-round so that the stresses are unevenly distributed and buckling results. The only way to determine the yield stress in such a structure is by measuring the pressure required to explode it, as buckling does not occur in a cylinder distended by internal pressure.

The gas vesicle is highly permeable to gases and the gas pressure inside it can be raised by slowly infiltrating the suspending medium with gas (Walsby, 1969). The gas vesicle will not collapse as long as the pressure difference between the gas above the suspension and that in the structure does not exceed p_c during the pressure rise; with this precaution gas vesicles can be loaded with gas at 5 MPa or more (Walsby, 1984).

I describe below experiments in which the gas pressures above such supersaturated suspensions were suddenly released, causing gas vesicles to explode. By finding the pressure required to do this, the yield pressure (p_y) of the gas vesicle could be determined. The experiments are similar to those performed on suspensions of bacteria by Hemmingsen & Hemmingsen (1980). They found that cells containing gas vesicles were blown apart by gas bubbles that nucleated from the expanding vesicle gas. In a detailed quantitative analysis of their experiments it was concluded that these bubbles would have arisen from exploded gas vesicles (Walsby, 1989) though there was no direct evidence of this.

Measurements of the critical pressure and yield pressure described below were made on gas vesicles isolated from the cyanobacterium *Microcystis* sp. They are similar in structure to those of *Anabaena* but they are somewhat narrower and, consequently, have a higher critical pressure (Walsby & Bleything, 1988). They also remain more stable when isolated from the cells (Walsby & Hayes, 1988) and do not show the anomalous instability, when held under pressure, that has been encountered with gas vesicles isolated from *Anabaena* (Walsby, 1980). This has permitted more accurate measurements to be made of their elastic compressibility and other properties.

The mechanical principles of the *Microcystis* gas vesicles should be generally applicable to those of other cyanobacteria, as they have a similar molecular structure. The main component, GVPa, is a highly conserved, small, hydrophobic protein (Tandeau de Marsac *et al.*,

1985; Walker *et al.*, 1984; Hayes *et al.*, 1986), which forms the ribs of the structure (Walsby & Hayes, 1988). The gas vesicles of *Anabaena* and *Microcystis* (Hayes *et al.*, 1988) and certain other cyanobacteria (see Damerval *et al.*, 1987, 1989) also contain a second, larger, hydrophilic protein, GVPc, which occurs on the outer surface. Gas vesicles are markedly weakened when GVPc is removed (Walsby & Hayes, 1988), but they regain their strength when this protein rebinds onto the structure, perhaps because it holds the ribs together (P. K. Hayes, B. Buchholz & A. E. Walsby, unpublished).

Methods

Gas vesicle isolation. The cyanobacterium *Microcystis* sp. strain BC 84/1 was grown in medium BG 11 of Rippka *et al.* (1979). Gas vesicles were isolated from cells lysed with lysozyme and were purified with the methods described by Walsby (1974) and Hayes (1988). They were stored at 5 °C in a solution of 6 mM-NH₄HCO₃ and 5 mM-NaCN.

Critical collapse pressures. The critical pressure distribution of the gas vesicles was determined by turbidity measurements (Walsby, 1980) with a modification of the pressure nephelometer of Walsby (1973) fitted with a Kistler 4041 A50 pressure transducer connected to a four-place digital display sensitive to 0.5 kPa (5 mbar). The transducer was calibrated against a mercury column and a pressure balance. The output of the nephelometer photocell was recorded on another four place digital meter. The percentage of gas vesicles collapsed at a given pressure was calculated as $100(T - T_b)/(T - T_c)$, where T is the initial turbidity of the suspension, T_b is the turbidity remaining after applying the pressure, and T_c is the turbidity remaining after all the gas vesicles were collapsed (Walsby, 1973). The turbidity was measured after the pressure at each step had been held for about 12 s. The statistical methods of Walsby & Bleything (1988) were used to calculate the median p_c , the mean p_c and the standard deviation of the critical pressure distribution. All the pressures given are gauge pressures (in excess of the ambient pressure). Critical pressures were determined with suspensions of gas vesicles that had been equilibrated with air at the ambient pressure; the pressure of the vesicle gas is then identical to the ambient pressure, because the gas vesicles are highly permeable to gas (Walsby, 1984).

