

GAS VACUOLES

Light Shielding in Blue-Green Algae

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INTRODUCTION

Gas vacuoles are small, cylindrical, gas-filled vesicles which occur in certain procaryotic cells. They are found in numerous blue-green algae, many photosynthetic bacteria, some halophilic bacteria, and some planktonic freshwater bacteria (3, 7-9, 11, 13). Because gas-vacuolate blue-green algae are often observed floating at the surface of water where high light intensity may damage cells, Lemmerman (4) first suggested that gas vacuoles may function as light-shielding organelles. Recently, it has been suggested that such light shielding (if it occurred) might be due to the optical properties and the intracellular distribution of the gas vacuoles (12, 16). Being gas filled, these vacuoles have a refractive index much lower than that of the cytoplasm which surrounds them, and therefore they scatter light. A suspension of gas-vacuolate cells becomes visibly less milky and more transparent when the gas vacuoles are collapsed. Similarly, when a milky-white, opalescent suspension of isolated gas vacuoles is subjected to sudden pressure, a completely transparent suspension results. These changes are due to a decrease in light scattering when gas vacuoles collapse. But, although light scattering by gas vacuoles could protect cells by scattering away a large portion of the incident radiation, it could also lead to increased rather than decreased irradiation of some cellular components if the light were back-scattered into these components. Whether gas vacuoles function as light shields would depend upon the distribution of the gas vacuoles inside the cells.

In this paper, we present spectrophotometric evidence that the presence of inflated gas vacuoles in *Nostoc muscorum* does actually reduce the amount of light absorbed by the photosynthetic pigments. This reduced absorption shows that the gas vacuoles may indeed function as light shields and

is attributable to the parietal distribution of the vacuoles within the cells of the particular strain of blue-green alga used in these experiments.

MATERIALS AND METHODS

Nonvacuolate cultures of *Nostoc muscorum* C. Agardh (strain UC142) were grown in aerated, liquid Allen's medium (1) at 25°C, 300 ft-c (warm white fluorescents). In order to induce gas vacuole formation, cultures were either transferred to 450 ft-c light intensity or diluted with 50 parts of distilled water (14). Gas-vacuolate cells were lysed by placing them in 1 M sucrose for 12 hr. Vacuoles were then isolated from the lysate by accelerated flotation using the method of Walsby and Buckland (17). Gas vacuoles (isolated or in cells) were collapsed by sudden application of hydrostatic pressure by using a small syringe fitted with a stopcock.

Absorption spectra were measured with a Cary 14 Recording Spectrophotometer equipped with a high intensity light source and a Scattered Transmission Accessory, Model 1462 (Cary Instruments, Monrovia, Calif.). Equivalent results were obtained with an Aminco-Chance Dual Wave-length Split Beam Recording Spectrophotometer equipped with an opal glass diffuser and with a Shimadzu Multi-purpose Recording Spectrophotometer, MPS-50L (both available from American Instrument Co., Inc., Silver Spring, Md.)

Freeze-etching was carried out as described elsewhere (2, 5, 6). Replicas were examined in a Siemens Elmiskop I.

RESULTS AND DISCUSSION

When a nonvacuolate *Nostoc* culture growing in liquid Allen's medium at a light intensity of 300 ft-c was moved to 450 ft-c intensity, the cells formed gas vacuoles. The cells never formed gas vacuoles in this medium at 300 ft-c or lower. As the culture grew at the higher light intensity and became more dense, the mutual shading (optical density) of the culture increased, and the gas

vacuoles gradually disappeared. These observations suggest that gas vacuoles may protect cells from the high light intensity until the culture becomes so dense that the cells shade each other and the effective light intensity per cell is reduced. But this evidence does not conclusively show that gas vacuoles are acting as light shields. In fact, gas vacuoles have also been reported to be induced or to form in greater abundance at very low light intensities in the blue-green algae *Oscillatoria redekei* (18) and *Anabaena flos-aquae* (15).

If gas vacuoles function as light-shielding devices, they must lower the amount of light reaching the photosynthetic pigments and thus decrease damage by photobleaching. By measuring the absorption spectra of *Nostoc* cells with inflated and with collapsed gas vacuoles, we can determine whether the presence of gas vacuoles affects the amount of light reaching the photosynthetic lamellae. Spectra were measured using spectrophotometers which corrected for a large portion of the light scattering by the samples so that the absorption by the photosynthetic pigments could be detected.

