The homologies of gas vesicle proteins

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In addition to GvpA, the main structural protein, an SDS-soluble protein has been found in gas vesicles isolated from six different genera of cyanobacteria. N-terminal sequence analysis of the first 30 to 60 residues of the gel-purified proteins showed that they were homologous to GvpC, a protein that strengthens the gas vesicle in *Anabaena flos-aquae*. The proteins from some of the organisms showed rather low homology, however, and this may explain why the genes that encode them have not been found by Southern hybridization studies. The gas vesicles of another cyanobacterium, *Dactylococcopsis salina*, contained two SDS-soluble proteins (M_r 17 000 and 35 000) that were identical in sequence for the first 24 residues but not thereafter; these two proteins showed no clear homology to GvpC. The sequence of GvpA, the main structural gas vesicle protein, was very similar in each of the organisms investigated. GvpA from the purple bacterium *Amoebobacter pendens* was different for the first 8 residues but 51 of the next 56 residues were identical to those of the cyanobacterial GvpA. Analysis of the GvpA and GvpC sequences provides support for the idea that the low diversity of GvpA reflects a high degree of conservation rather than a recent origin followed by lateral gene transfer between different bacteria.

Introduction

Gas vesicles provide many micro-organisms with buoyancy. They occur in at least five of the eleven phyla in the Bacteria (the cyanobacteria, green sulphur bacteria, green non-sulphur bacteria, purple bacteria and Gram-positive bacteria), and two of the phyla in the Archaea *sensu* Woese *et al.* (1990) (the extreme halophiles and methanogens). In cyanobacteria the gas vesicle has the form of a hollow cylindrical tube closed by hollow conical end caps (Bowen & Jensen, 1965; Jost, 1965). Both the end caps and the central cylinder are made up of ribs 4.6 nm wide, which, in gas vesicles of *Anabaena flos-aquae*, have a crystallographic structure with subunits repeating at intervals of 1.15 nm (Blaurock & Walsby, 1976). Direct amino acid sequencing has shown that these gas vesicles contain a small, mainly hydrophobic protein of 70 amino acid residues, which corresponds in size to that of the crystallographic unit cell (Walker *et al.*, 1984; Hayes *et al.*, 1986). Similar proteins have been demonstrated in gas vesicles of five other genera of cyanobacteria (Walker *et al.*, 1984; Hayes *et al.*, 1986), and two species of halobacteria (Walker *et al.*, 1984; Surek *et al.*, 1988).

Tandeau de Marsac *et al.* (1985) isolated the gene (*gvpA*) that encodes this protein, which is now termed

GvpA, from *Calothrix* sp. PCC 7601. Downstream from *gvpA* was a second gene that encoded an identical protein and an open reading frame, *gvpC*, encoding a putative protein of 162 amino acids that had four contiguous repeats of 33 amino acid residues (Damerval *et al.*, 1987).

Damerval *et al.* (1989) showed by Southern hybridization that of 24 gas-vacuolate strains of cyanobacteria all contained a gene homologous to *gvpA* from *Calothrix* sp. but only 12 contained sequences homologous to *gvpC* from the same organism. They concluded that either the product of *gvpC* was not essential for gas vesicle formation, or the gene had diverged to the extent that it could no longer be detected by DNA:DNA hybridization.

Hayes *et al.* (1988) isolated a gene homologous with the *Calothrix gvpC* from *Anabaena flos-aquae* and showed that its product, the protein GvpC, was a component of the gas vesicle. GvpC accounted for only 2.9% of the protein molecules present in the structure, with GvpA making up the remainder (Walsby & Hayes, 1988; Hayes *et al.*, 1988). Walsby & Hayes (1988) showed that the GvpC could be removed from the gas vesicle by rinsing with detergent; the structure remained intact but was weakened. It was concluded that GvpC was located on the outside of the gas vesicle and it was suggested that its function might be to strengthen the ribbed structure formed by GvpA. This was confirmed by Hayes *et al.* (1992), who demonstrated that GvpC could be

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reattached to gas vesicles that had been stripped of their native GvpC, and that this restored their strength.

The fact that the basic gas-filled structure of the gas vesicle survives when GvpC is removed may indicate that this protein is not absolutely essential for its formation. We have analysed the proteins in gas vesicles isolated from a number of cyanobacteria to determine whether the GvpC-like proteins were universal constituents of these structures. We investigated representatives of three of the five taxonomic sections (I, III and IV) described by Rippka *et al.* (1979). Some Section V organisms (branched filaments) may produce gas-vacuolate hormogonia but are not available in culture; no gas-vacuolate organisms are known from Section II.

