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Nature of the Energy Requirement for the Irreversible Adsorption of Bacteriophages T1 and ϕ80 to *Escherichia coli*

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The nature of the energy requirement for irreversible adsorption of phages T1 and ϕ80 was studied by using various specific energy inhibitors and mutants lacking either the Ca^{2+}, Mg^{2+}-adenosine triphosphatase or the ability to produce cytochromes in the absence of added 5-aminolaevulinic acid. It was found that irreversible adsorption could be energized both through the electron transport chain and from adenosine 5'-triphosphate via the Ca^{2+}, Mg^{2+}-adenosine triphosphatase, indicating the involvement of the energized membrane state. These results and the discovery that phages T1 and ϕ80 adsorb reversibly to the isolated *tonA* gene product are discussed in terms of the possible involvement of functions expressed by the *tonB* gene region in irreversible adsorption and the relationship to iron transport.

It has been shown that some phages adsorb to the host cell by a two-step process. The first step is reversible (15, 21) and possibly involves an electrostatic interaction between the phage tail fibers and the host cell wall, whereas the second step is irreversible adsorption, after which infective phage particles cannot be eluted from the cell by dilution or agitation. For phages T1 and ϕ80, unlike other T phages, these processes can be simply differentiated (15). It has been long known that phage T1 requires energy for irreversible adsorption, whereas the other T phages have no such requirement. For instance, it was shown that irreversible adsorption of T1 was inhibited by 10 mM sodium azide (10) or by low temperature (21) and did not occur with cells disrupted in various ways (10, 29) or treated with heat or ultraviolet light (15). It was further shown (14, 15) that *tonB* mutants could adsorb T1 reversibly but not irreversibly, whereas *tonA* mutants could perform neither function. A number of other phenomena being studied in this laboratory, such as enterochelin, citrate, and ferrichrome iron transport, killing of cells by colicins B, I, V, M, and phage T5 adsorption, are blocked in *tonA* or *tonB* mutants (as summarized in 18). Therefore, it was decided to study the nature of the energy requirement of T1 and ϕ80 adsorption to relate it to the energization of these other processes. Recent advances in the study of the energy requirement for amino acid and sugar transport in whole cells (5, 6, 13, 20, 25), as well as the development of mutants defective in oxidative phosphorylation (9, 25) and the electron transport chain (16), have provided an adequate means for the study of this problem.

MATERIALS AND METHODS

Bacterial strains and media. *Escherichia coli* K-12 strains Ymel (*rha fadA but-12 lacI supE57 supF58*λ^+), BH212 (*rha fadA but-12 lacI supE57 supF58 uncA*λ^+) and A1004c (*rha fadA but-12 lacI supE57 supF58 ilv metE hemA*λ^+) were all supplied by H. Schairer (Tübingen). These strains had the same properties as other previously described *uncA* and *hemA* strains (16, 25).

Tryptone broth contained 1% tryptone (Difco 0123-01) and 0.5% NaCl. The addition of 1.5 and 0.65% agar (Difco 0140-01), respectively, made plates and soft agar overlays. For strains Ymel and BH212, minimal media was M9 media supplemented with 0.5% glucose or 0.4% succinate. For strain A1004c, Cohen-Rickenberg minimal medium as modified by Anraku (1) was used, supplemented with 0.5% maltose, 20 μg of the required amino acids per ml, 0.3% Casamino Acids, and, when needed for aerobic growth, 10 μM 5-aminolaevulinic acid. Adsorption buffer was 0.01 M tris(hydroxymethyl)aminomethane-hydrochloride (Tris), pH 7.4, containing 6×10^{-4} M $MgSO_4$ and 10 mg of gelatin per liter as described by Takeda and Yura (28).

