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Evolution

Basic protein enhances the incorporation of DNA into lipid vesicles: Model for the formation of primordial cells

(membrane/lysozyme/prebiotic/DNA encapsulation)

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ABSTRACT **DNA can be encapsulated into lipid vesicles formed by sonication. The presence of a basic protein, lysozyme, enhances the incorporation 100-fold above the level expected by random trapping. This is demonstrated by the ability of the lipid vesicles to protect DNA from digestion with DNase. Such an enhancement of nucleic acid incorporation into vesicles by basic polypeptides and the sharply increased concentration of these macromolecules in the internal volume may have been advantageous in prebiotic evolution.**

A critical process in the formation of the first cells was the isolation of macromolecules away from the external environment. Recent speculations about the RNA origin of life suggest that the first enzymes were RNA and that the first polypeptides played only a structural role (1). This led us to conjecture that one early role of protein might have been to help package nucleic acids within lipid membranes. The encapsulation of DNA by large lipid vesicles has been reported (2, 3), but the amount of DNA encapsulated was in simple proportion to the internal volume: the DNA must have been encapsulated by random trapping. The interaction of nucleic acid with phospholipid is an unfavored event because of the polyanionic nature of DNA or RNA, the hydrophobicity of lipid, and the negative charges associated with the phospholipid head groups. We propose that basic polypeptides might have mediated vesicle formation around nucleic acids by neutralizing charge, by condensing the linear strands, and by acting as an amphipathic template for lipid-bilayer formation. The experiments reported here show that basic proteins are efficient mediators of DNA encapsulation within lipid membranes: the presence of lysozyme causes DNA to be segregated into lipid vesicles 100-fold that expected by random trapping.

MATERIALS AND METHODS

Lysozyme, histone, bovine serum albumin, trypsin, and Ficoll were obtained from Sigma. Micrococcal nuclease was obtained from Boehringer Mannheim. Asolectin (a chloroform/methanol extract of soybean lipids) was obtained from Avanti Polar Lipids (Birmingham, AL). Linear [^{32}P]5′-DNA was a gift from S. Swanberg and D. Paul.

Vesicles were formed by a variation of the method of Dixon and Hokin (4). A solution of 50 μg of asolectin in 10 μl of chloroform was dried in the bottom of a Microfuge tube under a nitrogen stream, washed two times with 25 μl of ether, and dried under nitrogen for 1 hr at 40°C. Ten nanograms of linear [^{32}P]5′-DNA, 1000 base pairs long, and 10 μg of protein were added in a final volume of 10 μl of 10 mM Tris·HCl, pH 7.4/1 mM EDTA, and sonicated in a bath sonicator (Laboratory Supplies, Hicksville, NY) for 6 min during which time the mixture became opalescent, indicative of the formation of vesicles.

The vesicles were challenged with micrococcal nuclease by making the preparations 10 mM $CaCl_2$ and adding 0.3 unit of micrococcal nuclease. The samples were incubated for 60 min at 37°C. The digestions were terminated by the addition of 1 μl of 250 mM EDTA followed by the addition of 2 μl of 20% $NaDodSO_4$ and boiling for 3 min. Samples were analyzed by gel electrophoresis and autoradiography.

Flotation of vesicles by Ficoll-gradient centrifugation was done by an adaption of the method of Fraley *et al.* (3). A vesicle sample with a volume of 100 μl was diluted with 30% Ficoll in 10 mM Tris·HCl, pH 7.4/1 mM EDTA to a final volume of 500 μl. The sample was overlaid with a Ficoll step gradient in 10 mM Tris·HCl, pH 7.4/1 mM EDTA: 1 ml of 20% Ficoll, 3 ml of 10% Ficoll, and 1.5 ml of buffer alone in 13 × 51-mm ultraclear centrifuge tubes. Samples were spun in a 50.1 swinging bucket rotor at 40,000 rpm for 30 min and decelerated without braking. The gradients were fractionated into 130-μl samples, and radiolabeled DNA was detected by Cherenkov radiation.

Trapped internal volume was measured by preparing 10-μl samples of vesicles with 250,000 cpm of ^{14}C-labeled sucrose. The samples were diluted with 90 μl of 10 mM Tris·HCl, pH 7.4/1 mM EDTA and spun on an Airfuge centrifuge at 95,000 rpm for 1 hr. The supernatant was removed, and the pellet was washed with 100 μl of 10 mM Tris·HCl, pH 7.4/1 mM EDTA. The pellets were solubilized with 10 μl of 1% $NaDodSO_4$ and counted. Samples were done in triplicate, and samples of radiolabeled sucrose without vesicles were used as controls.

