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Short Communication

Characterization of Arginine as a Receptor Component for Coliphage T2H and Regions for Production of Phage Virion

(arginine/coliphage T2H/receptor)

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The number of phage T2H particles decreased with an increase in the amounts of authentic D-arginine and the lipoprotein preparation from *Escherichia coli* B. The number of phage particles, preincubated with D-arginine or lipoprotein preparation, did not decrease with the addition of indole which inhibits the adsorption of phage T2H to the host cell. These results suggest that D-arginine defines the characteristics of the adsorption of phage T2H or occupies the determinant group of the receptor site for the phage on the cell surface of *E. coli* B.

The radioactivity of [^{3}H]-thymidine-labeled phage T2H DNA was coincident with the NADH oxidase activity in the inner membrane fraction from the cell envelope of *E. coli* B, by centrifugation in a sucrose density gradient. It is quite possible that the phage DNA which binds to the receptor site on lipoprotein moiety in the outer membrane may reach specific loci on the inner membrane within 20 min of a latent period, after which production of the phage virion would occur.

The cell envelope of *E. coli* is a complex, multilayered structure composed of the cytoplasmic membrane and the cell wall which itself includes the peptidoglycan layer and the outer membrane (1). The outer membrane consists of lipopolysaccharide, lipoprotein, protein and phospholipid (2), and also contains receptors for various coliphages (3—6). The receptor for phage T2 is composed of lipoprotein (3), and we recently found that phage T2H was inactivated by basic D-amino acids such as D-arginine and D-lysine (7). However, it has not been determined whether or not D-arginine is the main component of the receptor for phage T2H. This paper deals with the characterization of D-arginine as a receptor component for the phage and with the determinant region for the production of phage virion.

Phage T2H suspended in a concentration of 5×10^6 plaque-forming units (PFU) per ml in 0.1 M Tris-HCl buffer (pH 7.4) was incubated with or without various 0.5 M substances tested at 37°C for 30 min. After incubation,

TABLE I. *Inactivation of Coliphage T2H by Various Substances*

Additions (0.5M)	Survival (%)	Additions (0.5M)	Survival (%)
None	100	D-Lysine	30
D-Ariginine	12	Cadaverine	96
L-Arginine	35	6-Amino hexanoic acid	75
Agmatine	95	Guanidine	90
L-Homoarginine	28	Ornithine	98

Phage T2H suspension (5×10^6 PFU/ml) was incubated with or without 0.5 M substances tested at 37°C for 30 min. The mixture was withdrawn after incubation, and the number of plaque-forming units (PFU) remaining was assayed as described previously (8).

the mixture was assayed for PFU as described previously (8). The results of inactivation of phage T2H by various substances are summarized in Table I. D-Arginine and D-lysine which were mainly contained in acid hydrolysate of lipoprotein (7) markedly inactivated the phage particles, whereas agmatine (decarboxylated arginine) and cadaverine (decarboxylated lysine) did not inactivated these particles. Guanidine, lacking a carboxyl group, was also inactive. It can be concluded that the presence of a negatively charged carboxyl group in these compounds is essential for viral inactivation. In the case of 6-aminohexanoic acid lacking the α-amino group (its two-dimensional structure resembles that of lysine), such did not lead to inactivation of the phage. However, the phage-inactivating ability of 6-aminohexanoic acid was more potent rather than that seen with decarboxylated compounds. On the other hand, L-homoarginine with one extra CH_2 group was more active than L-arginine, while ornithine which has one less CH_2 group than does lysine was completely inactive. It seems likely that α-amino and CH_2 groups are responsible for the irreversible conformational change which occurs after the binding reaction to the structure of the phage.

