Effect of the Lambda *S* Gene Product on Properties of the *Escherichia coli* Inner Membrane

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The *S* gene of bacteriophage λ is a late gene required for cell lysis, but unlike the other two lysis genes, *R* and *R*₂, it does not code for an endolysin. Earlier studies have shown that the *S* gene product inhibits respiration and macromolecular synthesis and makes the inner membrane permeable to sucrose. In this study, the effect of the *S* gene product on a number of *Escherichia coli* membrane functions (active transport, permeability, respiration, and transhydrogenase and ATPase activity) were measured, and a product of the λ *S* gene was identified in the inner membrane fraction by two-dimensional polyacrylamide gel electrophoresis. The results of these experiments indicate that the λ *S* product is present in the inner membrane, that it increased the permeability of the membrane for all of the small molecules that were tested, and that its action is reversible. The simplest explanation of these results is that the *S* gene product forms a hydrophilic pore through the inner membrane, allowing small molecules and λ lysozyme to pass through.

The infection of *Escherichia coli* by bacteriophage λ terminates with the lysis of the cell and the release of the completed phage into the medium. Lysis has been shown to require the products of three λ late genes: *R*, *R*₂, and *S*. The *R* gene codes for an enzyme called λ lysozyme which catalyzes the hydrolysis of specific bonds in the peptidoglycan layer of the cell wall. This enzyme has been purified (4) and sequenced (11). It has been suggested that the *R*₂ gene codes for a lysozyme with a different specificity than the product of the *R* gene, but this has not been proven (30). Lysozyme is synthesized in an active form before lysis occurs (9) and is present in an active form in *S* mutants. These results show that the *S* protein must function to allow lysozyme to act rather than to activate lysozyme. The *S* protein has not been identified, and its exact role in the lytic process has not been determined.

In an early study on the role of the *S* gene, Adhya et al. (1) showed that both synthesis of macromolecules and respiration are inhibited in cells infected with λ *R* mutant phage and that these inhibitions occur at the same time as lysis in cells infected with wild-type λ phage. These inhibitions do not occur when λ *RS* mutant phage are used. The inhibitions do not appear to be due to phospholipase activity, as Reader and Simonovitch have shown (22, 23) that the phospholipase activity which increases at the time of lysis in cells infected with λ *R*⁻ is a host

enzyme. Furthermore, the phospholipase activity can be inhibited and lysis will still occur. The studies of Reader and Simonovitch also showed that the λ *S* protein causes the *E. coli* inner membrane to become permeable to sucrose (23).

Mukherjee and Mandal (17) suggested that the λ *S* gene product is a soluble protein which removes phospholipid from the inner membrane but does not degrade it.

Finally, Rolfe and Campbell have published a number of studies (6, 24) from which they concluded that there may be two pathways for lysis. One pathway is independent of the λ *S* protein and is normally blocked by another λ gene product, whereas the other requires the *S* protein.

There is evidence for a product similar to that of the λ *S* gene in P2 infection (15), S13 infection (3), and T4 infection (12, 16). Nelson and Butler (18) have shown for T4 that the phage itself contains a phospholipase. Strains with mutations in the *t* gene, which codes for a product that resembles the product of the λ *S* gene in its activity, lack this enzyme, but it is not certain that the *t* gene codes for the phospholipase.

In this study, the effects of the *S* gene product on *E. coli* membrane function were studied in whole cells and in normal and everted membrane vesicles. All of the results of these studies indicate that the product of the *S* gene is present in the inner membrane and causes a large increase in its permeability.

MATERIALS AND METHODS

Bacterial strains. *E. coli* CSH7 and UV159 were used as nonpermissive hosts for λ phage carrying amber mutations; *E. coli* QD5003 was used as a permissive host.

Bacteriophage. λ cI857 S7, λ cI857 R60, λ cI857 R60 S7, and λ cI857 S(Ts) were obtained from Jeffrey Roberts. A λ cI857 S(Ts) R amber phage was isolated from a population of λ cI857 S(Ts) phage mutagenized as described by Sato (25) by using the following selection. Strain CSH7 was lysogenized with the mutagenized phage. The lysogenic cells were induced by being heated at 45°C for 8 min and grown in tryptone broth medium at 42°C for 120 min. The cells were chilled, allowed to sit at 4°C for 30 min, and centrifuged. The cell pellet was washed with 0.01 M Tris, pH 8.0, and lysed by treatment with 0.01 M Tris, pH 8.0, containing 100 μ g of lysozyme per ml and 10^{-2} M EDTA. The released phage were used to lysogenize strain CSH7, and the selection described above was repeated. The final phage preparation was plated on strain QD5003, and individual plaques were tested on CSH7 and QD5003 to screen for amber R mutants. The selected phage was tested to insure that it contained both an amber R mutation and the original S(Ts) mutation.