RESULTS AND DISCUSSION

DNase Analysis of Encapsulation. Our assay follows the ability of lipid, formed into vesicles by sonication, to protect labeled DNA against subsequent digestion with nuclease. We used DNA rather than RNA because of a concern about endogenous RNases in the lipid preparations. In preliminary experiments we tried histones; however, such basic proteins can protect DNA against DNase directly in the absence of lipid, so we turned to lysozyme, a basic protein that does not have specific DNA-binding properties.

[^{32}P]5′-DNA was added to a mixture of phospholipids, either alone or with protein, and the sample was sonicated to form vesicles. The protein was present in 1000-fold weight excess. After these vesicles were challenged with micrococcal nuclease, the DNA was assayed for protection from digestion by gel electrophoresis and autoradiography.

Fig. 1 shows the results of such experiments. The labeled DNA, alone, without nuclease treatment, runs as a single band (lane A). Thus the sonication treatment did not damage the DNA (observed for both 1000- and 4300-base pair-long fragments). Samples of DNA sonicated with lysozyme alone (lane B) or lipid alone (lane C) and subsequently treated with

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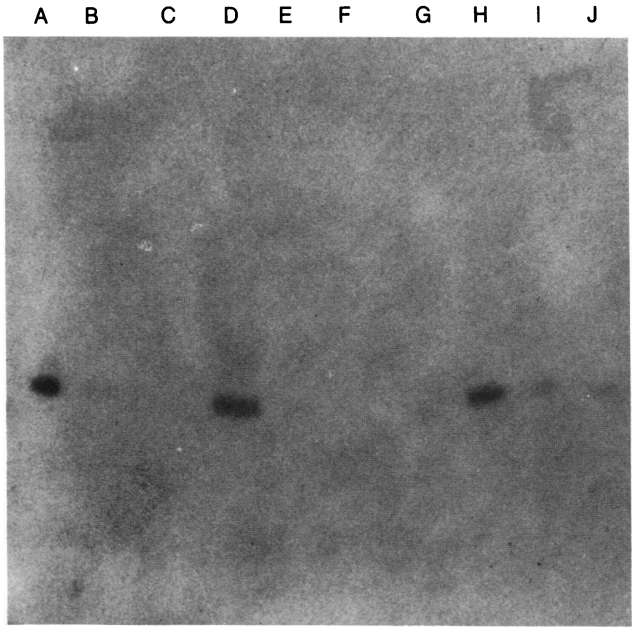

FIG. 1. Lysozyme-mediated vesicle encapsulation protects DNA from nuclease digestion. Linear [^{32}P]5′-DNA, 1000 base pairs in length, was sonicated with lipid and protein to form vesicles and challenged with micrococcal nuclease. The samples were run on a 0.7% agarose gel, dried, and autoradiographed with intensifier screen. Lane A, DNA; lane B, DNA and lysozyme with nuclease digestion; lane C, DNA and lipid with nuclease digestion; lane D, DNA, lipid, and lysozyme with nuclease digestion; lane E, DNA, lipid, and bovine serum albumin with nuclease digestion; lane F, DNA, lipid, and lysozyme treated with 1% Triton X-100 before nuclease digestion; lane G, DNA added after the sonication of lipid and lysozyme followed by nuclease digestion; lane H, one μg of trypsin added to DNA, lipid, and lysozyme after sonication followed by nuclease digestion; lane I, one μg of trypsin added before sonication to DNA, lipid, and lysozyme followed by nuclease digestion; lane J, 200 mM $MgCl_2$, DNA, lipid, and lysozyme with nuclease digestion.

nuclease were completely digested. However, when DNA was sonicated with lysozyme and lipid together (lane D), at least 50% of the DNA was protected (verified by densitometry scans of the autoradiograph). The sonication of DNA together with lipid and an acidic protein, bovine serum albumin, did not result in protection (lane E). These data show that under these conditions DNA is not efficiently encapsulated by lipid vesicle formation unless a basic protein (lysozyme) is present. Acidic protein (bovine serum albumin) cannot perform this function.

If the DNA was added to previously sonicated lysozyme/lipid mixtures, it was not protected from nuclease digestion (lane F), and the addition of the non-ionic detergent Triton X-100 (which disrupts vesicles) to lysozyme-lipid vesicles led to the digestion of the DNA (lane G). Incubation of the lipid, lysozyme, and DNA mixture with trypsin prevented protection only if the trypsin was added before sonication (lane I) but not afterward (lane H). These data demonstrate that the protection is dependent upon the integrity of the vesicles. Furthermore, the DNA and protein must both be present during vesicle formation.

The addition of 200 mM $MgCl_2$ to the lysozyme, lipid, and DNA mixture prevented the protection from nuclease (lane J). This suggests that the interaction of DNA with lysozyme is charge dependent, but more than a simple charge neutralization of the nucleic acid (which would be provided by the Mg^{2+} counter-ions) is required for lipid encapsulation of the DNA.