Compressibility. Measurements of the compressibility and gas content of gas vesicle suspensions were made by the methods described by Walsby (1982). A compression tube with an open-ended capillary was filled with the gas vesicle suspension and thermally equilibrated in a pressure tube with a thermostatted water jacket (Oliver & Walsby, 1988). Pressure was transmitted to the gas vesicle suspension via the open capillary. The resulting reduction in volume of the gas vesicles was indicated by the change in position of the meniscus in the capillary, after due allowance was made for changes in volume of the suspending water and the capacity of the glass tube. The volume of gas-vesicle gas in a suspension was determined from the irreversible change in volume after applying and the releasing a pressure (1.3 MPa) that collapsed all of the gas vesicles.

Yield pressure of the gas vesicles. Suspensions of gas vesicles were equilibrated with nitrogen or helium gas at high pressure in a stainless steel tube attached to a gas-handling system constructed from 3 mm diameter, 1 mm bore Teflon tubing connected with Swagelok pressure connectors to Whitey miniature forged-body needle valves and shut-off

Fig. 1. Schematic diagram of the apparatus used to equilibrate gas vesicles (V), in aqueous suspension, with gas (G) at high pressure, and then subject them to rapid decompression, causing them to explode: A, stainless steel pressure tube; B Bourdon-tube pressure gauge; C, cylinder of compressed gas; D, E, sections of microbore tubing used for D, decompression of the gas vesicles, and E, expansion of the decompressed suspension; F, pressure connector; S, reciprocal shaker; T, pressure transducer with 10 MPa digital display; 1–10, shut-off valves or needle valves (6, 8, 9) used to regulate gas flow.

valves (Fig. 1). The section of the apparatus between valves 3 and 5 was mounted on a shaker with a 35 mm stroke, oscillating at 1 Hz. The apparatus was operated as follows.

(1) With valve 1 closed, 5 ml of gas vesicle suspension (V) was placed in tube A, the pressure connector F was inserted and the shaker was started. (2) With valves 2, 3, 4 and 5 open, and 6, 7, 8, 9 and 10 closed, the pressure in the regulator of gas cylinder C was raised to 5 MPa or more. (3) Valve 10 was then opened. (4) By adjusting inlet valve 6 the gas pressure in tube E and the rest of the system was raised at a rate not exceeding 0.4 MPa h^{-1} , monitored on the output of the pressure transducer T. (5) When the final pressure was reached, valve 6 was closed and the suspension was equilibrated for a further 30 min. A sample of the suspension was removed from tube A for explosion as follows. (6) Tube A was tilted so that the suspension covered the opening to valve 1. (7) Valve 1 was opened. (8) Valves 3 and 4 were closed. (9) Valve 9 was opened briefly, reducing the pressure on gauge B by about 0.02 MPa (0.2 bar), and then closed. (10) Valve 3 was opened slightly, causing $40 \mu\text{l}$ of suspension V to seep through valve 1, filling the tubing D, and then valve 3 was closed. (11) Valve 2, and then valve 1, were both closed, and the following two steps were performed in quick succession. (12) Valves 3 and 9 were opened fully so that the gas pressure dropped completely. (13) Valve 2 was opened rapidly so that the pressure was released on the gas vesicles. When the pressure drop was sufficient the gas vesicles exploded. The suspension outgassed, expanding into the tubing E between valves 2 and 3. (14) The compression fitting holding the tubing to valve 1 was unscrewed and the tubing end placed in an Eppendorf sample tube. (15) Outlet valve 9 was closed and by adjusting valve 8 the pressure was raised slightly to blow the 'exploded' sample into the sample tube. (16) The sample tube was removed and valve 8 was opened fully, to blast out any remaining drops of suspension; it was then closed. (17) The tubing was reconnected to valve 1. (18) Inlet valve 8 was operated to raise the gas pressure on gauge B to the same pressure as shown on transducer T. (19) Valve 4 was opened and the shaker restarted. (20) Valve 9 was operated to reduce the pressure by 0.5 MPa . The suspension in tube A outgassed until it was in equilibrium with the new pressure. (21) The cycle from step (6) to (21) was then repeated. (22) Finally, the pressure was slowly

reduced to the ambient pressure and a final sample of suspension V was taken. The relative gas vesicle content of each of the samples was then determined by pressure nephelometry.

