The Dimensions of Cyanobacterial Gas Vesicles in Relation to Their Efficiency in Providing Buoyancy and Withstanding Pressure

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The lengths and cylinder diameters of gas vesicles isolated from eight cyanobacteria were determined by electron microscopy. From these measurements the volumes and buoyant densities of the gas vesicles were calculated. A quantitative analysis was made of the effects of length and diameter on efficiency in providing buoyancy. In seven of the cyanobacteria the mean length exceeded that which would give 90% of the maximum efficiency. It is postulated that the mean diameter (d) in each organism is constrained by the requirement for a particular critical pressure (p_c) to withstand the combination of turgor pressure and hydrostatic pressure experienced in the natural habitat, such that $d = 59 (p_c/\text{MPa})^{-0.56}$ nm. The mean diameter of gas vesicles from *Anabaena flos-aquae* was estimated to be 84.0 nm with a standard deviation of 4.1 nm. The weakest 10% of the vesicles had a diameter 6.2 nm wider than the strongest 10%, indicating that the variation was not an artifact of measurement. The intrinsic variation in width accounts for about a third of the observed critical pressure variation. The width of new gas vesicles formed in cells of *Anabaena flos-aquae* grown in 100 mM-sucrose, which reduced turgor pressure by half (to 0.17 MPa), was only marginally greater than in normal conditions, and it is concluded that the mean width is genetically determined and not directly altered by pressure.

INTRODUCTION

Many planktonic cyanobacteria and other aquatic bacteria derive their buoyancy from gas vesicles, which are hollow structures formed from proteins known as gas vesicle protein (GVP). The economy of providing buoyancy by gas vesicles can be related to the amount of protein that is required to enclose a given amount of gas space. The width of gas vesicles varies in different groups of prokaryotes; those of the halobacteria are widest and, as they have the same thickness of wall protein, they must have the lowest buoyant density and provide buoyancy with greatest economy (Walsby, 1971). The critical collapse pressure of a gas vesicle varies inversely as a function of its width, and this factor has clearly influenced the evolution of gas vesicles in different prokaryotes. Narrower, stronger gas vesicles are found in organisms that possess high cell turgor pressures and live in deep waters (Walsby, 1972). A higher ratio of GVP to gas space, and lower efficiency in providing buoyancy, is therefore the penalty that must be paid for the increased strength required to withstand greater pressures.

The gas vesicles of cyanobacteria were once considered to have approximately the same cylinder diameter, 65 to 75 nm (e.g. Bowen & Jensen, 1965; Jost, 1965; Smith & Peat, 1967; Jost & Jones, 1970), a range that might have been explained by the error of measurement by electron microscopy (Walsby, 1972). Hayes & Walsby (1986) have recently demonstrated, however, that the gas vesicles of five genera of cyanobacteria show significantly different cylinder diameters, ranging from 64 nm to 107 nm, and that the mean critical pressures of these gas vesicles are inversely related to their widths. There was, moreover, found to be a correlation between the critical pressure of gas vesicles in each organism and the pressure that they were likely to encounter in the natural habitat. Hayes & Walsby (1986) proposed that there had been natural

Abbreviation: GVP, gas vesicle protein.

selection of gas vesicles with the maximum width allowed by the requirement to withstand pressure.

We have extended the investigation of Hayes & Walsby (1986) by making length as well as width measurements on the gas vesicles of eight different cyanobacteria. From these data we have been able to determine the buoyant densities of gas vesicles, which can be related directly to their efficiency in providing buoyancy. The measurements made by Hayes & Walsby (1986) suggested that within each organism there was a small variation in gas vesicle cylinder diameter but they did not eliminate the possibility that these differences were artifacts caused by shrinkage or stretching during the preparation for electron microscopy. We have now demonstrated that genuine differences in diameter do exist, and that they account for part of the range of critical pressure of gas vesicles in a given organism.

If there are variations in the width of gas vesicles in a given organism it is possible that the width of individual gas vesicles might be regulated in some way. Economies in gas vesicle production could be achieved by making wider and hence weaker gas vesicles that survive under combinations of low hydrostatic and turgor pressure. We have therefore investigated the effect of lowering cell turgor pressure on the size of gas vesicles subsequently produced.

METHODS

Cyanobacteria and culture conditions. The cyanobacteria used are listed in Tables 1 and 2. They were grown in illuminated incubators at 20 °C, except for *Dactylococcopsis salina*, which was grown at 32 °C. The culture media used were medium B + N of Armstrong *et al.* (1983) for *Nostoc muscorum*, medium BG11 of Rippka *et al.* (1979) for *Microcystis* sp. and *Calothrix* sp., and the media described by Walker *et al.* (1984) for the other cyanobacteria.