Methods

Organisms and culture conditions. Gas vesicles were isolated from the bacteria listed in Table 1, which gives information on the taxonomic groupings and strain numbers from the following collections: Culture Collection of Algae and Protozoa (CCAP) at the Institute of Freshwater Ecology, Ambleside, Cumbria, UK; Pasteur Culture Collection (PCC), Institut Pasteur, Paris, France; Culture Collection of Algae (CYA) at the Norwegian Institute for Water Research, Oslo, Norway. *Anabaena flos-aquae* was grown in the medium of Walsby & Booker (1980). *Aphanizomenon flos-aquae* and *Calothrix* sp. were grown in medium B + N of Armstrong *et al.* (1983). *Dactylococcopsis salina* was grown at 32 °C in the medium described by Walsby *et al.* (1983), but with lower concentrations of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (0.08 g l⁻¹), K_2HPO_4 (8 mg l⁻¹) and Na_2CO_3 (2 mg l⁻¹), and a higher concentration of Na_2EDTA (3 mg l⁻¹). *Microcystis* sp. was grown in medium BG 11 of Rippka *et al.* (1979). The green strain of *Oscillatoria agardhii* was grown in the medium of Van Liere & Mur (1978). *Spirulina* sp. was grown in medium containing the following ingredients in 1 litre: NaCl (1 g),

NaNO_3 (2.5 g), CaCl_2 (0.04 g), $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (0.01 g), Na_2EDTA (0.08 g), K_2SO_4 (1 g), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.2 g), K_2HPO_4 (0.5 g), NaHCO_3 (16.8 g), and 1 ml of the trace element mixture of Stanier *et al.* (1971). All cultures were grown under warm white fluorescent light and bubbled continuously with air. Cells of *Amoebobacter pendens* were supplied by Professor Norbert Pfennig at the University of Konstanz, Germany.

Gas vesicle isolation. Cultures were left to stand at low irradiance (<4 $\mu\text{mol m}^{-2} \text{s}^{-1}$) for up to 72 h. Floating cells or filaments were drawn off from the liquid surface using a cannula attached to an evacuating Buchner flask. Cells of *Aphanizomenon flos-aquae* and *Anabaena flos-aquae* were lysed by the addition of 0.7 M-sucrose, and incubated at 37 °C for 2 h. Cells of *Amoebobacter pendens*, *Microcystis* sp., *Oscillatoria agardhii* CYA 29 and *Spirulina* sp. were lysed by the addition of lysozyme (at concentrations of 0.75, 4, 4 and 5 mg ml⁻¹, respectively), 0.05 M-Tris pH 8.0, and 0.1 M-EDTA, and incubated at 37 °C overnight. Cells of *Dactylococcopsis salina* were lysed by the addition of 4 mg lysozyme ml⁻¹ in 3% (w/v) NaCl, and 2% (v/v) Triton X-100. Lysis was confirmed under the light microscope. Gas vesicles were collected by accelerated flotation (Walsby, 1974). After three successive centrifugations, gas vesicles were filtered through 1.0 μm pore size Nuclepore filters, concentrated as described above, and then stored at 4 °C in 0.05% (w/v) ammonium bicarbonate solution (or 3%, w/v, NaCl for *Dactylococcopsis salina*) containing 5 mM-NaCN to act as a preservative (Powell *et al.*, 1991).

Electrophoretic purification of gas vesicle proteins. Collapsed gas vesicles were resuspended in 2.5% (w/v) SDS, 0.05% (w/v) bromophenol blue and 0.05% (w/v) xylene Cyanol FF, and loaded into wells of 15% (w/v) polyacrylamide gels (Laemmli, 1970). Protein bands were stained with Coomassie blue. The size of the protein in each band was estimated by comparison with proteins of known M_r (Rainbow, Amersham).

For sequencing studies, gels were lightly stained (1 min) and the required bands were cut out and eluted in 30 mM-Tris (pH 8.5), 0.5 mM-EDTA, 1 mM-sodium thioglycollate and 0.1% (w/v) SDS. A current of 8–10 mA per tube was applied over 15 h (BioRad MiniProtein II Electro-eluter module). N-terminal amino acid sequences were obtained using an Applied Biosystems 477A protein sequencer.