Results

Critical pressure distribution of the gas vesicles

Gas vesicles in cells of *Microcystis* sp., suspended in 0.5 M -sucrose solution to remove turgor pressure, had the critical pressure distribution shown in Fig. 2, curve A, with a median p_c of 0.780 MPa , a mean p_c of 0.787 MPa and a standard deviation of 0.140 MPa , similar to the values obtained by Walsby & Bleything (1988). Gas vesicles were isolated and purified from the cell suspension. Eleven days after the start of the purification procedure they were concentrated into 2.3 ml of suspension; a sample diluted 540-fold in $5 \text{ mM-K}_2\text{HPO}_4$ showed a critical pressure distribution (Fig. 2, curve B) with a median p_c of 0.890 MPa , a mean p_c of 0.867 MPa and a standard deviation of 0.136 MPa . The turbidity readings were virtually steady after the 12 s applications of pressure at each step (but see below).

Stability of isolated gas vesicles during storage

The gas vesicle suspensions stored at 5°C remained intact over long time periods. Gas vesicles isolated from *Anabaena flos-aquae* appeared essentially unchanged in 10 years of storage, but they were mechanically much

Fig. 2. Critical pressure distributions, determined by pressure nephelometry, of *Microcystis* gas vesicles: A, gas vesicles inside cells suspended in 0.5 M-sucrose (means and standard deviations of five measurements); B, suspension of purified gas vesicles, isolated from the cells used in measurement of bulk elastic modulus (means of three measurements); C, the same suspension after storage for 4 weeks, used to investigate stability under a sustained pressure of 0.8 MPa (arrow) initially causing 61% gas vesicle collapse; D, isolated gas vesicles stored at 5°C for over 2 years (means of four measurements).

weaker and collapsed on application of <0.2 MPa. The *Microcystis* gas vesicles also became weaker during storage: the median critical pressure decreased from 0.89 MPa to 0.80 MPa after 4 weeks and, in another sample, to 0.61 MPa after 2.4 years (Fig. 2, curve D). Most of the following experiments were performed on samples within 5 weeks of their preparation.

Stability of isolated gas vesicles under sustained pressure

In a sample of the original suspension left under pressure of 0.50 MPa for 12 s, the turbidity change indicated that 2.4% of the gas vesicles collapsed. Another sample of the same suspension left for over 20 min at the same pressure lost less than 3% of its gas vesicles. This indicated that the critical pressure was virtually independent of the exposure time. A more sensitive test of this was made (with the same preparation after 4 weeks' storage) by applying a pressure in the region of the steepest gradient on the critical pressure distribution (Fig. 2, curve C). The suspension was subjected to a pressure raised from 0 to 0.80 MPa in 6 s, and then held without change for 5 min while the turbidity change was recorded at frequent intervals (see Fig. 3). Immediately the initial pressure rise had stabilized (i.e. at 6 s) the turbidity change indicated that 60.6% of the gas vesicles had collapsed. After a further 12 s, the time interval routinely used between pressure increments, the percentage collapsed reached 61.6% and the value stabilized at 64.4% after 3.5 min (see Fig. 3).

