Deoxyribonucleic Acid in *Nitrobacter* Carboxysomes

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Received for publication 23 July 1979

Carboxysomes were isolated from *Nitrobacter winogradskyi* and *Nitrobacter agilis*. The icosahedral particles contained double-stranded deoxyribonucleic acid (DNA). In the presence of ethidium bromide and cesium chloride, the particle-bound DNA had a buoyant density of $\rho_{25} = 1.701 \text{ g/cm}^3$. Electron microscopy revealed the DNA to be a 14-$\mu$m circular molecule.

Polyhedral inclusion bodies (carboxysomes) isolated from *Thiobacillus neapolitanus* and from different *Nitrobacter* strains are 100 to 120 nm in diameter, possess a shell 3 to 4 nm thick, and contain the enzyme ribulose-1,5-bisphosphate carboxylase (2, 5-8). The shell of the *Nitrobacter* carboxysomes is icosahedral (5). Biedermann and Westphal (1) recently reported that sodium dodecyl sulfate electrophoresis of purified bodies from *Nitrobacter* revealed seven polypeptides. Two of the polypeptides showed a migration pattern similar to that of the large and small subunits of ribulose-1,5-bisphosphate carboxylase. Since no other enzymatic activity thus far has been demonstrated, the remaining five proteins were hypothesized to be components of the icosahedral shell.

Nucleic acid was suspected in *Nitrobacter* carboxysomes because of the relatively high density ($\rho_{25} = 1.296$), a 260 nm/280 nm absorption ratio of 1.2, and the visualization of a DNase-sensitive component by electron microscopy (2, 10).

This report provides direct evidence for DNA inside the carboxysomes.

**MATERIALS AND METHODS**

*Nitrobacter winogradskyi* and *N. agilis* (K.) were grown lithotrophically in 36 liters of mineral nitrite medium. The composition of the medium and the growth procedures were as previously described (9). After maximum growth was attained the cells were held in the growth medium for 1 month in the absence of nitrite. Older cells contain more polyhedral inclusions than actively growing cells. The cells were harvested by centrifugation, resuspended and recentrifuged twice with TM-buffer (0.01 M Tris-hydrochloride, 0.01 M MgCl$_2$, 2H$_2$O, pH 7.5), and finally resuspended in 10 ml of buffer. The cells were disrupted by sonication at 0°C (Lehfeldt sonic oscillator). The carboxysomes were isolated by differential centrifugation and by 10 to 40% (wt/wt) linear sucrose gradients as previously described (2). The membrane-contaminated particles were layered onto a 10 to 60% (wt/wt) sucrose gradient and centrifuged for 36 h at 220,000 $\times$ g. The gradients were scanned with an ISCO gradient fractionator. A band which consisted of pure carboxysomes as revealed by electron microscopy was present at 55% sucrose. Biedermann and Westphal (1) demonstrated by sodium dodecyl sulfate electrophoresis that these bodies were composed of only seven polypeptides. The particle-containing fractions were collected and diluted to twice the volume with buffer, and the bodies (0.5 to 1.0 mg of protein) were sedimented by centrifugation for 1 h at 200,000 $\times$ g.

For DNA isolation, the particle pellet was resuspended into 5 ml of TES buffer (0.03 M Tris-hydrochloride–0.05 M sodium chloride–0.005 M ethylenediaminetetraacetic acid–tetrasodium salt, pH 8.0) containing 20 mg of both RNase (Serva) and DNase (Serva) per ml. The intact particles were incubated for 2 h at 20°C to remove contaminating nucleic acid of the *Nitrobacter* cells which might have been attached to the outside of the shell. The particles were then sedimented at 50,000 $\times$ g for 1 h. The pellet was resuspended into 3 ml of TES buffer containing 0.1% sodium dodecyl sulfate. To completely disrupt the particle structure the salt concentration was increased to 1 M NaCl. Ethidium bromide (0.75 ml of a 1% [wt/vol] solution) and cesium chloride (8.2 g) were added, and the total volume was adjusted to 9.5 ml. Tubes were centrifuged at 80,000 $\times$ g for 48 h in a 75 Ti fixed-angle rotor at 15°C. The gradients were fractionated from the bottom and passed through a 0.1-ml cuvette in a Zeiss spectrophotometer to measure the adsorption at 260 nm simultaneously. One of the 30, approximately 0.3-ml fractions contained 260 nm of absorbing material. After dialysis against 1,000 volumes of TES buffer the material was precipitated by the addition of 95% ethanol at 0°C. The precipitate was held for 6 h at 20°C and then centrifuged at 30,000 $\times$ g for 30 min. The pellet material was scanned by UV spectroscopy (Perkin-Elmer Spectrophotometer, Hitachi 200).