Methods. Oxygen consumption was measured at 26°C with an oxygen electrode. Transport activities were measured as described previously (28). Everted vesicles were prepared by the procedure of Hertzberg and Hinkle (10). Normal vesicles were prepared by incubating cells (washed two times with 0.01 M Tris, pH 8.0) with 0.01 M EDTA, 100 μ g of lysozyme per ml, and 0.01 M Tris, pH 8.0, for 30 min at 21°C. $MgCl_2$ (3×10^{-2} M) and pancreatic DNase and RNase (10 μ g/ml) were then added, and the incubation was continued for 20 min. The lysate was centrifuged at $800 \times g$ for 20 min to remove whole cells. The supernatant was centrifuged at $40,000 \times g$ for 60 min to obtain the vesicle pellet, which was suspended in 0.1 M KPO_4 (pH 6.6). The transport activity of the vesicles was determined by the procedure of Kaback (13). Transhydrogenase and ATPase assays were carried out as described by Futai (7). NADH oxidase was assayed spectrophotometrically (340 nm) at room temperature in a final volume of 1.0 ml containing 250 mM sucrose, 20 mM inorganic potassium phosphate, 2 mM $MgCl_2$, and 0.1 mM NADH, pH 7.4. Lysozyme (8), glucose-6-phosphate dehydrogenase (14), and acid hexose phosphatase (19) were assayed as previously described.

Lysogenic strains were grown at 30°C in tryptone broth containing 0.2% fructose to a density of 3×10^8 cells/ml, and the prophage were induced by heating the culture at 45°C for 8 min. After induction the culture was grown at 37°C for 70 min and harvested. The uninduced cells were grown at 30°C to 6×10^8 cells/ml and harvested. Strain CSH7 was grown in tryptone broth containing 0.2% fructose, 0.2% maltose, and 10^{-2} M $MgCl_2$ at 37°C to a density of 3×10^8 cells/ml, and λ R⁻ phage were added to give a multiplicity of 5. The culture was shaken slowly for 10 min and then normally for 60 min, and the cells were harvested. Control cells, grown as above, were harvested at a density of 6×10^8 cells/ml. All samples were washed and assayed for transport as previously described (28). The concentrations of proline, glutamine, fructose,

and α -methyl glucoside used in the transport assay were 8.3×10^{-6} , 8.3×10^{-6} , 1.66×10^{-5} , and 1×10^{-5} M, respectively. The specific activities were about 10 μ Ci/ μ mol except for fructose, which was 2 μ Ci/ μ mol. Fructose and succinate were used as the energy source for proline and glutamine uptakes, but no energy source was provided for fructose or α -methyl glucoside uptake. Vesicles prepared from 1-liter cultures of induced CSH7(λ R⁻) and CSH7(λ R⁻ S⁻) cells were assayed for proline transport as previously described (14). The proline concentration in the assay was 8.3×10^{-6} M.

The inner membrane, outer membrane, and cytoplasmic fractions used for gel electrophoresis were prepared by washing the cells two times with minimal medium by centrifugation and then suspending them in a buffer (pH 7.8) containing 1 mM Tris-hydrochloride, 1 mM $MgCl_2$, and 2 mM phenylmethylsulfonyl fluoride to inhibit proteases. The cells were broken with a French pressure cell at 8,000 lb/in², and unbroken cells were removed by centrifugation ($6,000 \times g$, 20 min). The supernatant was centrifuged at $105,000 \times g$ for 60 min. The pellet was washed once by centrifugation with 1 mM Tris-hydrochloride containing 1 mM $MgCl_2$ (pH 7.8) and suspended in 10 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) buffer (pH 7.4) at a protein concentration ≤ 5 mg/ml. Inner and outer membranes were purified by sucrose gradient centrifugation (26). The supernatant from the 60-min centrifugation at $105,000 \times g$ was used as the cytoplasmic fraction.