Flotation Analysis of Encapsulation. As an alternative demonstration that the DNA is associated with vesicles, we analyzed vesicles prepared with radiolabeled DNA and either lysozyme or bovine serum albumin by centrifugation on Ficoll step gradients. After centrifugation, we observed two opalescent bands—one at the interface between the sample and 20% Ficoll and the other between the 10% Ficoll and the buffer layers. The radiolabeled DNA was detected by Cherenkov counting each fraction from the gradient. Fig. 2 shows that in the lysozyme-prepared vesicles, 50% of the radiolabeled DNA comigrated with the first opalescent band, floating with the vesicle population on the Ficoll step gradient. However, the DNA remained at the bottom of the gradient when the vesicles were made with bovine serum albumin.

Both samples were examined by electron microscopy of thin sections from Airfuge-pelleted vesicles. They contained a heterogeneous mixture of vesicles including single lamellar and multilamellar structures. Vesicles prepared with lysozyme had diameters between 1000 and 2000 Å, whereas those made with bovine serum albumin ranged from 2500 to 4000 Å.

These data together show that lysozyme mediates an enhanced segregation of DNA into vesicles in a charge-dependent fashion and that once vesicles are formed, macromolecules are excluded. The observations that the protection is eliminated by detergent and that the DNA must be present before sonication argue against a nonspecific protection of the DNA by lipid.

Lysozyme Enhances DNA Encapsulation 100-Fold Above That Expected by Random Trapping. Fraley *et al.* (3) and Deamer and Barchfield (2) have reported encapsulation of DNA by the formation of large vesicles in which the fraction of DNA incorporated was equal to the fraction of total volume trapped by the vesicles. Half of the DNA added was thus incorporated, because the large average diameter of these vesicles (0.4 micrometers) resulted in 50% of the total volume being trapped.

The internal volume of the vesicles in our experiments was 0.5% of the total volume (determined by ^{14}C-labeled sucrose trapped volume studies and corroborated by calculations based on the total amount of lipid and the average vesicle diameter). Fifty percent of the DNA was protected, well above the expectation from random trapping during sonication. This implies that lysozyme provides some specific enhancement for vesicle encapsulation beyond charge neu-

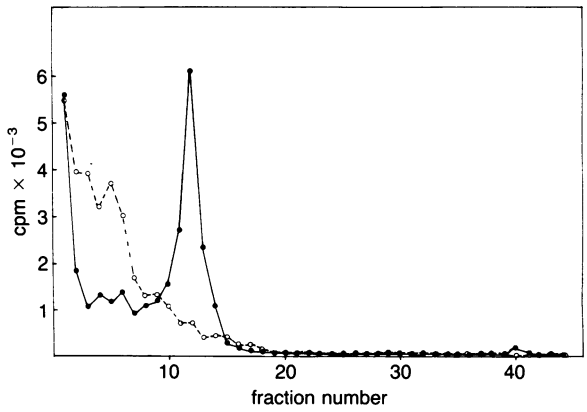

FIG. 2. Flotation of vesicles by Ficoll-gradient centrifugation. Ficoll gradient samples were prepared as described. Two opalescent bands were observed at the interfaces between the sample and 20% Ficoll and between 10% and 0% Ficoll; the location of the peak of ^{32}P radioactivity corresponds to the former opalescent band. The gradients were fractionated into 130-μl aliquots, and radiolabeled DNA was detected by Cherenkov radiation. Vesicles were prepared with [^{32}P]5′-DNA, lipid, and lysozyme (●) or with [^{32}P]5′-DNA, lipid, and bovine serum albumin (○).

tralization, which would only account for incorporation by random trapping. In fact, the concentration of DNA within the internal environment of the vesicles was increased 100-fold.

The preferential enclosure of DNA by lipid mediated by lysozyme may have a practical application. Vesicle fusion has been used to introduce DNA into animal cells (3, 5). Enhanced incorporation of DNA may increase the efficiency of this method of DNA transfection and permit one to use vesicles as targeting vehicles.

Significance for Prebiotic Evolution. If protein-mediated encapsulation occurred during early cell formation, why would such an event favor prebiotic evolution? Encapsulation segregates macromolecules away from the external environment. Protein-mediated encapsulation creates high local concentrations of protein and nucleic acids within the vesicular volume compared with random trapping. This would enhance the interaction of molecules with low affinities, potentiating the formation of aggregates with biological function. Permeable membranes or polypeptide pores would permit the entry of small molecules. The fusion and budding of vesicles might provide a means by which functional aggregates could be shuffled to obtain new prebiotic species. Most importantly, the sequestering of self-replicating nucleic acids into membrane-bound structures would provide the separate entities on which natural selection could operate.

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