Chemicals. *N,N'*-dicyclohexylcarbodiimide (DCCD), carbonyl cyanide-*m*-chlorophenolhydrazone (CCCP), *para*-chloromercuribenzene sulfonic acid (pCMBS) and *para*-chloromercuribenzoate (pCMB) were purchased from the Sigma Chemical Co., St. Louis, Mo., potassium cyanide, sodium azide, and disodium hydrogen arsenate from E. Merck, Darmstadt, West Germany, and 2,4-dinitrophenol (DNP) from Serva, Heidelberg, West Germany. 2-Trifluoromethyltetrachlorobenzimadazole (TTFB) was a gift from E. Bamberg (Konstanz).

Growth of phages and bacteria. Phages T1 and ϕ80 were propagated by the technique of Deeb et al. (12) using tryptone instead of Penassay broth, followed by the precipitation of phage with 10% polyethylene glycol.

Strain Ymel was maintained on tryptone agar plates, whereas strain BH212 was maintained on M9 agar containing 0.5% glucose. Strain A1004c was maintained on M9 agar containing 0.5% glucose, 20 μg of isoleucine per ml, 20 μg of valine per ml, and 20 μg of methionine per ml, and 10 μg of 5-aminolaevulinic acid per ml, on which medium it could grow aerobically.

Strains Ymel and BH212 were grown overnight with shaking at 37 C in M9 media containing 0.5% glucose. They were then diluted 1:15 and 1:11, respectively, and grown for 2 to 3 h (three to four generations) at 37 C with shaking to an optical density (OD) of 0.4 to 0.6 at 578 nm, centrifuged down at 20 C, washed once with 0.01 M Tris buffer, pH 7.6, and resuspended in adsorption buffer to an OD_{578} of 0.5. Before arsenate treatment, the cells were washed three times in Tris buffer before resuspension in adsorption buffer. Cultures of strain BH212 were frequently tested for revertants by plating the washed culture on M9 agar with succinate as the sole carbon source.

For the growth of strain A1004c, a single colony was inoculated into Cohen-Rickenberg medium containing maltose, required amino acids, and Casamino Acids, and the culture flask was placed in a desiccator. Air was extracted from the desiccator with a rotary vacuum pump for 30 to 60 min and the cells were grown anaerobically at 37 C to late-log or stationary phase. The culture was then diluted to an OD_{578} of 0.15 in the same medium and again grown in an evacuated desiccator at 37 C until it had reached an OD_{578} of approximately 0.5. The culture was then centrifuged, washed, and then resuspended in adsorption buffer as described above for strains Ymel and BH212.

Washed cultures were streaked on M9 agar containing succinate and required supplements to test for revertants. Although the subsequent adsorption experiment was performed in the presence of air, the *hemA* mutant A1004c grown without added 5-aminolaevulinic acid lacks cytochromes and mimics anaerobic growth.

Adsorption assay. Phage adsorption was assayed at 37 C after pretreatment of the cells (5×10^8 cells/ml) with one of the following inhibitors, the pretreatment times being given in parentheses: DCCD (30 min), arsenate (15 min), DNP (10 min), azide (10 min), CCCP (8 min), pCMBS (8 min), pCMB (8 min), cyanide (7 min) or TTFB (7 min). After these periods of incubation, 0.1 ml of a solution of phage T1 or ϕ80 (MOI = 0.4 to 0.6 diluted in adsorption buffer) was added to 0.9 ml of treated cells, incubated with gentle shaking at 37 C for 10 min, and then diluted 10^{-2} in medium unsuitable for adsorption (tryptone broth) and strongly agitated for 5 s on a Vortex Genie mixer (Scientific Industries Inc., Springfield, Mass.) to remove all phages reversibly adsorbed to the cells. After 3 min at 37 C, the bacteria together with the irreversibly adsorbed phages were centrifuged down and the supernatants were diluted and assayed for unadsorbed phages.

