Key words: cyanophage As-1/adsorption/liposomes/phospholipids

AS-1 Cyanophage Adsorption to Liposomes

(Accepted 18 February 1982)

SUMMARY

The cyanophage AS-1 adsorbs to vesicles of known lipid composition. The lipids used included: phosphatidylcholine (from soybean); monogalactosyldiacylglycerol, digalactosyldiacylglycerol, sulfoquinovosyldiacylglycerol, and phosphatidylglycerol (from Anacystis nidulans); cholesterol. Adsorption required the presence of phosphatidylcholine and cholesterol but not the algal lipids.

The cyanophage AS-1 infects the unicellular algae, Synechococcus cedrorum and Anacystis nidulans (Safferman et al., 1972). It has been shown that AS-1 cyanophage adsorbs to spheroplasts prepared from synchronous cultures of S. cedrorum using lysozyme treatment (Desjardins & Barkley, 1972). Attempts to study the adsorption and release of nucleic acid of cyanophage AS-1 were carried out in this study using liposomes prepared from well-defined lipids.

Liposomes were prepared using L-a-phosphatidylcholine (PC: 1,2-diacyl-sn-glyceryl-3-phosphocholine; Sigma, P5638 from soybean), cholesterol (C) (Sigma, CH-S 99+%%) and different fractions of the lipids extracted from A. nidulans. Lipids were extracted from A. nidulans (UTEX-625) cultivated under standard conditions (Barkley, 1976). Seven-day-old liquid cultures were centrifuged for 10 min at 10000 g. Chloroform/methanol (50%, v/v) was added to the cell pellet, kept for 30 min at room temperature and immediately treated with water/chloroform according to the method of Bligh & Dyer (1959). The chloroform phase was separated and filtered, and dried by low-pressure evaporation. The lipid film was redissolved in methanol/dichloroethane (9:1) and passed through a Lipidex-5000 (Packard Instruments) column (19 × 1.2 cm). Fractions of 4 ml were collected and aliquots were analysed by thin-layer chromatography (TLC).

TLC analysis of the lipid fractions of A. nidulans showed the presence of digalactosyldiacylglycerol (DGDG), monogalactosyldiacylglycerol (MGDG), phosphatidylglycerol (PG) and sulfoquinovosyldiacylglycerol (SL). Fraction number 3 contained DGDG, SL and PG. Fraction number 4 contained DGDG, SL, PG and MGDG. Fraction number 5 contained PG and MGDG. These lipid fractions were separated from the algal pigments, which were retained on the column.

Purified preparations of AS-1 cyanophage were added to liposomes prepared with PC + C alone or mixed with the lipid fractions number 3, 4 or 5 of A. nidulans. The mixtures were kept for 60 min at room temperature. After this period the preparations were placed on to grids, stained with 4% uranyl acetate and analysed under the electron microscope (EM) (Hitachi HU12). The EM analyses have shown attachment and sheath contraction of AS-1 cyanophage only to the liposomes prepared with PC + C alone or in mixtures with fractions number 3, 4 or 5 (Fig. 1 and 2). There was no case of attachment or sheath contraction of the AS-1 cyanophage when mixed with liposomes prepared only with fractions number 3, 4 or 5 alone or in any combination of these fractions.

These results show that the attachment of AS-1 cyanophage to spheroplasts can be simulated by using artificial lipid vesicles. The stimulus for attachment and sheath contraction depends on the presence of soybean PC and cholesterol in the vesicle. Further experiments will delineate the specific lipid requirement.
Fig. 1. Virus AS-1 attachment to lipid vesicles containing algal lipids. (a) AS-1 virus particles. (b, c) Attachment of virus particles to lipid vesicles. These were prepared by mixing chloroform solutions of PC (10.6 mg), cholesterol (1 mg) and mixed lipids from *A. nidulans* (MGDG, DGDG, SL and PG; fraction 4 total 7.2 mg), removing the solvent under reduced pressure, and adding 4 ml of a solution 0.05 M with respect to tris–HCl pH 7.4 and 0.075 M with respect to NaCl. The mixture was placed in a bath sonicator (Bransonic 52) for 3 min to suspend the lipid. The AS-1 was added after the vesicle preparation. Bar marker represents 0.1 μm for (a) to (c).
Fig. 2. Virus AS-1 attachment to PC + C vesicles. Lipid vesicles were prepared as described in the legend to Fig. 1 except that only PC and C were used in the preparation. AS-1 was added after vesicle preparation. Bar marker represents 0.1 μm for (a) to (d).
Short communications

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(Received 12 August 1981)