Gas vesicle isolation. Intact gas vesicles were isolated by the methods described previously for species of *Dactylococcopsis*, *Oscillatoria*, *Anabaena*, *Aphanizomenon* (Walker *et al.*, 1984), *Microcystis* and *Calothrix* (Hayes & Walsby, 1986). Gas vesicles of *Nostoc muscorum* were prepared after lysing the cells with 0.7 M-sucrose, as for *Anabaena* (Walsby, 1974).

Electron microscopy. Isolated gas vesicles were prepared for electron microscopy by making platinum/carbon-shadowed replicas as described by Hayes & Walsby (1986). The gas vesicle replicas were photographed in a JEOL 1200-EX electron microscope at a magnification of about 20000. The exact magnification of each micrograph was determined by photographing a calibrated diffraction grating replica immediately after each micrograph. A particular marked square of the diffraction grating grid (Agar Aids grating with nominal spacing of 2160 lines mm⁻¹) was brought into focus with the mechanical stage, 'z' control of the electron microscope; this avoided refocusing with the lens current controls, which otherwise would have caused small magnification changes. The magnification of this 'sister' negative was determined by measuring the distance across the image of diffraction grating rulings with the vernier microscope. The images of gas vesicles on the corresponding micrograph negative, which were between 1.2 and 2.5 mm wide, were measured using a dissecting microscope with a calibrated eyepiece graticule. In this way errors generated by intermediate photographic enlargements were avoided.

Critical pressure of gas vesicles. The critical collapse pressure of gas vesicles of the various cyanobacteria was determined from pressure-sensitive turbidity measurements made with a pressure-nephelometer (Walsby, 1973). Measurements were made with the cells suspended in 0.5 M-sucrose, to remove turgor pressure, as described by Walsby (1980). The sucrose solution was supplemented with 5 mM-K₂HPO₄ as this was found to improve the stability of the gas vesicles under sustained pressure. Pressure was measured with a 10 cm diameter test gauge calibrated against a Kistler 5 MPa pressure transducer, which was itself calibrated against a mercury column for pressures of 0 to 0.1 MPa, and a pressure balance for 0.1 to 2.5 MPa. The median critical pressure $\bar{p}_c$, causing 50% of the gas vesicles to collapse, was determined from graphs of collapse-pressure curves of the type shown in Fig. 1. The mean critical pressure was calculated as follows. The percentage of gas vesicles collapsing between one pressure and the next was calculated (f_i). The mean of these two pressures (x_i) was determined and the products $f_i x_i$ and $f_i x_i^2$ calculated. The same procedure was followed for each of the pressure intervals and the sums $\sum f = n$ (= 100), $\sum fx$ and $\sum fx^2$ were calculated. The mean critical pressure was then $\bar{p}_c = \bar{x} = \sum fx/n$ and standard deviation $SD = \{[n \sum fx^2 - (\sum fx)^2]/n(n-1)\}^{1/2}$.

*Growth of *Anabaena flos-aquae* with reduced turgor pressure.* Cultures were harvested by mild centrifugation and the filaments exposed to a pressure of 1.0 MPa (10 bar) to collapse their gas vesicles. The filaments were then resuspended in culture medium (Walsby & Booker, 1980) supplemented with sucrose at concentrations of 100, 200 and 300 mM in one experiment and 25, 50, 100, 125 and 150 mM in the next. Sucrose, which does not permeate the cells of this species, gave a reduction in cell turgor pressure that was sustained for several days, as recorded previously (Walsby, 1980). These cultures were left to re-form gas vesicles, incubated at 20 °C under continuous illumination at an incident photon irradiance of 10 $\mu\text{mol m}^{-2} \text{s}^{-1}$ as measured with a Macam quantum sensor measuring between wavelengths of 400 and 700 nm. Gas vesicle re-formation was monitored by pressure nephelometry. Cell turgor pressure was measured immediately prior to isolation of gas vesicles from these cultures.