Each fraction was solubilized with sodium dodecyl sulfate and treated with Nonidet P-40 by the procedure of Ames and Nikaido (2). Each solubilized sample was then fractionated on a nonequilibrium isoelectric focusing gel which used the pH range 3.5 to 10 ampholines system described by O'Farrell et al. (21). After electrophoresis for 1,600 V h, the gel was removed and electrophoresed in the second dimension on a 7.5 to 14% polyacrylamide gradient slab gel by the method of O'Farrell et al. (21) for 7 h at 160 V.

Gels were stained with silver (20) and photographed.

RESULTS

The uptake of substrates by each of the three major classes of *E. coli* active transport systems (29) was measured, and in each case the S gene product strongly inhibited active transport (Table 1). The uninduced lysogens had the same transport rates as did the parent strain for proline, glutamine, and fructose, but a somewhat lower rate for α -methyl glucoside transport. The induced λ R⁻ S⁻ lysogen had normal levels of glutamine, fructose, and α -methyl glucoside transport and retained 40% of the normal level of proline transport. When the S gene product was present, either through induction of the λ R⁻ prophage or by infection with λ R⁻ phage, transport of both proline and fructose was inhibited by more than 90%, α -methyl glucoside transport was inhibited 84%, and glutamine transport was inhibited 73%. These values are the average of the inhibition in the induced λ R⁻ lysogen and in λ R⁻-infected cells. The transport

TABLE 1. Transport inhibition by the λ S protein

Sample	Transport rate (nmol/min per mg of protein)			
	Proline	Glutamine	Fructose	α -Methyl glucoside
CSH7	4.0	0.9	27	3.0
CSH7(λ R^-)	3.9	1.1	25	2.27
Induced CSH7(λ R^-)	0.13	0.2	0.6	0.60
λ R^- -infected CSH7	0.3	0.4	0.5	0.12
CSH7(λ R^- S^-)	3.2	0.9	23	1.31
Induced CSH7(λ R^- S^-)	1.33	0.9	26	2.14
λ R^- S^- vesicles	3.2			
λ R^- vesicles	0.4			

inhibition did not appear to be caused by the *E. coli* phospholipase activity which is activated by the λ S gene (23), since transport was still inhibited in cells to which 10^{-3} M EGTA was added 30 min after induction and maintained throughout the transport assay. This concentration of EGTA did not inhibit transport in control cells but did inhibit phospholipase activity (data not shown).

Transport inhibition by the S protein was also observed in membrane vesicles. Vesicles prepared from an induced λ R^- lysogen did not transport proline, whereas vesicles from an induced λ R^- S^- lysogen showed normal transport (Table 1). These vesicles retained only membrane-bound transport systems, so that glutamine, fructose, and α -methyl glycoside transport were not measured.

The kinetics of transport inhibition are shown in Fig. 1. Transport inhibition in an induced λ R^- lysogen occurred at about the time that lysis would occur in an induced wild-type lysogen (i.e., 40 min after induction). A direct comparison of the time of lysis and the time of transport inhibition was carried out with a wild-type lysogen grown in two different media (Fig. 2). In both media the inhibition of transport could not be distinguished from the lytic event, as the specific activity of transport did not show a drastic drop before or at the time that lysis began. This means that the two events must be occurring within a few minutes of each other.

Results were obtained providing direct evidence for a permeability change in the membrane when the S protein is present (Table 2). Spheroplasts made from cells which contain the S protein could not be stabilized by sucrose, glycine, or NaCl, indicating that these molecules can pass through the inner membrane. Polyethylene glycol (PEG) (6,000 average molecular weight) did not give stabilization at a concentration of 10%; however, 10% PEG (20,000 average

molecular weight) did stabilize spheroplasts containing the S protein.

The presence of the S protein allowed the nucleotide pool of the cell to pass through the membrane into the medium (Fig. 3). There were no radioactive nucleotides in the medium from either the 35-min or 60-min sample of the λ R^- S^- -induced lysogen or from the 35-min sample of the λ R^- -induced lysogen. The medium from the 60-min sample of the λ R^- lysogen did contain nucleotides. These results show that when the S gene product is present, the cell becomes permeable to nucleotides, including the nucleotide triphosphates which are the major component of the nucleotide pool of the cell.