Table 1. Soluble proteins in gas vesicles isolated from cyanobacteria and a purple bacterium

Organism	Strain	Section*	Estimated M_r †	Reference to strain
<i>Anabaena flos-aquae</i>	CCAP 1403/13f	IV	28400‡	Walsby & Hayes (1988)
<i>Aphanizomenon flos-aquae</i>	CCAP 1401/1	IV	29700	Walker <i>et al.</i> (1984)
<i>Calothrix</i> sp.	PCC 7601	IV	20000§	Damerval <i>et al.</i> (1987)
<i>Dactylococcopsis salina</i>	PCC 8305	I	35200	Walker <i>et al.</i> (1984)
	CCAP 1417/1		17300	
<i>Microcystis</i> sp.	CCAP 1450/13	I	25200‡	Walsby & Hayes (1988)
<i>Oscillatoria agardhii</i> (green)	CYA 29	III	21700	Walsby & Bleything (1988)
	CCAP 1459/36			
<i>Spirulina</i> sp.	CCAP 1475/10	III	22100	P. Newton (unpublished)
<i>Amoebobacter pendens</i>	Konstanz 5813	—	ND	Eichler & Pfennig (1986)

* The systematic sections of cyanobacteria defined by Rippka *et al.* (1979).

† The M_r values estimated from the mobilities of bands in the SDS-PAGE system used. The discrepancies with the known values (§, §) indicate that the value given for other species may also be overestimated. ND, Not determined.

‡ The M_r indicated by the deduced amino acid sequence (Hayes *et al.*, 1988) is 21985.

§ The M_r indicated by the deduced amino acid sequence (Damerval *et al.*, 1987), including N-terminal Met, is 19179.

Results

Electrophoretic separation of gas vesicle proteins

When entire gas vesicles from each of the cyanobacteria were analysed by SDS-PAGE, Coomassie blue stained three components: (1) the residual protein that failed to penetrate the gel, (2) a minor component that lodged at the junction of the stacking and separating gels, and (3) usually a single band in the separating gel with a mobility indicating an M_r in the range of 19000 to 30000 (Fig. 1). Previous studies on gas vesicles from *Anabaena flos-aquae* showed that the protein in the well was GvpA and the protein in the mobile band was GvpC (Hayes *et al.*, 1988; Walsby & Hayes, 1989). Western blotting studies previously suggested that the protein at the top of the separating gel was GvpA (Walsby & Hayes, 1989). To obtain confirmation of this, entire gas vesicles isolated from *Anabaena flos-aquae* were run on SDS-polyacrylamide gels, and the protein lodged at the top of the separating gel was eluted and sequenced. The sequence obtained was Ala-Val-Glu-Lys-Thr-Asn-Ser-Ser-Ser-Ser-Leu-Ala, which is the N-terminal sequence of GvpA from *Anabaena flos-aquae* (Walker *et al.*, 1984, and see Fig. 2). In similar gels that had been stained for a longer time it was possible to see, by examination with a hand lens, that this band comprised a family of very narrow

Fig. 1. SDS-polyacrylamide gel of proteins from isolated gas vesicles: lane 1 'Rainbow' size markers with M_r values; lane 2 *Microcystis* sp. showing, from the top, GvpA in the well, GvpA at the top of the running gel, GvpC, and a trace of lysozyme; lane 3, *Dactylococcopsis salina* showing two mobile bands.

Fig. 2. Amino acid sequences for GvpA aligned to show the high degree of sequence homology within this family of proteins. Boxed and shaded areas show where the amino acid residues are identical for 50% or more of the proteins. Sequences for *Anabaena flos-aquae*, *Calothrix* sp. (PCC 7601) and *Pseudanabaena* sp. (PCC 6901) have been derived from their gene sequences (P. K. Hayes, unpublished; Tandeau de Marsac *et al.*, 1985; Damerval *et al.*, 1991). Partial amino acid sequences for *Aphanizomenon flos-aquae*, the red *Oscillatoria agardhii* CYA 18 (PCC 7821; CCAP 1459/37) and *Microcystis* sp. are from Walker *et al.* (1984) and Hayes *et al.* (1986). Residues of uncertain identity at positions 37 and 42 in the *Aphanizomenon* and red *Oscillatoria* sequences have been previously listed as P or P/E (Walker *et al.*, 1984). (See Table 1 for other strain numbers).