Elastic compressibility of the gas vesicle suspension

A sample of the original (undiluted) suspension, containing approximately $60 \mu\text{l ml}^{-1}$ of gas vesicle gas, was

Fig. 3. Collapse of gas vesicles, indicated by the turbidity change, with time after applying a pressure at A of 0.80 MPa; the broken line indicates the final percentage of gas vesicles collapsed; at B the pressure was raised to 1.45 MPa.

exposed to a hydrostatic pressure of 0.55 MPa for 20 min to ensure that in subsequent repeated applications of 0.50 MPa no further gas vesicles would collapse and that the only volumetric change that occurred would be due to elastic compressibility. Analysis of the collapse-pressure curve (Fig. 2, curve B) above 0.55 MPa indicates that the critical pressure distribution of the gas vesicles remaining would have had a mean p_c of 0.882 MPa and standard deviation of 0.098 MPa.

Fig. 4. Reversible changes in the position of the meniscus in the capillary of a compression tube containing 2155 μl of water (○) or the same volume of a *Microcystis* gas vesicle suspension containing 124 μl of gas space (●). The points at 0.1 MPa intervals are means of three or four measurements; the 95% confidence limits do not extend beyond the symbols.

A compression tube of capacity 2158 μl was filled with this suspension and equilibrated at 20 °C. The meniscus in the capillary of the tube stabilized at 95 mm from the open end, indicating that the volume of the suspension was 2155 μl . The pressure in the pressure tube was raised in steps of 0.05 MPa to a final value of 0.25 MPa, or steps of 0.10 MPa to a final value of 0.50 MPa, and then released. The position of the meniscus was noted at each step (Fig. 4) before proceeding to the next. The procedure was repeated several times. There was no significant change in the compressibility during the measurements; application of 0.40 MPa gave a change in meniscus position of 25.89 mm at the start and 25.90 mm at the end of the series of readings, which showed that no gas vesicles were collapsed during the repeated compressions. The results (Fig. 4) showed that there was a linear change in meniscus position (M_s) with pressure (P), described by the regression equation $M_s = (-0.16 + 66.12 P/\text{MPa})$ mm, with a correlation coefficient of $r = 0.9999$ for $n = 28$ measurements. As a 1 mm change is equivalent to a volume decrease of 0.0314 μl , the results indicate an overall volume change of $dV_t = -2.077 \mu\text{l MPa}^{-1}$ for the 2155 μl of suspension in the compression tube. The overall contraction is made up of volume changes resulting from compression of the gas vesicles (dV_g), the suspending water (dV_w) and, in a counteracting manner, the compression tube (dV_i) such that

$$dV_t = dV_g + dV_w - dV_i \quad (1)$$

(Walsby 1982). The values of the last two terms were determined by making similar measurements with the tube filled with water. The results (Fig. 4) again indicated a linear change in meniscus position with pressure, described by the regression equation $M_w = (-0.07 + 29.27 P/\text{MPa})$ mm, with a correlation coefficient of $r = 0.9998$ for $n = 8$ measurements. This indicated an apparent volume change of $dV'_i = -0.920 \mu\text{l MPa}^{-1}$ for the 2155 μl of water in the compression tube.

When the compression tube is subjected to pressure P (equal on the inside and outside) its capacity (V_i) also undergoes a relative contraction that is given by

$$dV_i/V_i = -P/K \quad (2)$$

where K is the bulk elastic modulus of glass, 4.6×10^4 MPa. The volume change expected for the tube of $V_i = 2155 \mu\text{l}$ is therefore $dV_i = -0.047 \mu\text{l MPa}^{-1}$.

Since this tube contraction has a counteracting effect on the movement of the water in the capillary, the actual volume change of the water is given by

$$dV'_w = dV'_t + dV_i \quad (3)$$

and hence the total volume change of the water was $-0.967 \mu\text{l}$. The compressibility of water, $-dV'_w/V_i P$, indicated by these measurements was $(0.967 \mu\text{l}/2155 \mu\text{l}) \text{MPa}^{-1} = 4.49 \times 10^{-4} \text{MPa}^{-1}$, which is within 1.6% of the published value, $4.56 \times 10^{-4} \text{MPa}^{-1}$ (Hodgman, 1963).