Isolation of the strongest and the weakest gas vesicles. Gas vesicles were isolated from *Anabaena flos-aquae* and the pressures required to collapse 10% and 90% of the gas vesicles in diluted samples of the preparation were determined by pressure nephelometry. Capillary tubes, 3 cm long and 2 mm internal diameter, were filled with undiluted samples of the gas vesicle preparation and then inverted under a 2 cm layer of water in the pressure nephelometer tube. One sample (A) was then exposed to a pressure that would cause 10% gas vesicle collapse and a second sample (B) to one that would cause 90% gas vesicle collapse. The water layer over the inverted tubes prevented equilibration of gas vesicles with gas during the pressure rise, which would otherwise have permitted some vesicles to survive supra-critical pressures at the gas–water interface (Walsby, 1971). The two samples, A and B, were then transferred to Eppendorf tubes, where they formed layers of depth (H) 5 mm, and centrifuged at an acceleration (g) of 1450 m s^{-2} for 2 h. The maximum pressure generated by centrifugation (calculated as $p = H\rho g$ where ρ is the density of the suspension, about 1000 kg m^{-3}) was therefore only 7.3 kPa, much less than the minimum critical pressure of the gas vesicles (about 250 kPa). The intact gas vesicles that survived previous application of pressure floated at the surface of each sample, forming a layer. The average gas vesicle of *Anabaena flos-aquae* has a sedimentation constant in water of $\sim 10^4$ Svedberg units (A. E. Walsby, unpublished calculations) and would therefore have floated up a distance of 100 mm under the conditions used, compared with the maximum distance of 5 mm required to reach the surface. There would not, therefore, have been significant loss of smaller, more slowly floating gas vesicles during this procedure. In the case of sample B these floating gas vesicles were removed in a small volume, resuspended in water and centrifuged again under the same conditions. The procedure was repeated twice. In this way the collapsed gas vesicles were removed from the sample and only the intact ones (representing the strongest 10%) remained. In the case of sample A, the clear suspension underneath the white surface layer of intact gas vesicles was removed to a fresh Eppendorf tube, which was also centrifuged under the same conditions. The clear 'subnatant' suspension contained only the collapsed gas vesicles (representing the weakest 10%), and was free of contaminating intact ones. The collapsed gas vesicles were then resuspended in phosphate buffer (0.01 M, pH 8.0) layered over a pad of CsCl (5.7 M) in a 16 ml centrifuge tube and centrifuged at $1.5 \times 10^6 \text{ m s}^{-2}$ (150000 g) for 3 h. The collapsed gas vesicles, which formed a visible band on the CsCl pad, were recovered with a microsyringe.

Calculation of gas vesicle dimensions and buoyant densities. The width, w , of the collapsed gas vesicle determined by electron microscopy is equivalent to πr , where r is the cylinder radius (Jost & Jones, 1970). Hence the cylinder diameter is given by $2w/\pi$. The cone height, h , was not determined separately for each gas vesicle type. For the purpose of the calculations given below it is assumed that h is similar to the mean value in gas vesicles of *Anabaena flos-aquae*, 52.8 nm. The length of the sloping edge of the cone is calculated from geometrical considerations as $(r^2 + h^2)^{1/2}$. The length, l , of the cylindrical portion of the gas vesicle is given by $L_c - 2s$ if it is assumed that the cones collapse perfectly to sectors of circles of radius s ; L_c is the overall length of the collapsed gas vesicle from collapsed cone tip to cone tip. The ideal end angle of the intact cone is given by $2\beta = 2(\tan^{-1} r/h)$ and the complementary base angle is $\alpha = \tan^{-1} h/r$. The overall volumes of the gas vesicle (V), the wall (V_w) and the contained gas space (V_i) can be calculated from the equations of Walsby & Armstrong (1979):

$$V = \pi r^2 (l + \frac{2}{3}h) \quad (1)$$

$$V_w = \pi \left\{ l(2rt - t^2) + \frac{2}{3} \left[r^2 h - \left(r - \frac{t}{\sin \alpha} \right)^2 \left(h - \frac{t}{\sin \beta} \right) \right] \right\} \quad (2)$$

and

$$V_i = V - V_w \quad (3)$$

where t is the wall thickness. The mean wall thickness of the *Anabaena* gas vesicle was determined (Walsby & Armstrong, 1979) by finding the weight of wall protein of known density, $\rho_w = 1320 \text{ kg m}^{-3}$ (Blaurock & Walsby, 1976), that surrounded a certain measured gas vesicle gas volume. The value obtained by Walsby & Armstrong (1979) is improved by substituting in their equations the revised value, $r = 42 \text{ nm}$; this yields a value of $t = 1.80 \text{ nm}$ for the mean wall thickness. This value is assumed to be the same in gas vesicles of all species; similarly, the buoyant density of the wall protein (ρ_w) is assumed to be the same in all species. (See Hayes & Walsby, 1986, for the supporting arguments.)