Kanner and Kozloff (9) reported that indole has a specific effect on the biological properties of phage T2 and inhibits the adsorption of the phage to the host cells. To determine whether or not the phage-inactivating and the phage-adsorbing abilities were blocked by indole, the mixture of phage suspension (5×10^6 PFU/ml) and 0.5 M D-arginine or 500 μg/ml of lipoprotein preparation, obtained by the method of Clarke *et al.* (10), was incubated at 37°C with or without 0.04 M indole, withdrawn at intervals, after which the number of PFU remaining was assayed. As shown in Fig. 1, the PFU in samples, preincubated with D-arginine or the lipoprotein preparation, did not decrease by addition of indole. These results indicate that indole blocks the phage-inactivating and -adsorbing abilities of D-arginine. We also reported that the receptor sites for T4D and gamma phages which are composed of glucose and glucose derivatives such as N-acetyl-glucosamine and glucosamine were blocked by wheat germ agglutinin and concanavalin A (6, 11). The adsorption of phage T2H to the host cell was not inhibited by these lectins

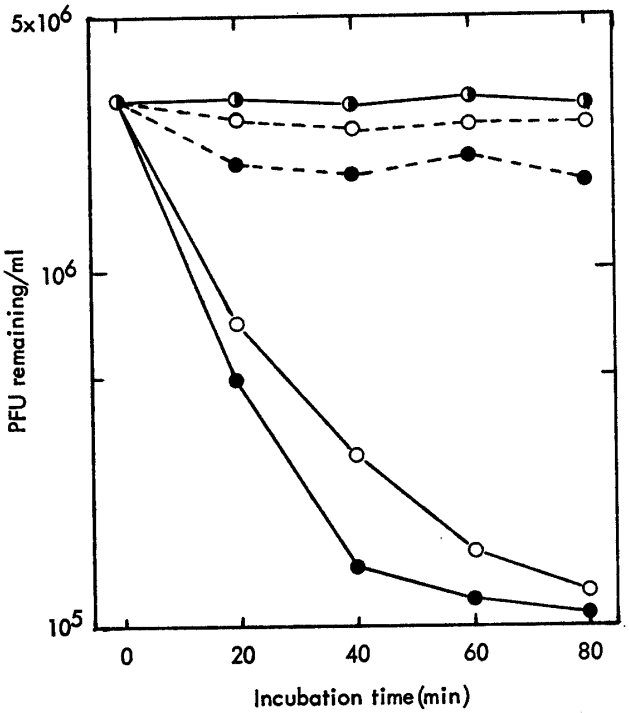

Fig. 1. Blocking of inactivation and adsorption of phage T2H by indole. The mixture of phage particles (5×10^6 PFU/ml) and 0.5 M D-arginine or lipopolysaccharide preparation (500 μg/ml) was incubated at 37°C with or without 0.04 M indole. As control, the phage particles were incubated at 37°C with indole (—◑—). The mixture was withdrawn at indicated intervals and the number of PFU was assayed. T2H+arginine (—○—), T2H+arginine+indole(- -○- -), T2H+lipoprotein(—●—), T2H+lipoprotein +indole (- -●- -).

(data not shown), From these findings, it is quite plausible that D-arginine defines the characteristics of the adsorption of phage T2H or occupies the determinant group of the receptor site on the cell surface of *E. coli* B.

To determine the penetrating phage T2H DAN associates with either the outer or the inner membrane from *E. coli* B, the cells were incubated at 37°C for 18 hr in amino acid-enriched Mg medium (12) containing 3 μCi of [^{14}C]-leucine. [^{14}C]-leucine-labeled lipoprotein fraction from the labeled cells was prepared by the method of Clarke *et al.* (10). The procedure for obtaining the labeled phage DNA was carried out by the method of Jazwinski *et al.* (13), that is, *E. coli* B cells, grown to $2-4\times10^8$/ml in the above medium containing 5 mCi of [^{3}H]-thymidine were multiplely infected with 4 to 6 phage particles/cell (MOI) and superinfected with the same MOI 5 to 8 min later. After 2—3 hr of incubation at 37°C, a maximum amount of phage-related material had accumlated intracellularly, and the cells were harvested by low speed centrifugation. The lysis of the cells was induced by chloroform, with repeated thawing. [^{3}H]-thymidine-labeled phage particles were purified and collected as described previously (14). The labeled phage particles were used at MOI of 5—8 to coinfect *E. coli* B cells (5×10^8/ml) for 20 min at 37°C. The cells were chilled to 0°C, washed and resuspended in the above buffer