Measuring attachment to partially purified tonA-protein. T5 phage receptor, partially purified on a Bio-Gel A50 column as previously described (8), was dispersed in 1 or 2% Triton X-100 for 30 min at 23 C. The presence of these amounts of Triton X-100 had no effect on the adsorption or stability of phages T1 or ϕ80. Receptor was suspended in 0.01 M sodium phosphate, pH 7.0, containing 5×10^{-4} M $MgSO_4$, in which buffer, phages T1 and ϕ80 could adsorb to cells, although not quite as well as in Tris adsorption buffer. Phage T1 or ϕ80 (0.1 ml, 2×10^4 to 3×10^4 plaque-forming units) was added to 0.4 ml of receptor, or as a control to 0.4 ml of buffer, for 6 min at 37 C. Log-phase cells of strain Ymel, which were washed and suspended in 0.5 ml of adsorption buffer (OD_{578} = 0.8), were gently added and the suspensions were slowly swirled at 37 C for 6 min. They were then diluted 1:10 in tryptone broth, vortexed, and incubated a further 3 min at 37 C. They were then centrifuged and the supernatant was plated for plaque-forming units with an overnight culture of strain Ymel. As a control, a solution of partially purified protein from a *tonA* mutant, containing inactive *tonA*-protein receptor (8), was substituted for the above preparation from a *tonA*$^+$ strain. The amount of protein in each case was approximately 4 mg/ml.

RESULTS

Irreversible adsorption to untreated cells. Garen and Puck (14, 15) showed that, given a phage-cell ratio of one or less, the two steps of T1 adsorption could be represented by the following equations:

$$P + C \underset{k2}{\overset{k1}{\rightleftharpoons}} PC \text{ ----------}$$

$$PC \xrightarrow{k3} X \text{ ----------}$$

where P represents T1 phage; C, the host cells; PC, reversibly adsorbed phage; and X, the irreversible phage-cell complex. The symbols $k1$, $k2$, and $k3$ are the specific rate constants of the reactions indicated. The first reaction can be forced to the left by dilution and agitation, yielding free phage in the supernatant. However, once the second reaction occurs, free infectious phage cannot be eluted from the cells. Therefore the adsorption assay described in Materials and Methods measures only phages bound to the cells irreversibly.

The results for irreversible adsorption to strains Ymel and BH212 in the absence of inhibitors were similar for both T1 and ϕ80. Strain Ymel adsorbed 84% of T1 (average of 13 determinations) and 83% of ϕ80 input phages

(average of 16 determinations), whereas strain BH212 adsorbed 85% of T1 (average of 14 determinations) and 87% of ϕ80 input phages (average of 19 determinations). It was shown that none of the solvents used to dissolve the inhibitors had any effect on phage adsorption. Since the phages are eluted from the cells by dilution into tryptone broth, cells suspended in this media were tested for adsorption of T1 and ϕ80 and were demonstrated to be unable to adsorb either phage reversibly or irreversibly. Cells which had been carefully disrupted by means which produce membrane vesicles were sometimes shown to be able to adsorb T1 and ϕ80 irreversibly. However, results were inconsistent and so intact cells were used in these experiments. Although only the results for strain Ymel are published here, all experiments in the presence or absence of inhibitors were repeated for another essentially wild-type *E. coli* K-12 strain, P400 (17), and yielded similar results.

All mutants used adsorbed T1 and ϕ80 normally, providing they were properly energized. For strain BH212, as with the wild-type strain Ymel, the internal energy source was sufficient to provide this energization. For the *hemA* mutant A1004c, 0.5% glucose was routinely added together with the phages to provide an energy source for the process of irreversible adsorption. Using this procedure, the levels of adsorption to strain A1004c were approximately the same as to strains Ymel and BH212, being for T1 83% (average of 11 determinations) and for ϕ80 82% (average of six determinations). The reason that glucose was added to the adsorption assay to strain A1004c was that the cells became partially starved during the thorough washing of the cells before the arsenate inhibition experiments. The ease with which *hemA* mutants can become starved of energy has been observed by other workers (R. Teather, personal communication).