The buoyant density of each type of gas vesicle is calculated as $\rho_g = \rho_w V_w/V$ (see Table 1). The ratio of wall mass to gas volume in each case is calculated as $\rho_w V_w/V_i$, also given in Table 1.

RESULTS

Gas vesicle dimensions

The mean lengths and widths of gas vesicles isolated from the eight species (from seven genera) of cyanobacteria are shown in Table 1. From these two dimensions are calculated the average volumes of the gas vesicles, their walls and gas spaces, and the overall densities of the gas vesicles.

Table 1. Dimensions and other characteristics of gas vesicles isolated from the eight cyanobacteria

Symbol	Quantity (and units)	Anabaena flos-aquae*										Calothrix sp.		Nostoc muscorum		Aphanizomenon flos-aquae		Oscillatoria CYA 29 (green)		Microcystis sp.		Oscillatoria PCC 7801 (red)		Method of determination†
		Dactylococcopsis salina																						
Collapsed vesicles	$\left\{ \begin{matrix} w \\ L_c \\ n \end{matrix} \right.$																							EM
	Width (nm)	171.8					129.2					128.9		122.1		104.9		102.5		97.3		EM		
	Length (nm)	339.4					418.2					748.6		712.1		426.6		448.7		354.2				
	No. measured	46					105					98		48		88		102		82			EM	
Intact vesicles	$\left\{ \begin{matrix} d \\ r \\ L \\ V \\ V_w \\ V_i \end{matrix} \right.$																							$2w/\pi$ w/π $L_c - 2(s-h)$ Eqn 1 Eqn 2 $V - V_w$
	Cylinder diameter (nm)	109.4					82.2					82.1		77.7		66.8		65.2		62.0				
	Cylinder radius (nm)	54.7					41.1					41.0		38.9		33.4		32.6		31.0				
	Overall length (nm)	292.9					390.0					720.5		686.5		407.2		430.2		337.3				
	Total volume (10 ⁶ nm ³)	2.092					1.696					3.433		2.929		1.181		1.201		0.806				
	Wall volume (10 ⁶ nm ³)	0.159					0.159					0.308		0.277		0.133		0.138		0.099				
	Gas volume (10 ⁶ nm ³)	1.933					1.537					3.125		2.652		1.047		1.063		0.707				
Ratios																								
ρ_g	Gas vesicle density (kg m ⁻³)	100					124					119		125		149		152		162		$\rho_w V_w/V$		
V_i/V_w	Gas volume per wall volume	12.16					9.67					10.15		9.57		7.87		7.70		7.14		V_i/V_w		
V_i/m_w	Gas volume per wall mass (μl mg ⁻¹)	9.21					7.32					7.69		7.25		5.96		5.84		5.41		$\rho_w W_w/V_i$		

* a, Calculated from b; b, data from Walsby & Armstrong (1979). † EM, electron microscopy.

Table 2. Median and mean critical pressures of gas vesicles from the eight cyanobacteria

Cyanobacterium	Critical pressure (MPa)		
	Median	Mean	SD
<i>Dactylococcopsis salina</i> BC 79/1	0.31	0.33	0.11
<i>Anabaena flos-aquae</i> CCAP 1403/13f	0.60	0.61	0.10
<i>Calothrix</i> sp. PCC 7601	0.57	0.58	0.08
<i>Nostoc muscorum</i> PCC 6719	0.56	0.57	0.08
<i>Aphanizomenon flos-aquae</i> CCAP 1401/1	0.60	0.60	0.08
<i>Oscillatoria agardhii</i> NIVA CYA 29 (green)	0.70	0.71	0.12
<i>Microcystis</i> sp. BC 84/1	0.77	0.77	0.12
<i>Oscillatoria agardhii</i> PCC 7801 (red)	0.98	0.99	0.13

The cylinder diameters of the gas vesicles, calculated from the widths of the collapsed gas vesicles, agree closely (to within 3%) with the estimates of diameter made of five of the species listed by Hayes & Walsby (1986), who calibrated their electron micrograph magnifications by using standardized latex beads. The diameter of the *Microcystis* sp. gas vesicles, 65.2 nm, is somewhat less than that found for another strain of this genus, 71.6 nm, by Jost & Jones (1970), who also used a diffraction grating to calibrate the micrograph magnifications. The species are arranged in order of gas vesicle width in Table 1.

There is no apparent relationship between the mean widths and lengths of gas vesicles in the different species. The shortest gas vesicles, those of *Dactylococcopsis salina* (length 293 nm), were the widest, with a diameter of 109 nm, but the next shortest, those of the red-coloured *Oscillatoria agardhii* PCC 7801 (length 337 nm), were the narrowest. Substantially the longest gas vesicles were those of *Nostoc muscorum* at 720 nm; Waaland & Branton (1969) reported previously gas vesicles over 1000 nm in length from this species.