The elastic modulus of the *Microcystis* gas vesicle

The compression tube was emptied of the gas vesicle suspension, which was diluted 98.94-fold with water. The compression tube was then refilled with samples of diluted suspension and the change in the meniscus position was measured after applying, and the releasing, a pressure of 1.3 MPa. Triplicate determinations showed an irreversible contraction of 39.89 ± 0.16 mm, indicating the collapse of 1.253 μl of gas vesicle gas space. The gas volume in the 2155 μl of the original undiluted suspension was therefore 124 μl .

According to the measurements of Walsby & Bleything (1988) the gas occupies 0.885 of the total volume of the gas vesicle in this strain of *Microcystis*. The total volume of gas vesicles in the suspension was therefore $V_g = 140 \mu\text{l}$ and the remaining volume occupied by water was 2015 μl . The expected volume change of this water would therefore have been $dV_w = -0.967 \mu\text{l MPa}^{-1} \times 2015/2155 = -0.904 \mu\text{l MPa}^{-1}$. Substituting the values for dV_t , dV_w and dV_i in equation (1) and rearranging gives $dV_g = (-2.077 + 0.904 - 0.047) \mu\text{l MPa}^{-1} = -1.220 \mu\text{l MPa}^{-1}$. Dividing by the volume of the gas vesicle gas space in the compression tube gives the compressibility of the gas vesicle as $dV_g/V_g =$

Fig. 5. The proportion of gas vesicles surviving rapid decompression after equilibration with gas at the pressure indicated. The different symbols indicate different experimental runs, using helium (■) or nitrogen (○, □, △) as the equilibrating gas.

$-0.00871 \text{ MPa}^{-1}$. From this we can calculate the elastic bulk modulus of the gas vesicle as

$$K_a = -V_g P/dV_g \quad (4)$$

Hence, putting $P = 1 \text{ MPa}$, $K_a = 115 \text{ MPa}$.

Elastic changes in gas vesicle volume detected by turbidity changes

The elastic compression of gas vesicles is known to cause a small reversible change in turbidity (Walsby, 1971). An attempt was made to measure this change and relate it to the volumetric change. The critical pressure distribution of a suspension, determined by pressure nephelometry, had a mean of 0.787 MPa and standard deviation of 0.143 MPa . The initial turbidity reading given by the suspension was amplified to give nearly the maximum reading on the photometer of the nephelometer; the overall turbidity change on collapsing all the gas vesicles was 1785 units. During the sequence of pressure rises the 0.55 MPa step was held on for 2 min and then released. The turbidity decreased by 116 units below the initial value, indicating 6.3% gas vesicle collapse. The 93.7% of gas vesicles remaining were exposed to repeated application, followed by release, of pressures from 0.1 to 0.5 MPa, resulting in reversible turbidity decreases of 1 to 6 units on the nephelometer. A regression analysis of the turbidity change (dT) and pressure (P) gave $T = (-0.6 + 13.1 P/\text{MPa})$ units, with a correlation coefficient of $r = 0.90$, on $n = 18$ measurements. The relative turbidity change was therefore $13.1/1669 \text{ MPa}^{-1}$, giving $dT/T = 0.0078$.

This reversible change is of the same order as the relative change in gas volume, $dV/V = 0.0098$, which generates the turbidity change. These turbidity changes are near the limit of detection and it cannot be concluded that the difference between the two measurements is significant. The reversible turbidity change caused by

the compressibility of intact gas vesicles will generate a small error in the estimate by nephelometry of the percentage of gas vesicles collapsed by a given pressure. At the median collapse pressure of 0.8 MPa , for example, the percentage of gas vesicles remaining would be underestimated by $50\% \times 0.8 \text{ MPa} \times 0.0078 = 0.3\%$.

Relation between gas vesicle volume and optical density

Changes in turbidity provide the most sensitive method of detecting gas vesicles and measuring the relative changes in gas vesicle content, but it is difficult to standardize nephelometric measurements. However, the turbidity of gas vesicle suspensions generates an optical density (apparent light absorbance) that is reproducible and can be used in standardizing the concentration of the suspensions.