Carboxysomes as well as the pure 260-nm absorbing material were spread on cytochrome c or benzylidemethylaminochrome BAC films according to the Klein Schmidt technique and observed in a Phillips 201 electron microscope (3, 4, 10).

Native DNAs of *Escherichia coli* K and *N. agilis* K were used for controls.
RESULTS

The isolated 260-nm absorbing material, as well as the material extruded from osmotically shocked bodies, was sensitive to DNase, but not to RNase or proteinases.

When \textit{N. agilis} carboxysomes were osmotically shocked and spread on either cytochrome...
c or BAC films, DNA filaments could be observed in the electron microscope (Fig. 1-4). Because of the stability of the bodies, the osmotic shocking procedure did not result in 100% breakage. Ruptured bodies appeared less dense than unruptured carboxysomes (Fig. 1). When the particle concentration was high it was difficult to identify a relationship between the filaments and the bodies. Occasionally the DNA seemed to be extruded from ruptured carboxysomes (Fig. 2). At low-particle concentrations, however, the filaments of DNA commonly originated from structures resembling ruptured bodies (Fig. 3). On rare occasions one could observe the splitting of the DNA into filaments with smaller diameters, which indicated that the DNA was double stranded (Fig. 4).

The DNA isolated from the carboxysomes of either N. winogradskyi or N. agilis had a buoyant density of $\rho = 1.701$ (ethidium bromide-cesium chloride) and absorption maximum of 263.5 nm. The somatic DNA of *Nitrobacter* had the same density, but a different absorption maximum (266.0 nm). Both DNAs were distinctly different in density when compared with *E. coli* DNA. When the isolated DNA was spread on a cytochrome c film and observed in the electron microscope, circular molecules, 14-μm in length, could be seen (Fig. 5). Some non-circular molecules were observed. We feel that these resulted from shearing. In contrast to the somatic DNA, the filament was characterized by a high frequency of loops.

**DISCUSSION**

Because of the extensive DNase treatment, the DNA associated with the carboxysomes must have been protected in some way. It seems most plausible to us that this would best be accomplished by having the DNA inside the carboxysome envelope. We have mixed *Nitrobacter* somatic DNA with pure carboxysomes and demonstrated that they can be easily separated by density gradient centrifugation, i.e., the DNA has no tendency to stick to the surface of the carboxysome. It is of course possible that the DNA was attached to the surface of the carboxysome but was protected in some way from the action of the enzyme.

It also appears, even though the density is the same, that this is a plasmid and not somatic DNA. The circular nature of the DNA, the different absorption maximum, and extensive loops support this contention. We have also demonstrated (unpublished data) that sublethal levels of acridine orange cause a marked decrease in the number of carboxysomes per cell. Furthermore, if it were trapped somatic DNA one might expect different amounts per carboxyosome. The constant density of the carboxysomes in any given preparation, as well as from several different isolations, supports the presence of a nonsomatic-type DNA.

We estimate that the DNA is less than 5% of the total body weight which is estimated to be $1.5 \times 10^7$. However, it appears that this DNA, assuming no repetitive sequences, could code for approximately 35 proteins of 50,000 molecular weight. We have no idea at this time what the DNA codes for, but it is tempting to speculate that it might code for envelope proteins. The virus-like structure of the envelope, i.e., the icosahedral structure, lends support to this possibility.

We realize that the DNA needs to be electrophoresed and that restriction enzyme mapping needs to be accomplished. These experiments are planned, but carboxysome and DNA yields are low and need to be improved considerably. In this regard we have demonstrated that the carboxysomes of *Thiobacillus intermedius* and *T. neapolitanus* (unpublished data) also contain DNA. Since these organisms grow more rapidly and give greater cell yields than *Nitrobacter* the isolation and characterization of the DNA should be more feasible. Furthermore, we are presently developing new methods of isolation.

**Fig. 5.** Electron micrograph of a circular filament of purified DNA from carboxysomes. Bar marker = 1.0 μM.
which will give greater carboxysome yields.

The function of the DNA in the carboxysome is unknown at this time, but we envision a possible role in assembly or stability.

ACKNOWLEDGMENTS

This research was supported in part by DFG grants BO 398/3 and 398/4 (E.B.) and by grant 1141 from the Scientific Affairs Division, North Atlantic Treaty Organization (J.M.S.).

LITERATURE CITED