The relative efficiency of the different gas vesicles in providing buoyancy can be gauged by the ratio of gas space to wall volume (V_i/V_w in Table 1), a measure of the amount of buoyancy provided by the gas vesicle protein. It is seen that, except for the case of the longest gas vesicles of *Nostoc muscorum*, this ratio falls in the same order as the gas vesicle width. Thus while the ratio increases with both width and length, it is width that has the dominant effect (see Discussion and Fig. 6).

Critical pressure of gas vesicles, related to their diameter

The mean critical pressure ($\bar{p}_c$) of the gas vesicles of each species is shown in Table 2. The values shown are those obtained for gas vesicles inside cells from which turgor pressure had been removed by addition of 0.5 M-sucrose to the culture medium. The values of $\bar{p}_c$ varied from 0.33 MPa for *Dactylococcopsis salina* to 0.98 MPa for the red-coloured strain of *Oscillatoria agardhii*, PCC 7801 (Fig. 1). A graph of mean critical pressure versus mean diameter of the gas vesicles from the different species (Fig. 2) shows the inverse relationship between strength and width referred to previously (Walsby, 1972). A double logarithmic plot of these data gives a straight line, as found for the smaller sample analysed by Hayes & Walsby (1986). Regression analysis gives the relationship

$$\ln(p_c/\text{MPa}) = 5.62 - 1.67 \ln(r/\text{nm}) \quad (4)$$

from which we may calculate the expected critical pressure of a gas vesicle of known radius as

$$p_c = 275 (r/\text{nm})^{-1.67} \text{ MPa} \quad (5)$$

The regression coefficient is -0.97 . From the same regression analysis we can calculate the diameter expected for a gas vesicle of a required critical pressure,

$$d = 59.2 (p_c/\text{MPa})^{-0.561} \text{ nm} \quad (6)$$

These relationships are used in the following discussion.

Fig. 1. Distribution of gas vesicle critical pressures in some of the cyanobacteria investigated, illustrating the wide range of variation: *Dactylococcopsis salina* (○); *Aphanizomenon flos-aquae* (●); green-coloured *Oscillatoria* CYA 29 (□); *Microcystis* sp. (■); red-coloured *Oscillatoria* 7801 (△).

Fig. 2

Fig. 3

Fig. 2. The inverse relationship between strength and cylinder diameter of gas vesicles in different cyanobacteria. The line of best fit was calculated from equation (5).

Fig. 3. Critical pressure distribution of gas vesicles isolated from *Anabaena flos-aquae*, showing pressures subsequently applied to collapse the weakest 10% and 90%.

Cylinder diameter of gas vesicles with different critical pressure

The critical pressure distribution of gas vesicles isolated from *Anabaena flos-aquae* was determined by pressure nephelometry (Fig. 3). (The isolated gas vesicles are slightly weaker ($p_c = 0.51$ MPa) than those inside cells, owing to gradual degradation.) From this collapse-pressure curve was determined the pressure required to collapse 10% of the gas vesicles (0.345 MPa) and 90% of the gas vesicles (0.635 MPa). These were the pressures then applied, separately, to more concentrated gas vesicle suspensions in capillaries. The distribution of cylinder diameter of the weakest 10% of the gas vesicles collapsed by the lower pressure was determined by electron microscopy on $n = 97$ vesicles (see Fig. 4a): the mean diameter was $\bar{d} = 85.61$ nm, the standard deviation was $SD = \pm 3.28$ nm, and the 95% confidence limit ($= 1.96 SD/\sqrt{n}$) was ± 0.65 nm. For the strongest 10% of the gas vesicles, which survived the higher pressure, measurements on $n = 84$ indicated $\bar{d} = 79.38$ nm, $SD = \pm 3.49$ nm and a 95% confidence limit of ± 0.74 nm (see Fig. 4b). Comparison by Student's t -test indicated a highly significant difference between the two distributions ($P < 0.001$). Measurements were also made on an unpressurized sample ($n = 61$) in which $\bar{d} = 83.92$ nm, $SD = \pm 3.36$ nm and the 95% confidence limit was ± 0.84 nm. There was again found to be a significant difference between this and both the weakest 10% and the strongest 10% gas vesicle samples ($P < 0.001$ in each case).