Absorption spectra of isolated gas vacuoles, inflated and collapsed, are given in Fig. 1. Inflated vacuoles exhibit a large amount of light scattering

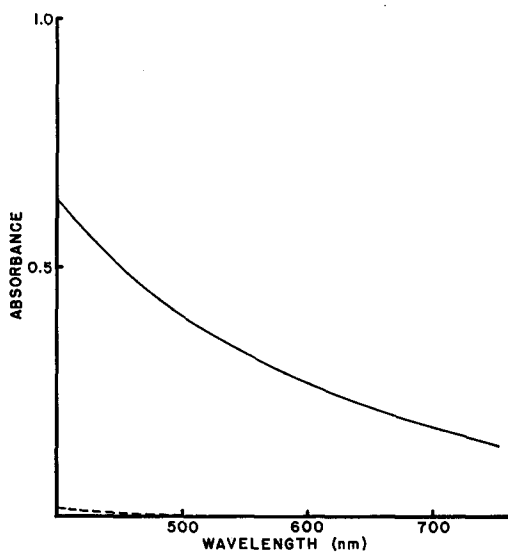


FIGURE 1 Spectra showing apparent absorption due to light scattering by isolated inflated gas vacuoles and isolated collapsed gas vacuoles. —, inflated isolated gas vacuoles. - -, collapsed isolated gas vacuoles.

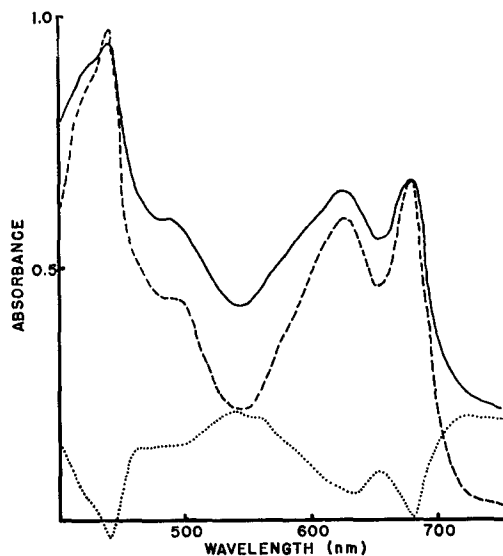


FIGURE 2 Absorption spectra of cells containing intracellular gas vacuoles. The two spectra were taken on the same sample of cells before and after collapsing the gas vacuoles. —, cells containing inflated gas vacuoles (a). - -, cells containing collapsed gas vacuoles (b)., difference $(a - b) = (c)$.

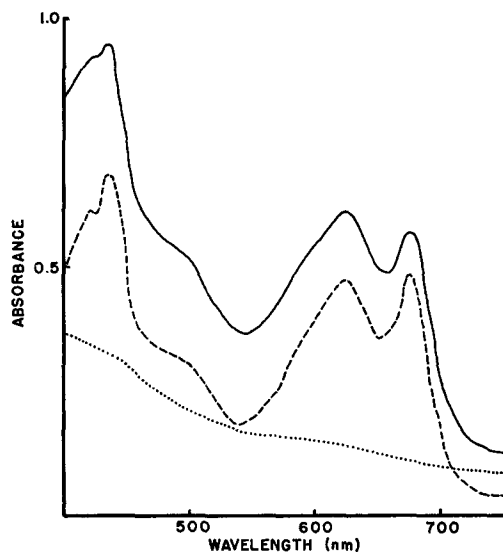


FIGURE 3 Absorption spectra of nonvacuolate cells mixed with isolated inflated gas vacuoles and nonvacuolate cells mixed with isolated collapsed gas vacuoles. —, cells plus isolated inflated gas vacuoles (a). - -, cells plus isolated collapsed gas vacuoles (b)., cells plus collapsed vacuoles versus cells plus inflated vacuoles (c).

whereas collapsed vacuoles do not. The spectrum of inflated gas vacuoles is typical of light scattering by small, nonabsorbing particles. The spectrum of collapsed gas vacuoles shows that they have no true absorption in the visible portion of the spectrum.

Absorption spectra of *Nostoc* cultures with intracellular gas vacuoles, inflated or collapsed, are shown in Fig. 2. The difference between these two spectra is also plotted. Microscopic examination of the cells whose vacuoles had been collapsed revealed no change in their gross morphology other than the disappearance of their gas vacuoles. Hence, the only difference between spectra of cells with inflated vacuoles and of cells with collapsed vacuoles should be due to loss of inflated gas vacuoles. It might, therefore, be expected that the difference spectrum between these samples would be identical in shape with that of isolated, inflated gas vacuoles. However the difference spectrum (Fig. 2 c) is a curve with depressions at 678, 630, and 440 nm, which mirror the absorption peaks of the photosynthetic pigments. Thus, the photosynthetic pigments absorb less light in cells with

inflated gas vacuoles than in cells with collapsed vacuoles.