Effect of cyanide and arsenate. As expected for a mutant unable to synthesize cytochromes, cyanide, which inhibits cytochrome *d* of the electron transport chain (23), caused no inhibition of irreversible adsorption of either T1 or ϕ80 to *hemA* strain A1004c, even at a concentration of 10 mM. However, while adsorption of these phages to strain Ymel (*uncA*$^+$ *hemA*$^+$) was only slightly reduced in the presence of 5 mM cyanide, the adsorption to the *uncA* strain BH212 was stongly inhibited by this concentration (Fig. 1). At a very high concentration of cyanide (10 mM), the inhibition of adsorption to Ymel became quite high, but nevertheless was lower than the result for strain BH212. The

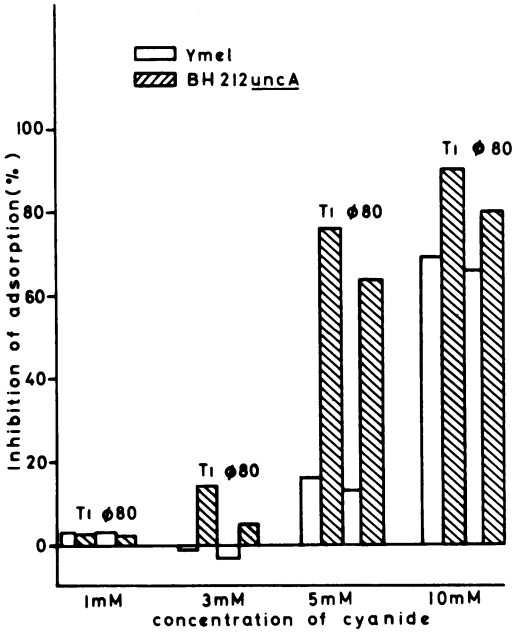

FIG. 1. *Inhibition of the irreversible adsorption of phages T1 and ϕ80 to strains Ymel (uncA$^+$) and BH212 (uncA) using various concentrations of potassium cyanide; expressed as mean percentage of inhibition (control in each experiment equals 0% inhibition; actual irreversible adsorption in the absence of cyanide was for strain Ymel 84% [T1] and 83% [ϕ80] and for BH212 85% [T1] and 87% [ϕ80]) for between two and four experiments by using 1 and 3 mM cyanide and between four and eight experiments by using 5 and 10 mM cyanide. Assay conditions were as described in the legend of Table 2 (see also text). Adsorption of either phage T1 or ϕ80 to strain A1004c was not inhibited by 10 mM cyanide.*

increased inhibition by cyanide of adsorption to the *uncA* mutant can be explained by this mutant's dependency on the electron transport chain for energization of the membrane, in contrast to the *uncA*$^+$ strain Ymel which, in addition, is able to derive energy from adenosine 5'-triphosphate (ATP) hydrolysis.

Preliminary experiments with cells starved with 5 mM DNP by the technique of Berger (5), indicated that these cells, which were unable to adsorb T1 or ϕ80 irreversibly, could be reenergized for irreversible adsorption using 0.5% glucose added at the same time as the phages. By using such starved preparations of strains BH212 *uncA* and Ymel, a difference in inhibition (i.e., greater inhibition of irreversible adsorption to the *uncA* strain) could be seen using 3 mM cyanide. The difference was equivalent to that noticed using unstarved cells and 5 mM cyanide.

The effect of arsenate, which depresses cellular ATP levels in phosphate-free media (20), was studied. Using this compound, slight or no inhibition of adsorption of phage T1 or ϕ80 to the strains Ymel or BH212 was noticed at any of the concentrations used (Table 1). However, the *hemA* strain A1004c can only couple energy derived from ATP to energy-requiring processes. In this strain, 1 mM arsenate caused 100% inhibition.

