INTERACTION BETWEEN ISOLATED DNA OF PHAGE λ AND Escherichia coli SPHEROPLASTS TREATED WITH STURINE

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Centrifugation of preparations of spheroplast membranes, treated and untreated with sturine and infected with DNA of phage λ, in a sucrose density gradient (30-55%) showed that sturine treatment of the spheroplasts increases the adsorption of phage DNA by about three times. Under these circumstances, about 50% of the phage DNA molecules absorbed by the spheroplasts are bound with the cytoplasmic membrane of the sturine-treated spheroplasts, and 50% of the DNA molecules are bound with the cell wall of spheroplasts untreated with sturine. It can be concluded from these results that the stimulating effect of sturine in transfection of E. coli spheroplasts by phage λ DNA is connected with a redistribution of phage DNA adsorbed on the spheroplasts from the cell wall to the cytoplasmic membrane, thereby facilitating penetration and attachment of the introduced DNA to the membrane.

KEY WORDS: transfection; spheroplasts; sturine; DNA–membrane complex.

There is as yet no unanimity regarding the mechanism of the stimulating action of basic proteins in transfection of bacterial spheroplasts. The lack of factual material on this question has led to the promotion of various hypotheses all of which require experimental verification [1, 3-5]. In particular, it has been shown that the treatment of spheroplasts with protamine increases the efficiency of binding isolated phage DNA with the recipient. During contact between the bacterial recipients and the protamine, structural changes have been observed in the surface of the spheroplasts, and the basic protein is found both on the surface of the spheroplasts, in the cytoplasmic membrane, and to a lesser degree, directly in the cytoplasm itself.

The object of this investigation was to study interaction between isolated biologically active DNA of phage λ and the membranous structures of Escherichia coli spheroplasts, either treated or untreated with the basic protein sturine.

EXPERIMENTAL METHOD

Phage λ C1857 DNA-^{3}H was used for transfection. DNA-^{3}H-labeled phage was prepared by thermoinduction of strain E. coli CR-34 (λ C1857) λ $B_1^-TL^-$thymine$^-$, lysogenic for this phage, obtained from Baldwin (USA), and grown in synthetic medium with thymidine-^{3}H (5 μCi/ml) [7]. Phage λ DNA-^{3}H was isolated by double phenolization followed by dialysis against 0.01 M Tris–HCl buffer, pH 7.8. The labeling efficiency was 36,000 cpm/μg DNA. The recipient was E. coli strain 7026, a K12 (lac^-, pro^-, B_1^-) derivative obtained from Beckwith (USA). Lysozyme spheroplasts were prepared and treated with sturine as described previously [6]. The combined sturine–protamine fraction from sturgeon milt was kindly provided by E. P. Yulikova (Moscow University).

The biological activity of the DNA was assessed from the number of infected spheroplasts. For this purpose DNA was added to 1 ml of a suspension of spheroplasts (10^9 spheroplasts/ml), after preliminary contact with sturine (3 h at 4°C), up to a final concentration of 0.5 μg/ml, and the mixture was incubated for 10 min at 37°C. The samples were then investigated by the agar layer method with E. coli 7026 as the indicator strain.

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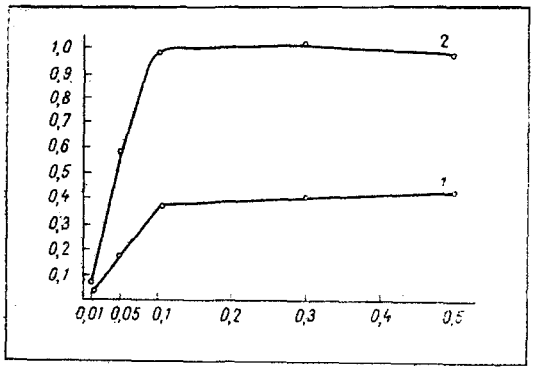

Fig. 1. Dependence of binding of phage λ DNA molecules by E. coli spheroplasts on quantity of phage DNA added: 1) untreated with sturine; 2) treated with sturine. Abscissa, quantity of phage DNA added (in μg/ml suspension containing 10^9 spheroplasts); ordinate, number of bound molecules of phage λ DNA per spheroplast.