Six replicate measurements of gas volume were made on a gas vesicle suspension using 2.4 ml capacity compression tubes. The suspension contained $0.5606 \pm 0.0098 \mu\text{l ml}^{-1}$ of gas. The suspension was diluted fivefold and eight measurements were made of the optical density at 500 nm, before and after collapsing the gas vesicles with a pressure of 1.4 MPa . The mean pressure-sensitive optical density (PSOD) of the original undiluted suspension was $1.135 \pm 0.016 \text{ cm}^{-1}$, and hence a PSOD of 1 cm^{-1} indicates a gas vesicle gas concentration of $0.494 \mu\text{l ml}^{-1}$. In the *Microcystis* gas vesicle the average ratio of gas volume to wall (protein) mass is $5.84 \mu\text{l mg}^{-1}$ (Walsby & Bleything, 1988); a PSOD at 500 nm of 1 cm^{-1} therefore indicates a gas vesicle protein concentration of $0.0845 \text{ mg ml}^{-1}$.

The yield pressure of *Microcystis* gas vesicles

Measurements of the yield pressure were made in four experiments on a suspension of *Microcystis* gas vesicles that initially had a critical pressure distribution similar to

curve B in Fig. 2, with a median critical pressure (p_c) of 0.80 MPa, a mean p_c of 0.78 MPa, and a standard deviation of 0.15 MPa. In these four experiments an average of 82% of the gas vesicles remained intact in samples recovered from the pressure tube A after the full cycle of gradual compression and decompression. It is assumed that the loss of gas vesicles occurred through collapse during the compression stages and that the weakest 18% of the gas vesicles had been lost; those that remained would therefore have had a mean p_c of 0.83 with standard deviation of 0.094 MPa. Fig. 5 shows the percentages of these remaining gas vesicles that withstand rapid decompression from different pressures. The graph indicates that 50% of the gas vesicles would have exploded when decompressed from a pressure of 4.3 MPa (43 bar). This is the estimate of the median yield pressure, p_y , of the gas vesicles.

Discussion

The gas vesicles isolated from *Microcystis* have proved to be more stable than those of *Anabaena*: they remained more stable when stored, and were more stable when held under sustained pressures. The *Microcystis* gas vesicles therefore provide better material for a range of experiments. The gas vesicles that had been freshly isolated had a slightly higher mean critical pressure than those in the cells, probably because some of the weaker gas vesicles in the preparation were lost during centrifugation; the highest critical pressures were no higher than those of gas vesicles in the cells.

Inside cells, gas vesicles have to withstand sustained pressures generated by cell turgor and the hydrostatic head of water. Isolated gas vesicles that had been subjected to a pressure of 0.55 MPa subsequently withstood a pressure of 0.5 MPa without further collapse, but for those subjected to pressures closer to their critical pressure there appeared to be some dependence on the duration of the pressure. The cause of this is not known.

Because the gas vesicles isolated from *Anabaena* were more sensitive to this time-dependent collapse it was not possible to investigate their elastic compressibility at pressures approaching their critical pressures; at pressures up to 0.1 MPa the average compressibility was 0.0155 MPa^{-1} and (in contravention of Hooke's law) it appeared to increase slightly with rising pressure (Walsby, 1982). More accurate measurements were made here with the *Microcystis* gas vesicles, which could be held without collapse at pressures up to 0.5 MPa; the compressibility, of 0.00871 MPa^{-1} , was perfectly linear up to this value. A lower compressibility is expected for the *Microcystis* gas vesicle because it is narrower and a given pressure therefore acts on a smaller cross-sectional area to develop a lower stress in the wall. A more useful

comparison is made between the elastic properties of the wall material of the two types of gas vesicle.