Unfortunately the Ca^{2+}, Mg^{2+}-adenosine triphosphatase (ATPase) inhibitor DCCD directly neutralized the phages (average of 12 determinations = 79.9% neutralization in 10 min, 37 C, with 50 μM DCCD), rendering this inhibitor unsuitable for use in this system. No other compound described in this paper was shown to have a direct effect on the phages.

Effect of uncouplers. Uncouplers of oxidative phosphorylation are thought to facilitate proton movement across the membrane and thus to dissipate the energized membrane state (7, 11). These compounds would be expected to inhibit the process of irreversible adsorption if it used this state directly as a source of energy. A number of compounds known to uncouple oxidative phosphorylation, TTFB, CCCP, DNP (11), and azide (7), were tested and yielded similar results for both phages (Table 2). All were shown to strongly inhibit irreversible adsorption to the three strains. At higher concentrations, the inhibition was complete. The results for CCCP may be partly due to its action as a sulfhydryl reagent (19), as both pCMB and pCMBS caused inhibition at the concentrations used. Furthermore, the effect of 50 μM CCCP could be reversed by the addition of 10 mM dithiothreitol (a thiol reagent) to the cells either 5 min before or 5 min after the addition of 50 μM CCCP. Thus these results implicate a sulfhydryl group as being important in the adsorption of these phages. Whether this sulfhydryl group is a part of the receptor for irreversible adsorption, or plays some part in the events which provide energy for the process, is not known.

TABLE 1. *Effect of arsenate on irreversible adsorption of phages T1 and ϕ80 to various strains*[a]

Arsenate concn (mM)	Inhibition of adsorption (%)[b, c]					
	Ymel		BH212 *uncA*		A1004c *hemA*	
	T1	ϕ80	T1	ϕ80	T1	ϕ80
1	−2	−5	—	—	100	100
5	2	1	8	5	—	—
10	6	3	10	13	—	—

[a] Log-phase cells were washed 3x in Tris buffer and resuspended in adsorption buffer (5×10^8 cells/ml). The given concentration of arsenate was added for 15 min at 37 C. Phages were then added (MOI = 0.4 to 0.6) for 10 min at 37 C. After this period of incubation, the solutions were diluted 10^{-2} to elute reversibly bound phages, the bacteria and irreversibly bound phages were precipitated by centrifugation, and the supernatant was diluted and assayed for unadsorbed phages.

[b] Percentage of inhibition in the absence of inhibitor equals 0%; actual levels of irreversible adsorption were for strain Ymel 84% (T1) and 83% (ϕ80); for BH212 85% (T1) and 87% (ϕ80); and for strain A1004c 83% (T1) and 82% (ϕ80).

[c] All figures are the average of three or more determinations. —, Not determined.

Nature of the adsorption to the tonA protein. There are two well-defined bacterial mutants resistant to phages T1 and ϕ80, these being *tonA* and *tonB*. The phages are unable to bind irreversibly to either mutant, although they can bind reversibly to *tonB* mutants. The recent purification (8) of the *tonA* gene product (called here tonA protein) provided a system for testing the nature of the interaction of the two phages with tonA protein.

The assay described in Table 3 depends on the fact that the rate of desorption of the phages from their receptor, after they have become reversibly bound, is very much slower than the rate of adsorption. The half-life of the reversibly bound phage T1-receptor complex (see reaction [1]) at the Mg^{2+} ion concentration used is approximately 15 min (14). Therefore, providing this result is similar for isolated receptor, few of the phages which adsorb to the receptor during the first 6 min will desorb in the next 6 min after cells have been added. Thus, these phages will be protected from irreversible adsorption during the second 6-min incubation. Upon dilution into tryptone broth, the phages bound reversibly to both cells and receptor will be eluted into the supernatant. In fact, as shown in Table 3, added tonA protein caused an increase of 21.7% in the reversibly bound T1 phages, as reflected in a lower percentage of irreversible adsorption to cells. For phage ϕ80 the increase was 19.6%. Further evidence that the binding was reversible was provided when, after preincubation of the phages with tonA protein and subsequent incubation with buffer, all phages were eluted from the protein upon dilution.