Table 2. Distance matrix analysis of *GvpA* sequence

The number in each box is the percentage of amino acid residues in the shorter sequence of each comparison that are identical to residues in the other sequence. For strain numbers see Table 1 and Fig. 1.

Organism	No.											
<i>Aphanizomenon</i>	1	100										
<i>Anabaena</i>	2	100	100									
<i>Calothrix</i>	3	98	99	100								
<i>Pseudanabaena</i>	4	94	94	93	100							
<i>Oscillatoria green</i>	5	94	95	93	97	100						
<i>Oscillatoria red</i>	6	95	95	93	98	98	100					
<i>Spirulina</i>	7	89	94	91	98	96	95	100				
<i>Microcystis</i>	8	96	97	95	94	92	91	91	100			
<i>Dactylococcopsis</i>	9	81	85	87	83	79	80	81	85	100		
<i>Amoebobacter</i>	10	80	78	77	78	77	77	77	78	72	100	
<i>Halobacterium</i>	11	61	55	57	54	57	59	64	60	64	58	100
Organism	No.	1	2	3	4	5	6	7	8	9	10	11

bands whose intensity decreased with distance from junction of the gels.

N-terminal amino acid analysis of three new *GvpAs*

The N-terminal amino acid sequences of *GvpA*, obtained by analysing entire gas vesicles, of the green-coloured strain of *Oscillatoria agardhii* CYA 29, *Spirulina* sp. and *Amoebobacter pendens* are shown in Fig. 2. Comparisons are made to sequences of *GvpA* in *Anabaena flos-aquae*, and other cyanobacteria previously investigated (Walker *et al.*, 1984; Tandeau de Marsac *et al.*, 1985; Hayes *et al.*, 1986; Damerval *et al.*, 1991) using distance matrix analysis (Table 2).

The SDS-soluble gas vesicle proteins

Gas vesicle proteins of *Aphanizomenon flos-aquae*, *Calothrix* sp., the green *Oscillatoria agardhii*, *Spirulina* sp. and *Microcystis* sp. were separated by SDS-PAGE. In each organism, a protein of M_r between 19000 and 30000 was observed (see Table 1).

Previous studies on *Dactylococcopsis salina* gas vesicles indicated only a single mobile band when gas vesicles were separated by SDS-PAGE (Hayes *et al.*, 1986). In this study, however, two mobile bands were consistently found, one of M_r 35000 as previously seen, and a second of M_r 17000. The two bands were obtained even after

rinsing the isolated gas vesicles with 2% (v/v) Triton X-100, 0.001% (w/v) SDS, and vigorous vortex-mixing in water. They were present in approximately equimolar quantities.

Contamination by lysozyme

In some of the samples (*Microcystis*, *Spirulina*, *Oscillatoria*, *Amoebobacter*) there was an additional minor band with a mobility indicating a M_r of about 14000, which ran coincidentally with lysozyme. This band was gel-purified from a sample of *Amoebobacter* gas vesicles; N-terminal sequence analysis gave the amino acid sequence Lys-Val-Phe-Gly-Arg-(unknown)-Glu-Leu-Ala-Ala-Ala-Met-Lys-Arg-His-Gly-Leu-Asp-Asn-Tyr-Arg-Gly-Tyr-Ser-Leu-Gly-Asn, which identified this protein as lysozyme (Jung *et al.*, 1980). It appeared that the lysozyme originally added to lyse the cells of these organisms had become adsorbed to the gas vesicles during the isolation procedure.

N-terminal amino acid analysis of the *GvpC*-like proteins

N-terminal amino acid sequences obtained from the proteins eluted from the single mobile bands from *Aphanizomenon flos-aquae*, *Oscillatoria agardhii* CYA 29, *Spirulina* sp., and *Microcystis* sp., and the two mobile bands from *Dactylococcopsis salina*, are shown in Fig. 3.

Fig. 3. Amino acid sequences for SDS-soluble proteins gel-purified from samples of gas vesicles. Boxed areas show where the amino acid residues are identical for 50% or more of the proteins, excluding the two *Dactylococcopsis* sequences. Sequences for *Anabaena flos-aquae* and *Calothrix* sp. were derived from their *gvpC* sequences (Damerval *et al.*, 1987; Hayes *et al.*, 1988).