The λ DNA–spheroplast membrane complex was isolated from the infected spheroplasts after washing them twice for 1 min at 12,000g, by osmotic lysis in 5 μM EDTA, pH 7.8. The membrane fractions were separated by centrifugation in a 30-55% sucrose gradient on a Spinco centrifuge with SW-39 rotor for 16 h at 35,000 rpm. Fractions of 0.2 ml were taken and their density determined with a refractometer. Radiometry of the samples was carried out with a Mark II liquid scintillation counter. Standard scintillation fluid (0.6% PPO and 0.01% POPOP in toluene) was used for counting the radioactivity on the filter.

EXPERIMENTAL RESULTS

The aim of the experiments was quantitative analysis of the binding efficiency of the phage DNA with the cell wall and the cytoplasmic membrane of spheroplasts either treated or untreated with sturine. A control of the biological activity of the phage DNA also was set up at all stages specified above with respect to determination of the binding efficiency of the phage DNA.

Experiments with infection of E. coli spheroplasts, treated or untreated with sturine, by isolated DNA-^{3}H from phage λ were carried out with consideration of the optimal conditions for transfection determined previously [6]: concentration of spheroplasts 10^9/ml, concentration of sturine 15 μg/ml. The optimal concentration of DNA was determined in experiments to study adsorption and transfection (Fig. 1); DNA was used in a concentration (0.5 μg/ml) ensuring stability of the results.

The proportion of added DNA to be bound with spheroplasts untreated with sturine was 18%. The distributions of phage λ DNA molecules absorbed by the spheroplasts on their membrane fractions is shown in Fig. 2: As will be seen, 50% of the DNA bound with the spheroplasts was distributed in the sucrose gradient at a density of 1.22, characteristic of the cell wall.

After treatment of the spheroplasts with sturine, 2.5-3 times more of the added DNA molecules bound with them than with spheroplasts untreated with sturine. Under these circumstances about 50% of the phage DNA bound with the spheroplasts was distributed in the sucrose density gradient at 1.16, corresponding to the density of the cytoplasmic membrane.

Verification of the biological activity of the phage DNA showed that after treatment of the spheroplasts with protamine the efficiency of transfection was increased a hundredfold: from $2\cdot10^2$-$3\cdot10^2$ infection sites/ml in the case of untreated spheroplasts to $2\cdot10^4$-$4\cdot10^4$ infected spheroplasts/ml after treatment with sturine.

It can be concluded from the results of these experiments that stimulation of transfection with phage λ DNA caused by treating E. coli spheroplasts with sturine is due not so much to an increase in the adsorption of phage DNA on the recipient as to a redistribution of DNA already adsorbed from the cell wall to the cytoplasmic membrane.

The very small increase in adsorption of phage DNA on sturine-treated spheroplasts obtained in these experiments compared with the 20-fold increase obtained in experiments to study adsorption of phage T2 DNA on bacterial spheroplasts [2] may perhaps be connected with differences in the size of the DNA and, in particular, with the fact that in the case of phage T2 DNA, whose molecular weight is much greater than that of

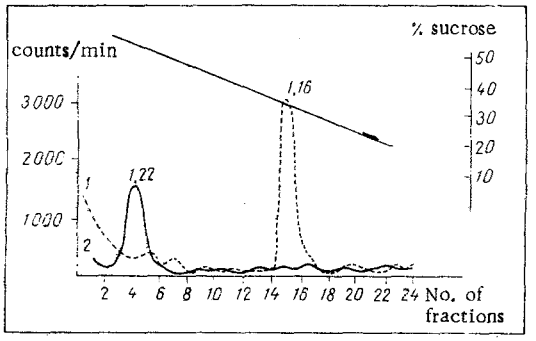

Fig. 2. Distribution of DNA of phage λ among membrane fractions of infected E. coli spheroplasts in sucrose density gradient: 1) spheroplasts treated with sturine; 2) spheroplasts untreated with sturine.

phage λ DNA, no transfection whatever is observed on spheroplasts untreated with protamine, and only treatment with protamine converts the spheroplasts into recipients for transfection by T2 DNA. In the case of phage λ DNA, on the other hand, treatment of the spheroplasts with protamine leads only to stimulation of the phenomenon of transfection which already takes place.

It was shown previously that treatment of spheroplasts with sturine for 3 h in the cold is essential for the redistribution of protein on the surface of the spheroplasts, in the membrane, and in the cytoplasm [6]. The results now obtained suggests that sturine is bound with the cytoplasmic membrane of the cell wall, and they also confirm the hypothesis that the action of sturine during transfection is aimed largely at facilitating penetration of phage DNA molecules into the spheroplasts and their binding to the membrane.

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