Fig. 4. Distributions of cylinder diameter of gas vesicles isolated from *Anabaena flos-aquae*: (a) those with the lowest 10% critical pressure, which were collapsed by a pressure of 0.345 MPa; (b) those with the highest 10% critical pressure, which survived a pressure of 0.635 MPa.

It can be concluded from the results of this experiment that *Anabaena flos-aquae* does produce gas vesicles of different diameters and that the range of diameter previously indicated cannot be totally accounted for by measurement error. The experiment also demonstrated that at least part of the range of critical pressure of the gas vesicles in this organism can be explained by differences in cylinder diameter. In an attempt to assess what proportion of the critical pressure variation it accounts for, the following calculation is made.

Analysis of the critical pressure distribution for *Anabaena flos-aquae* gas vesicles (in cells without turgor pressure, suspended in 0.5 M-sucrose) gave the mean critical pressure as $\bar{p}_c = 0.615$ MPa and standard deviation $SD = 0.095$ MPa. [The mean critical pressure differs from the median value (pressure giving 50% collapse) of 0.600 MPa, because the distribution is skewed towards the higher pressures.] The critical pressures one standard deviation away from the mean therefore lie at 0.520 MPa and 0.711 MPa. Analysis of the cylinder diameter of gas vesicles in cells grown in similar conditions gave $\bar{x} = 83.97$ nm and $SD = 4.14$ nm, so that the diameters for gas vesicles one standard deviation away from the mean were 79.83 nm and 88.11 nm. If the cylinder diameter were the only cause of variation in $\bar{p}_c$ we should predict from the relationship given by equation (5) that

when $d = 83.97$ nm, $p_c = 0.542$ MPa;

when $d = 79.83$ nm, $p_c = 0.589$ MPa, or 0.047 MPa more than that of the calculated value for the mean diameter;

when $d = 88.11$ nm, $p_c = 0.500$ MPa, or 0.042 MPa less than that of the calculated value for the mean diameter.

In actuality the critical pressures one standard deviation from the mean differ by 0.095 MPa, or twice that predicted by equation (5). It is therefore concluded that variation in width can account for only part of the critical pressure variation.

Gas vesicle formation under reduced turgor pressure

Samples of the pressure-treated cultures of *Anabaena flos-aquae* grew, and re-formed gas vesicles, in culture media supplemented with sucrose at concentrations up to 125 mM but not at higher concentrations. Measurements were made of the median apparent critical pressures ($\tilde{p}_a$) of the gas vesicles in these cultures and also of the actual critical pressures ($\tilde{p}_c$) determined in samples mixed with equal volumes of 1.0 M-sucrose to remove all turgor pressure. The results are

Fig. 5. Median critical pressures of gas vesicles of *Anabaena flos-aquae* formed in cells grown in medium enriched with sucrose at different concentrations. ●, Critical pressure ($\bar{p}_c$) measured with cells suspended in medium further enriched with 0.5 M-sucrose to remove turgor pressure completely; ○, apparent critical pressure ($\bar{p}_a$) measured with cells in the growth medium used; □, turgor pressure, calculated as $\bar{p}_c - \bar{p}_a$.

shown in Fig. 5. The value of $\bar{p}_c$ decreased slightly with increasing sucrose concentration of the growth medium, from 0.595 MPa at 0 mM to 0.570 MPa at 125 mM. The value of $\bar{p}_a$ showed a marked increase, from 0.243 MPa at 0 mM to 0.433 MPa at 125 mM-sucrose, indicating that the cell turgor pressure ($p_t = \bar{p}_c - \bar{p}_a$) decreased from 0.352 to 0.137 MPa over this range. This result is similar to that obtained previously using cultures in which the original gas vesicles were not collapsed (see Fig. 2 and Table 1 of Walsby, 1980); the turgor pressure falls and is maintained at a lower value because the sucrose does not penetrate the cells of this cyanobacterium, even after several days (Walsby, 1980).

In this experiment the decrease in critical pressure ($\bar{p}_c$) of the gas vesicles could be a result of the decrease in turgor pressure under which they formed. A plot of critical pressure versus turgor pressure gives the relationship

$$\bar{p}_c = 0.558 \text{ MPa} + 0.106 p_t$$

with a correlation coefficient of $r = 0.97$.

Gas vesicles were isolated from the culture grown in medium enriched with 100 mM-sucrose and their cylinder diameters were determined by electron microscopy. The mean diameter (of $n = 84$ gas vesicles) was $\bar{x} = 85.56$ nm with $\text{SD} = \pm 3.10$ nm and a 95% confidence limit of 0.69 nm. Student's t -test shows this to be significantly different from the value obtained under normal conditions, at the $P = 0.002$ level of probability, although the difference in the mean value is small, only 2%.