The Young's modulus (Y) of the wall of a cylindrical structure, like a gas vesicle, can be calculated from the (rearranged) equation of Newman & Searle (1957)

$$Y = K_a r (4\sigma - 5) / 2t \quad (5)$$

For the *Microcystis* gas vesicle, K_a , the elastic bulk modulus, is 115 MPa; r , the cylinder radius, is 32.6 nm; t , the mean wall thickness, is taken to be 1.8 nm (see Walsby & Bleything, 1988); and σ , the Poisson ratio, 0.33 (see Walsby, 1982). Equation 5 then gives $Y = 3.8 \text{ GPa}$. By substituting the appropriate values for the *Anabaena* gas vesicle of $K_a = 64.5 \text{ MPa}$, $\sigma = 0.33$ (Walsby, 1982) $r = 42 \text{ nm}$ and $t = 1.8 \text{ nm}$ (Hayes *et al.*, 1986), the value calculated for its wall material is $Y = 2.8 \text{ GPa}$. This somewhat lower value must be related to the fact that the *Anabaena* gas vesicle became weaker when isolated from the cells.

When cyanobacterial gas vesicles collapse, the cylinders form rectangular flattened envelopes. The form of the collapsed structure indicates that the gas vesicle undergoes instability failure; the cylinder goes out-of-round and buckles so that breakage lines propagate parallel to the axis. In a cylinder of homogeneous wall structure the expected buckling pressure is given by

$$p_b = [Yt^3(q^2 - 1)]/[12(1 - \sigma^2)r^3] \quad (6)$$

where Y , t , r and σ have their previous meanings and q is the number of half-waves in the buckled form (Allen & Bulson, 1980). In a cylinder, like the gas vesicle, with an axial ratio of about 10, $q = 2$, whence $q^2 - 1 = 3$, and

$$p_b = [Yt^3]/[4(1 - \sigma^2)r^3] \quad (7)$$

(see Walsby, 1982). Substituting the values of Y , t , r and σ for the *Microcystis* gas vesicles gives $p_b = 0.18 \text{ MPa}$. This predicted buckling pressure is only 0.2 of the observed mean critical pressure ($p_c = 0.882 \text{ MPa}$ for the sample analysed); it confirms that the gas vesicle structure must be stiffened in such a way as to postpone buckling (Walsby, 1982). The theoretical buckling pressure is close to the decreased critical pressure of 0.23 MPa that is observed when the outer covering of GVPc is removed from the *Microcystis* gas vesicle (Walsby & Hayes, 1988). This suggests that GVPc provides the stiffening. It may do this by linking the ribs together (Walsby & Hayes, 1988; Hayes *et al.*, 1988).

The mean critical pressure of the *Microcystis* gas vesicles used in the explosion experiments, $p_c = 0.83 \text{ MPa}$, was, in turn, only 0.2 of their yield pressure when the excess pressure was on the inside, $p_y = 4.3 \text{ MPa}$. This confirms that, although buckling is postponed, instability failure under excess external pressure occurs well before the yield pressure is reached.

An estimate of the yield stress of the proteinaceous gas vesicle wall can be obtained from the value of p_y . An excess pressure (p_i) inside a cylinder generates a longitudinal stress (s_l) in the wall, running parallel to the principal axis, equal to $p_i r/2t$. For reasons of geometry the transverse stress (s_t), at right angles to the cylinder axis, is twice this, $p_i r/t$. Failure of the cylinder, therefore, occurs when the transverse stress reaches the yield stress of the wall material, when

$$s_y = p_y r/t \quad (8)$$

Substituting the values given above for the *Microcystis* gas vesicles, it is calculated that $s_y = 78$ MPa. It is interesting to compare the mechanical properties of the gas vesicle wall material with those of synthetic polymers that have been investigated in bulk quantities. The principal protein of the gas vesicle wall, GVPa, is largely in the form of β -sheet in which β -chains of peptides, $[-CO-NH-CHR-]$, are hydrogen-bonded to adjacent chains (Blaurock & Walsby, 1976; see Fig. 6 of Walsby & Hayes, 1989). The most apt comparison is with the polymer nylon, which has an analogous structure with β -chains of $[-CO-NH-(CH_2)_n-]$ hydrogen-bonded in a similar way. The Young's modulus of dense nylon is within the range 2 to 4 MPa and its yield stress is 83 MPa (Ashby & Jones, 1980). These values are very close to those determined here for the gas vesicle protein. The agreement indicates that the mechanics developed by engineers for the understanding of large structures can be used for the understanding of biological structures with molecular dimensions.

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