Table 3. Distance matrix analysis of GvpC-like proteins

The first number in each box is the percentage of amino acid residues in the shorter sequence of each comparison that are identical to residues in the other sequence, and the second number (in *italics*) is the percentage of residues that are similar. Similar residues are as defined by Devereux *et al.* (1984): neutral, weakly hydrophobic (P, A, G, S, T); hydrophilic, acid amine (Q, N, E, D); hydrophilic, basic (H, K, R); hydrophobic (L, I, V, M); hydrophobic, aromatic (F, Y, W). For strain numbers see Table 1 and Fig. 1.

Organism	No.									
<i>Aphanizomenon</i>	1	100								
		100								
<i>Anabaena</i>	2	92	100							
		97	100							
<i>Calothrix</i>	3	51	54	100						
		71	73	100						
<i>Microcystis</i>	4	42	45	42	100					
		55	60	66	100					
<i>Oscillatoria green</i>	5	24	20	16	36	100				
		36	36	36	52	100				
<i>Spirulina</i>	6	20	23	23	31	24	100			
		34	40	49	57	52	100			
<i>Dactylococcopsis</i> 17	7	12	15	17	23	8	23	100		
		32	35	38	42	40	49	100		
<i>Dactylococcopsis</i> 35	8	13	16	16	17	8	20	50	100	
		27	27	34	38	36	57	57	100	
<i>Halobacterium</i>	9	5	5	8	8	0	9	9	5	100
		24	28	19	15	19	29	20	17	100
Organism	No.	1	2	3	4	5	6	7	8	9

Also shown are the N-terminal portions of the deduced amino acid sequences of GvpC from *Calothrix* sp. and *Anabaena flos-aquae* which have been obtained from the gene sequences (Damerval *et al.*, 1987; Hayes *et al.*, 1988). The presence of GvpC in *Anabaena flos-aquae* gas vesicles was previously confirmed by N-terminal sequencing of the gel-purified protein (Hayes *et al.*, 1988), and in *Calothrix* by SDS-PAGE, which revealed a 20000 M_r band (Damerval *et al.*, 1991), which is close to the M_r of the *gvpC* gene product, 19179 (Damerval *et al.*, 1987). The N-terminal sequences of these seven proteins are compared to one another by distance matrix analysis in Table 3.

Discussion

GvpA

Our results confirm the presence of two types of protein in the gas vesicles of *Anabaena flos-aquae*. The identification of the series of bands at the top of the stacking gel as GvpA indicates that this protein forms aggregates that are not easily disrupted. Walker & Walsby (1983) found that the *Anabaena* gas vesicle membranes could be completely dissolved to form a clear solution in 80% (v/v) formic acid, but only the M_r 22000 band, now known to be GvpC, was subsequently obtained by SDS-PAGE. Damerval *et al.* (1991) showed that a single mobile band of M_r 7000 could be obtained from gas vesicles of *Pseudanabaena* sp. PCC 6901 after dissolving them in formic acid; this is probably GvpA, but its identity should be confirmed by N-terminal sequence analysis.

Our three new GvpA sequences provide further evidence of the high degree of conservation of this protein (Table 2). It is of interest that the sequences of the four Section III cyanobacteria – *Pseudanabaena*, *Spirulina* and the two *Oscillatoria* strains – are identical up to residue 40, and are distinct from all of the others in having valine at residue 5 and serine at residue 40.

The first eight residues of the *Amoebobacter pendens* GvpA differ from the cyanobacterial sequence but of the next 56 residues 51 are identical to the consensus sequence; the five changes are all conservative (four of them Ile→Val, and the fifth Arg→Lys) and two of them occur in other cyanobacteria. In fact this section shows more similarity to the consensus cyanobacterial sequence than the corresponding portion of *Dactylococcopsis* GvpA. The *Amoebobacter* GvpA shows greater homology to the cyanobacterial consensus sequence (80%) than to the sequence of *Halobacterium halobium* GvpA (58%).

Although gas vesicles of different cyanobacteria (and other organisms) are remarkably similar in overall shape and fine structure, they do differ in cylindrical diameter

(Hayes & Walsby, 1986; Walsby & Bleything, 1988). These differences in gas vesicle diameter could be determined by the amino acid sequence of GvpA or GvpC, or by other gene products that might be involved in assembly of the structure. Diameter is important because it determines the critical collapse pressure of the gas vesicle and its efficiency in providing buoyancy (Walsby & Bleything, 1988).