To test that the binding was to tonA protein and not to a contaminating protein in the preparation, a similar preparation from a *tonA* mutant was tested and shown not to inhibit subsequent adsorption to bacteria.

DISCUSSION

The results described in this paper indicate that irreversible adsorption of phages T1 and

TABLE 2. *Effect of uncouplers and sulfhydryl reagents on the irreversible adsorption of phages T1 and φ80 to strains Ymel, BH212 uncA, and A1004c hemA*[a]

Inhibitor	Concn	Inhibition of adsorption (%)[b, c]					
		Ymel		BH212 *uncA*		A1004c *hemA*	
		T1	φ80	T1	φ80	T1	φ80
TTFB	31 μM	81	91	89	84	99	100
	62 μM	100	100	100	100	100	100
DNP	0.25 mM	4	4	—	—	—	—
	0.5 mM	72	74	83	73	—	—
	1.0 mM	93	87	95	89	—	—
	5.0 mM	100	100	100	100	—	—
AZIDE	10 mM	65	56	76	77	88	90
	50 mM	100	100	100	100	—	—
CCCP	5 μM	73	82	83	76	83	—
	50 μM	100	100	100	100	—	—
pCMBS	50 μM	69	48	—	—	—	—
pCMB	100 μM	100	100	—	—	—	—

[a] The given concentrations of inhibitors were added to log-phase cells in adsorption buffer (5×10^8 cells/ml) at 37 C for various periods of time (see text). Phages (MOI = 0.4 to 0.6) were subsequently added for 10 min at 37 C. The solutions were then diluted 10^{-2} to elute reversibly bound phages, the bacteria and irreversibly bound phages were precipitated by centrifugation, and the supernatant was diluted and assayed for unadsorbed phages.

[b] Percentage of inhibition in absence of inhibitors equals 0%. Actual levels of irreversible adsorption were for strain Ymel 84% (T1) and 83% (φ80); for BH212 85% (T1) and 87% (φ80); and for A1004c 83% (T1) and 82% (φ80).

[c] All figures are the average of between two and seven, but usually four, determinations. —, Not determined.

φ80 can be energized both through the Ca^{2+}, Mg^{2+}-ATPase and the electron transport chain. Evidence for this is in the fact that both an *uncA* mutant deficient in the Ca^{2+}, Mg^{2+}-ATPase and a *hemA* mutant deficient in the electron transport chain can provide energy for the adsorption process. Furthermore, cyanide, an electron transport inhibitor, causes greater inhibition in the *uncA* mutant lacking the Ca^{2+}, Mg^{2+}-ATPase than in the strains which have a functional Ca^{2+}, Mg^{2+}-ATPase, whereas uncouplers have a pleiotropic effect in all strains.

Another method of energy coupling utilizes ATP through a pathway other than Ca^{2+}, Mg^{2+}-ATPase-mediated hydrolysis (5, 6, 27). Since arsenate severely depresses the cellular ATP level (20), it would cause inhibition of irreversible adsorption in all three strains, if ATP were the sole source of energy. The fact that arsenate causes inhibition only in the *hemA* mutant, where the only route of energization is through ATP, together with the potent effect of uncouplers, indicates that the above mechanism is not important.

The above evidence suggests that the direct source of energy for adsorption is probably the energized membrane state. One way commonly used to directly test whether energy is provided through the energized membrane state is to see if a net movement of protons across the membrane accompanies the addition of substrate to the cells. Unfortunately we were unable to successfully test this due to the impossibility of obtaining a high enough molarity of phages to give a measurable effect. A further complication is that phage T1 irreversible adsorption gives rise to infection, and an early result of this is leakage of cellular constituents (21). However, despite this lack of direct evidence, the results with inhibitors and mutants provide compelling evidence for the involvement of the energized membrane state as the source of energy for irreversible adsorption.