We have analysed, as previously, whether the difference in diameter is sufficient to explain the small difference in critical pressure. Using equation (5) it is calculated that for a vesicle of 83.97 nm diameter the expected p_c is 0.542 MPa whereas for one of 85.56 nm, it is 0.525 MPa, a difference of 0.017 MPa. This is very close to the difference observed, 0.015 MPa ($\bar{p}_c = 0.595$ MPa in the absence of sucrose, and 0.580 MPa in 100 mM-sucrose; see Fig. 5). Thus the increase in diameter can account for all of the decrease in strength. The main point, however, is that a substantial reduction in turgor pressure, which in 100 mM-sucrose is only half that in the absence of sucrose (see Fig. 5), brings about little change in either the diameter or the critical pressure of the gas vesicles that form.

DISCUSSION

The principal function of the gas vesicle is to provide buoyancy (Walsby, 1972). The amount of buoyancy provided depends on the volume of gas space in the gas vesicle, while the cost of providing this depends on the amount of protein required to surround the gas space. Since the

Fig. 6. The ratio of gas volume (V_i) to wall volume (V_w) of gas vesicles of various radii (r) and lengths (L). (a) Effect of changing radius for three length classes: 115 nm (= 2 cones + 2 ribs) (---); 470 nm (mean for the eight species investigated) (—); and 1000 nm (about the maximum in most species) (- - - -). (b) Effect of changing length for three width classes: radius 20 nm (- - - -), 39.4 nm (mean for the eight species) (—), and 60 nm (----). The arrows indicate the lengths at which the ratio reaches 90% (↓) and 95% (↑) of the value obtained at infinite length.

unit costs of making gas vesicle proteins are likely to be the same, the efficiency of gas vesicles in different organisms must depend on the ratio of gas space to protein, expressed in Table 1 in terms of the ratio of the volumes V_i/V_w , calculated by using equations (1), (2) and (3).

Fig. 6(a) shows how the ratio V_i/V_w changes with increasing width. The ratios have been calculated for the cases of gas vesicles of three length classes: $L = 115$ nm, which is the length of the two conical end caps plus a central cylinder of only two ribs, $L = 470$ nm, which is the mean length for the gas vesicles of the eight cyanobacteria listed in Table 1, and $L = 1000$ nm, about the longest usually encountered. In each case the ratio V_i/V_w increases almost linearly with the cylinder radius r . This analysis therefore provides quantitative support for the suggestion that wider gas vesicles enclose more gas space for a given amount of wall material and provide buoyancy at a lower cost (Walsby, 1978). It follows that for reasons of economy natural selection should support the evolution of gas vesicles of the maximum width allowed by the required critical pressure.

Fig. 6(b) shows how the ratio V_i/V_w changes according to length, for three width classes: $r = 20$ nm, which is slightly less than that of the narrowest gas vesicles known, in *Trichodesmium* sp. (Gantt *et al.*, 1984), $r = 39.4$ nm, which is the mean for the eight cyanobacteria in Table 1, and $r = 60$ nm, which is slightly more than the radius of the widest, in *Dactylococcopsis salina*. In each case the initial part of the curve, for lengths up to 106 nm, shows the V_i/V_w ratio for the biconical stages. The method of calculation assumes that the cone height is the same, $h = 52.8$ nm, in each case, so that the cone angles differ. (It is seen that as the gas vesicles then extend as cylinders the slope of the curve rises more steeply. This occurs because in the method of calculation it is assumed that the wall of the cone has the thickness t measured normal to the plane of the wall, so that its thickness considered normal to the long axis of the gas vesicle is greater by $1/\sin\alpha$. The actual thickness of the cone wall is not known precisely.) The main points to emerge from this analysis are that as length increases the ratio V_i/V_w increases, and so the efficiency in providing buoyancy increases. The ratio asymptotically approaches a maximum value, however. On each of the curves is indicated, by a downward-pointing arrow, the point at which V_i/V_w reaches 90% of its maximum value; this occurs at $L = 280$ nm for the case of $r = 20$ nm, $L = 355$ nm for $r = 39.4$ nm, and $L = 485$ nm for $r = 60$ nm. By similar calculations it can be shown that the mean length of the gas vesicles exceeds the 90% value for seven of the eight cyanobacteria investigated; the exception is in *Dactylococcopsis* (see Table 1).