GvpC

Preparations of gas vesicles isolated from seven genera of cyanobacteria have now been shown to contain SDS-soluble proteins. In four of these genera, *Anabaena*, *Aphanizomenon*, *Calothrix* and *Microcystis*, comparison of the sequences of these proteins indicates significant homology (Fig. 3, Table 3); in the other three genera the homology is less clear-cut.

Only 20% of the residues in the *Oscillatoria* protein are identical to those in the *Anabaena* GvpC, but 36% are identical to those in *Microcystis* GvpC. A number of the different residues indicate conservative changes; including these conservative changes yields 52% residues that are similar to those in *Microcystis* GvpC. For the *Spirulina* protein there are only 23% of residues identical to *Anabaena* GvpC and 31% to *Microcystis* GvpC, but the percentages of similar residues are 40% and 57%, respectively. These comparisons suggest that the proteins of these two species are also part of the GvpC family. Further evidence that they are true components of the gas vesicle might be provided by showing that they can restore the strength of the gas vesicles on being reabsorbed by them, or by showing that the encoding genes form part of a *gvp* operon.

The two SDS-soluble proteins obtained from *Dactylococcopsis salina* gas vesicles show little homology with the other GvpCs and we have no direct evidence that these two proteins are the homologues or even the functional analogues of GvpC. The remarkable feature of the two sequences is that the first 24 residues are identical; a gene duplication must have been responsible for this.

The results of this survey provide evidence that SDS-soluble outer-proteins are widespread, if not universally present, on the gas vesicles of cyanobacteria. Recent studies on the mechanical properties of gas vesicles (Walsby, 1991) indicate that GvpC increases the critical pressure of the structure by postponing buckling. This property would be especially important in wide gas vesicles, such as those of the halobacteria (Walsby, 1971). There is no direct information at present on whether halobacterium gas vesicles contain a functional analogue of GvpC, but in the *gvp* operon of *Halobacterium halobium* there is a gene downstream from *gvpA* that encodes a protein which, like the cyanobacterial GvpCs,

has a high content of acidic amino acids and a repeating structure, in this case seven repeats of length between 32 and 40 residues (Jones *et al.*, 1991; Horne *et al.*, 1991). The N-terminal sequence of this protein shows no homology to the cyanobacterial GvpCs (see Table 3) although the repeating sections show some weak homology with those in *Calothrix* GvpC (Jones *et al.*, 1991). It is not known if it is this protein or GvpA that constitutes the mobile band when gas vesicle proteins of halobacteria are separated by electrophoresis (see Simon, 1981; Surek *et al.*, 1988).

It remains now to question whether there are cases where gas vesicles lack GvpC. The observation (Damerval *et al.*, 1989) that Southern hybridization fails to find homologues of the *Calothrix gvpC* gene in certain cyanobacteria could be accounted for by the low degree of conservation of the encoded proteins. This would explain, for example, why homologous genes were found in species of *Anabaena* and *Microcystis*, where there is distinct homology between the proteins, but not in species of *Oscillatoria*, where the homology is less. Damerval *et al.* (1991) found no evidence of GvpC in gas vesicles isolated from *Pseudanabaena*, however, and they found no *gvpC*-like gene immediately downstream of the single *gvpA* gene in this organism. It is possible, of course, that this outer protein could have been lost during the isolation procedure, and that the encoding gene could be located further away from *gvpA*. Nevertheless, there is no direct evidence of GvpC in the *Pseudanabaena* gas vesicles and as Damerval *et al.* (1991) have pointed out, they may derive their stability from their small dimensions. A direct investigation of the relationship between the diameter and critical pressure of the *Pseudanabaena* gas vesicle is now required, using the methods developed by Walsby & Bleything (1988).

Evolution of GvpA and GvpC

Gas vesicles are found in two groups of micro-organisms, the Bacteria and Archaea, that diverged at an early stage of evolution (Woese, 1987; Woese *et al.*, 1990). If gas vesicles existed before the divergence of these two groups, the high degree of homology between extant GvpAs would indicate that there had been a very low rate of mutation in the *gvpA* genes (Damerval *et al.*, 1987). Alternatively, the gene may have developed more recently, mutated at a higher rate, and then become transferred laterally between the different organisms (Walker *et al.*, 1984). The much smaller degree of homology of GvpC is consistent with a long evolution of its encoding gene. It is unlikely that GvpC predated GvpA, and we therefore favour the idea that both genes are ancient and that the slower mutation rate of GvpA

must be due, at least in part, to this small protein having to fulfil a number of requirements to produce a gas-filled structure.

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