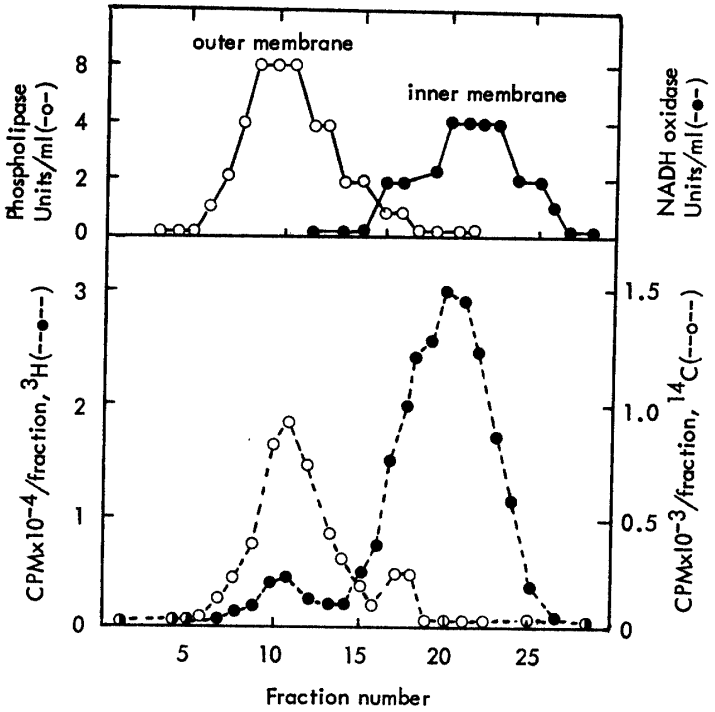

Fig. 2. Association of [^{3}H]-thymidine-T2H DNA and [^{14}C]-leucine-lipoprotein with inner and outer membrane from *E. coli* B. The mixture of the membranes containing [^{3}H]-T2H DNA and the [^{14}C]-lipoprotein preparation was applied to a linear sucrose density gradient (10—70%), fractionated by ultracentrifugation, and 6 drops were collected from the bottom of the tube. Aliquots were spotted on filter discs, dried and the radioactivities were counted in 10 ml of toluene-based scintillation fluid. The measurements of NADH oxidase and phospholipase activities were done by the method of Jazwinski *et al.* (16) and Osborn *et al.* (15), respectively.

at 5×10^{9}/ml. To isolate the outer and inner membranes from the labeled phage-infected cells, the cells were disrupted by sonic treatment. The membrane fraction was purified by differential centrifugation, collected by centrifugation at 105,000 × g for 30 min, resuspended in a minimal volume of the buffer containing 1 % Triton X, and then stirred for 60 min (8, 15). The mixture of the above lysate containing [^{3}H]-thymidine-labeled DNA and [^{14}C]-leucine labeled lipoprotein preparation was applied to a linear sucrose density gradient (10—70 %). The gradient was centrifuged at 105,000 × g for 18 hr at 4°C, and then 6 drops were collected from the bottom of the tube. Fractionated aliquots were spotted on Whatman 3 MM filter discs, dried and radioactivities were counted in 10 ml of toluene scintillation fluid (4 g PPO and 0.1 g dimethyl-POPOP in 1,000 ml of toluene). The position of the inner membrane is usually defined by NADH oxidase activity (16) while that of the outer membrane by phospholipase activity (15). As shown in Fig. 2, the radioactivity of [^{3}H]-thymidine-phage DNA coincided with the NADH oxidase activity in the inner membrane fraction, while that of [^{14}C]-leucine-lipoprotein to the phospholipase activity in the outer membrane fraction. Jazwinski *et al.* (16) reported that coliphage M13 which bound to the receptor on the cell surface