To determine whether the decreased absorbance by the photosynthetic pigments in the presence of gas vacuoles was due not only to the presence of gas vacuoles but also to the peripheral location and grouping of gas vacuoles within cells, we measured the absorption spectra of a mixture of nonvacuolate *Nostoc* cells and isolated, inflated gas vacuoles. The gas vacuoles were then collapsed and the spectrum was remeasured. These absorption spectra are presented in Fig. 3. A difference spectrum of cells plus isolated, inflated gas vacuoles vs. cells plus collapsed, isolated gas vacuoles closely resembles that of isolated, inflated gas vacuoles. Thus, when isolated gas vacuoles are mixed with nonvacuolate *Nostoc* cells, there is no change in the absorption of the photosynthetic pigments, and the absorption due to the pigments and the gas vacuole light scattering are additive. The decrease in absorbance by the photosynthetic pigments in cells with intracellular inflated gas vacuoles must, therefore, be due to the arrangement of the gas vacuoles in

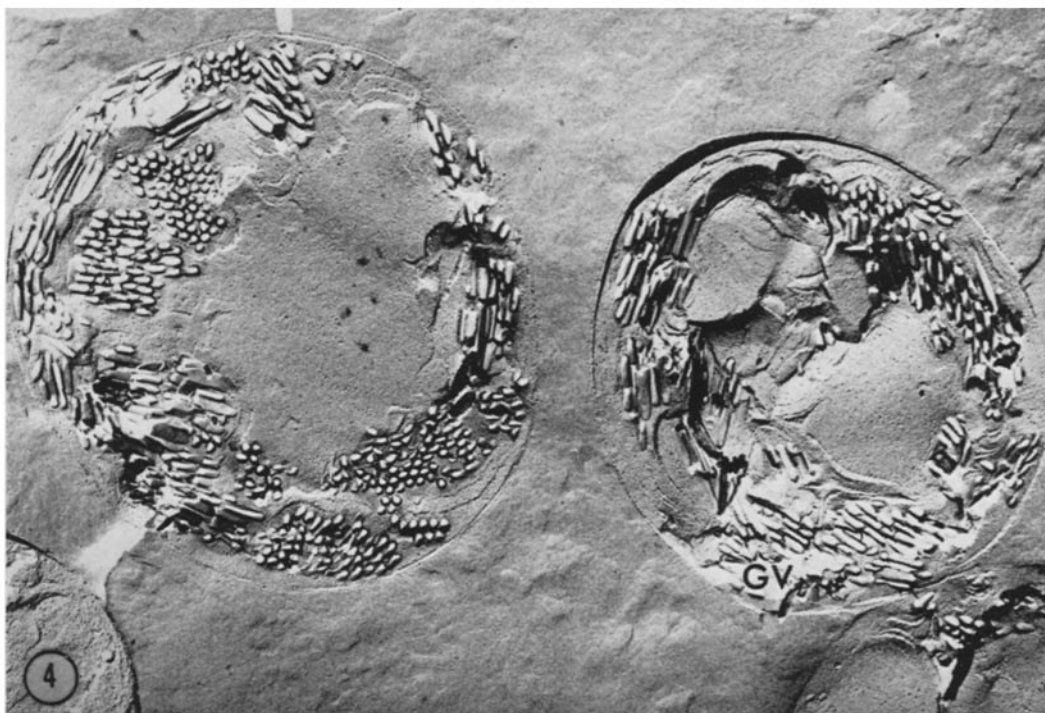


FIGURE 4 Electron micrograph of gas-vacuolate *Nostoc muscorum* cells. Gas vacuoles (GV) are arranged in ordered groups and are located at the periphery of the cell. $\times 14,000$.

parallel groups situated in various orientations external to and among the photosynthetic lamellae in the *Nostoc* cells. In such positions, they could interact with incident light before it reached the photosynthetic pigments.

Electron micrographs of freeze-etched *Nostoc* cells show that the groups of gas vacuoles are located at the periphery of the cell (Fig. 4). In this location, they are external to or scattered among the photosynthetic lamellae. Here they may scatter incident light away from the photosynthetic apparatus. Furthermore, they are present in ordered arrays so that their scattering effect may be mutually reinforced. Since the amount of light scattering increases in the ultraviolet region and since the nucleoplasm of blue-green algae is centrally located, we would also predict, as others have (12, 16), that gas vacuoles reduce the amount of harmful radiation reaching the ultraviolet-absorbing nucleic acids of the cell.

The location, position, and abundance of gas vacuoles within a cell may determine whether or not they serve as light shields as well as flotation devices. In the *Nostoc* which we investigated, and in other planktonic blue-green algae such as *Trichodesmium* (12) and some *Anabaena* strains (unpublished observations), the gas vacuoles are arranged around the periphery of the cell and can function as light shields. On the other hand, there may be blue-green algae in which gas vacuoles act solely as flotation devices and would not prevent incident light from striking the photosynthetic membranes. Some examples of this sort include *Anabaena flos-aquae* (10, 15) in which the gas vacuoles are randomly distributed in the cytoplasm and *Oscillatoria redekei* (18) in which the gas vacuoles form in conical groups near the cell's transverse walls. In the *Nostoc* strain which we investigated, as well as in other blue-green algae in which the gas vacuoles occupy the periphery of the cell, the gas vacuoles can play a dual role as both flotation devices and, as shown by our measurements, light-shielding structures.

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