It is indeed interesting that the energized membrane state exists in the cytoplasmic membrane, whereas phage adsorption occurs at the outer membrane. Electron micrographs have demonstrated that phage T1 and other phages bind to points of adhesion between the cell wall and the cytoplasmic membrane (2), and evidence for φX174 suggests that the irreversible binding site lies there (3). Therefore it is possi-

TABLE 3. *Reversible adsorption of phage T1 to partially purified tonA protein*[a]

First[b] addition	Second[c] addition	Irreversible adsorption (%)[d]
Triton X-100 + buffer	Bacteria	68.3 ± 4.3[e]
tonA protein in Triton X-100 + buffer	Bacteria	46.6 ± 4.9[e]
tonA protein in Triton X-100 + buffer	Buffer	0
Triton X-100 + buffer	Buffer	0
Protein preparation from *tonA* mutant in Triton X-100 + buffer	Bacteria	68.0 ± 5.9[e]
Protein preparation from *tonA* mutant in Triton X-100 + buffer	Buffer	0

[a] Results for phage φ80 were essentially identical.

[b] Four-tenths milliliter of the solution of Triton-solubilized protein or Triton X-100 was added to 0.1 ml of phage T1 for 6 min at 37 C.

[c] For the second addition, 0.5 ml of either a log-phase culture of strain Ymel in adsorption buffer or the buffer itself was added to 0.5 ml of the solution described in the first column. After 6 min at 37 C, the solution was diluted 1:10 to elute reversibly bound phages from cells and/or receptor. After centrifugation, an increased plaque count in the supernatant of those tubes which had received bacteria as the second addition (reflected in a decreased irreversible adsorption in column 3) implied that the solution in column 1 had bound T1 reversibly.

[d] Expressed as mean ± standard deviation.

[e] Mean for 11 determinations.

ble that the supply of energy from the cytoplasmic membrane to the phage receptor is channelled through these regions. It may even be that the adhesion sites contain some of the energy-producing components of the cytoplasmic membrane. Another possibility is that phages T1 and φ80, when reversibly adsorbed to their receptor, have their tails inserted into the cytoplasmic membrane, and that the energy requirement could be for unplugging the tails (a step postulated by Benz and Goldberg [4]) or for the transport of deoxyribonucleic acid. It should be noted that the initiation of deoxyribonucleic acid uptake is indistinguishable from a postulated irreversible binding reaction for phage T1, since, as concluded by Christensen and Tolmach (10), they are either a part of the same reaction or one follows the other very closely in time. Tolmach (29) has summarized the evidence that a covalent bond forms upon irreversible binding. A further possibility is that the receptor for irreversible adsorption is inaccessible to the phages unless energy is provided, as has been suggested for the β-galactoside carrier protein (24, 26).

Phages T1 and φ80 appear to use the tonA protein as their reversible adsorption receptor (Table 3), which explains why *tonA* mutants show resistance to the phages. Since they can adsorb reversibly but not irreversibly to bacteria mutated in the *tonB* region (15), it appears that one or more functions of the *tonB* region (since many *tonB* mutants have been shown to be deletion mutants) are involved in the irreversible binding of phages T1 and φ80. The energy requirement of irreversible adsorption described in this paper leads to the possibility that one role may be the coupling of the energized membrane state to this process. Experiments are being undertaken to determine if this is the role of the *tonB* region.

One might expect that other functions associated with the *tonA* and *tonB* loci might have the same type of energy dependence as phage adsorption. One such recently discovered function is ferrichrome uptake (18), which is blocked in *tonA* and *tonB* mutants. Wayne and Neilands (30) showed that ferrichrome competed for the reversible binding sites for phage φ80, which are shown here to be tonA protein. Work is proceeding in our laboratories to determine the nature of the energy requirement for ferrichrome uptake to determine if phages T1 and φ80 utilize this uptake system for entering the cell.

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