of *E. coli* K-12 undergoes an eclipse and that the DNA penetrates the inner membrane. In the case of phage T2H, it is concluded that the phage DNA which bound to the receptor site on the lipoprotein moiety from the outer membrane may reach specific loci of the inner membrane within 20 min of a latent period for the production of the phage virion. Otherwise, the lipoprotein fraction may be derived from the outer membrane from the cell wall of *E. coli* B.

REFERENCES

1) Glauert, A. M. and Thornley, M. J. (1969) The topography of the bacterial cell wall. *Annu. Rev. Microbiol.* **23**, 159–198
2) Osborn, M. J. (1969) Structure and biosynthesis of the bacterial cell wall. *Annu. Rev. Biochem.* **38**, 501–538
3) Weidel, W. (1958) Bacterial virus (with particular reference to adsorption/penetration) *Annu. Rev. Microbiol.* **12**, 27–48
4) Michael, J. G. (1968) The surface antigens and receptors in *Escherichia coli* B. *Proc. Soc. Exp. Biol. Med.* **128**, 27–48
5) Randall-Hazelbauer, L. and Schwartz, M. (1973) Isolation of bacteriophage lambda receptor from *Escherichia coli*. *J. Bacteriol.* **116**, 1436–1446
6) Watanabe, T. (1976) Role of lipopolysaccharide in adsorption of coliphage T4D to *Escherichia coli B*. *Can. J. Microbiol.* **22**, 745–751
7) Watanabe, T. and Saito, H. (1978) Inactivation of coliphage T2H by basic amino acids. *Microbiol. Immunol.* **22**, 167–172
8) Watanabe, T. and Shiomi, T. (1975) Inhibiting materials for gamma phage adsorption to the cell wall of *Bacillus anthracis, Pasteur* No. 2–H. *Jpn. J. Microbiol.* **19**, 115–121
9) Kanner, L. C. and Kozloff, L. M. (1964) The reaction of indole and T2 bacteriophage. *Biochemistry* **3**, 215–223
10) Clarke, K., Gray, G. W., and Reavely, D. A. (1967) The extraction of cell wall of *Pseudomonas aeruginosa* with aqueous phenol (material from the phenol layer). *Biochem. J.* **105**, 755–758
11) Watanabe, T. and Shiomi, T. (1976) Effect of plant lectins on gamma phage receptor sites of *Bacillus anthracis*. *Jpn. J. Microbiol.* **20**, 147–149
12) Kellenberger, E. (1968) Studies on the morphopoiesis of the head of phage T-even. V. the components of the T4 capsid and of other capsid-related structure. *Virology* **34**, 549–561
13) Jazwinski, S. M., Lindberg, A. A., and Kornberg, A. (1975) The lipopolysaccharide receptor for bacteriophage ϕX 174 and S13. *Virology* **66**, 268–282
14) Watanabe, T., Morimoto, A., and Shiomi, T. (1975) The fine structure and the protein composition of gamma phage of *Bacillus anthracis*. *Can. J. Microbiol.* **21**, 1889–1892
15) Osborn, M. J., Gander, J. E., Parisi, E., and Carson, J. (1972) Mechanism of assembly of the outer membrane of *Salmonella typhimurium*. Isolation and characterization of cytoplasmic and outer membrane. *J. Biol. Chem.* **247**, 3962–3972
16) Jazwinski, S. M., Marco, R., and Kornberg, A. (1973) A coat protein of the bacteriophage M13 virion participates in membrane-oriented synthesis of DNA. *Proc. Natl. Acad. Sci. USA* **70**, 205–209