Previous investigations by Walsby (1971) showed that there was no decrease in critical pressure of *Anabaena* gas vesicles as they increased in length. Without the penalties of strength encountered with increasing width, the gas vesicles in most of these cyanobacteria achieve lengths that provide buoyancy with high efficiency. In considering why the gas vesicles are not even longer, extracting the last few percent of efficiency, it should be recalled that gas vesicles grow slowly from biconical initials (Waaland & Branton, 1969; Lehmann & Jost, 1971) and that the length distribution encountered is that of a population of gas vesicles in various stages of maturation. In each case (except *Dactylococcopsis*) the longest (and hence oldest) gas vesicles encountered have a V_i/V_w ratio exceeding 95% of the maximum for the width class. There are also, of course, space limitations on the length of the gas vesicles that can be accommodated in the cells of some of these cyanobacteria. It is also possible that very long gas vesicles might be more easily damaged during the cytoplasmic movements that must occur during cell division.

We have demonstrated that the width of the gas vesicles in *Anabaena flos-aquae* does vary, with the diameter of the weakest 10% being on average over 6 nm wider than that of the strongest 10%. The width of the gas vesicle is, in a sense, determined by the number of gas vesicle protein (GVPa) molecules that form each rib of the structure. In *Anabaena flos-aquae*, molecules of the major protein (GVPa) form a periodic repeat at intervals of 1.15 nm along the ribs (Blaurock & Walsby, 1976). A rib of 84 nm diameter therefore contains 229 GVPa molecules, and there will, on average, be 17 more GVPa molecules present in the rib of the weakest gas vesicles compared to the strongest ones. It is clear, then, that gas vesicle width in this species is not specified by a precise, invariable number of protein molecules, in the way that the width of the tobacco mosaic virus is (Klug, 1972), but rather it would appear that there is a loose preference for the molecules to assemble into ribs of certain size. This may be determined by the shape of the GVP molecule (Walsby, 1978), which will be a product of its amino acid sequence. It might eventually be possible to test this idea by transferring the genes for gas vesicle proteins (Tandeau de Marsac *et al.*, 1985; Damerval *et al.*, 1987) from different cyanobacteria into some other organism in which the genes are expressed and the proteins assembled into gas vesicles.

We have recently demonstrated that in *Anabaena flos-aquae* there is, in addition to the main protein GVPa, a second protein, GVPc, which forms 9% of the mass of the gas vesicle (Hayes *et al.*, 1988). This minor component (which is homologous with the gene product of *gvpC* isolated by Damerval *et al.*, 1987, from *Calothrix*) is located on the outer surface of the *Anabaena* gas vesicle and its removal results in a marked decrease in critical pressure of the structure (Walsby & Hayes, 1988). It therefore appears that the association of GVPc with the gas vesicle inside cells may be one of the factors that accounts for the part of the variation in critical pressure not explained by variation in cylinder diameter, as discussed above.

It has been shown that the critical pressure of gas vesicles in different organisms, and within *Anabaena flos-aquae*, is inversely correlated with the width of the structure, but changing the hydrostatic pressure (in the form of turgor pressure) has little effect on the width of gas vesicles that form under it. What is proposed is that hydrostatic pressure in the natural habitat influences the *natural selection* of gas vesicles of different critical pressures, and hence of different widths according to the relationship described in equation (6). The principle can be illustrated by reference to the gas vesicles of the two extremes. The gas vesicles of *Dactylococcopsis salina* are strong enough ($\bar{p}_c = 0.33$ MPa) to withstand the combination of turgor pressure and hydrostatic pressure (0.14 MPa) in Solar Lake, Sinai, where this organism grows (Walsby *et al.*, 1983a). If such gas vesicles were to be produced in the cells of the red *Oscillatoria agardhii* from Lake Gjersjøen, Norway, however, over half of them would be collapsed by turgor pressure alone (0.36 MPa), and the remainder would be collapsed by the hydrostatic pressure (>0.4 MPa) generated at holomixis (Walsby *et al.*, 1983b). It is therefore clear why gas vesicles that are narrower and stronger ($\bar{p}_c = 0.99$ MPa) have evolved in this strain of *Oscillatoria*. Considering the reverse situation, gas vesicles from the *Oscillatoria* produced in the cells of *Dactylococcopsis* in Solar Lake would obviously survive the lower pressures, but they would require the production of 70% more gas vesicle protein to provide the same amount of gas space for buoyancy (see Table 1). We therefore see why gas vesicles that are wider and more efficient have

evolved in *Dactylococcopsis*. It is proposed that in each of the other organisms described, the evolved morphology of the gas vesicles has been similarly influenced by the requirement for a particular critical pressure.

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