The altered membrane permeability caused the inhibition of respiration reported by earlier workers (1, 23) (Table 3). Respiration stimulated by glycerol, fructose, or glucose was drastically inhibited by the S protein, although respiration stimulated by succinate or lactate, which are substrates of membrane-bound dehydrogenases, was only inhibited 50%. Determination of the kinetics of succinate respiration showed that it is the K_m of respiration which is altered, not the V_{max} (Table 3). The K_m for succinate respiration in cells containing the S protein, 2.3×10^{-3} M, was nearly identical to the K_m of succinate dehydrogenase, 2.0×10^{-3} M, measured in membrane vesicles permeabilized with toluene. The lower K_m for succinate respiration in con-

FIG. 1. Cells containing λ R^- (●, ▲, ■) or λ R^- S^- (○, △, □) were grown and induced as described in the text. Transport activities were measured as described, except that samples were removed at the indicated times and chloramphenicol (20 μ g/ml) was added to each sample as it was removed. Chloramphenicol was also present during the washing and during the transport assays. Symbols: (○, ●) proline; (△, ▲) galactose; (□, ■) fructose.

FIG. 2. Cells of CSH7(λ^+) were grown at 30°C in either tryptone broth or minimal medium containing 0.2% fructose, 10^{-3} M D-fucose, and 0.2% Casamino Acids. The cells were induced at time zero and transferred to 37°C. Transport of α -methyl glucoside was measured by adding 1.2 ml of the tryptone broth culture to 20 μ l of radioactive α -methyl glucoside and removing 0.5-ml samples at 20 and 40 s after the addition. The samples were filtered, washed, and counted as previously described (28). Galactose transport was measured on the culture growing in minimal medium in the same way by using 10 μ l of 10^{-4} M [14 C]D-galactose (specific activity, 3.7×10^6 cpm/ μ mol). The optical density at 600 nm (OD_{600}) of the culture was measured just before the transport assay. Symbols: ($\square$) Cells in minimal medium; ($\circ$) cells grown in tryptone broth; ($\circ$, $\square$) OD_{600} ; ($\bullet$, $\blacksquare$) counts per minute transported per OD_{600} ($\times 10^{-3}$).

trol cells was a result of the active transport of succinate, which was inhibited when the *S* protein was present. Respiration of glucose and other sugars required not only transport but also metabolism to produce NADH, the actual respiratory substrate. The leakage of ATP and metabolic intermediates from the cell probably pre-

FIG. 3. CSH7(λR^-) and CSH7($\lambda R^- S^-$) cells were grown at 30°C in 5 ml of tryptone broth containing 0.2% maltose and 10^{-2} M $MgCl_2$. When the OD_{600} was 0.15, KPO_4 (pH 7.5) was added to give a final concentration of 10^{-3} M, along with 100 μ Ci of $^{32}PO_4$. The cells were grown for 1 h at 30°C, induced by heating at 45°C for 8 min, and transferred to 37°C. Samples (1 ml) were removed at 35 and 60 min after induction and filtered through a 0.45- μ m nitrocellulose filter, collecting the supernatant in a flask containing one drop of 70% formic acid. The filters and filtrates were treated as described previously (5). The final step was chromatography on polyethyleneimine plates to separate nucleotides. The autoradiogram run on the dried chromatograph was exposed for 2 days for CSH7($\lambda R^- S^-$) and 7 days for CSH7(λR^-). The origin is at the bottom. For each sample the extract was run on the left and the medium on the right. Lanes: (a) CSH7($\lambda R^- S^-$), 35 min; (b) CSH7($\lambda R^- S^-$), 60 min; (c) CSH7(λR^-), 35 min; (d) CSH7(λR^-), 60 min.

vented NADH formation. The results with succinate and D-lactate showed that the *S* protein does not directly inhibit respiratory chain activity.

Additional evidence suggesting that the *S* protein only alters membrane permeability and does not inhibit membrane proteins came from stud-

TABLE 2. Spheroplast stabilization by different compounds^a

Cells	OD_{600} (% of control) after addition of:						
	No addition	20% sucrose	1 M glycine	0.2 M NaCl	10% PEG (MW 6,000)	10% PEG (MW 20,000)	20% PEG (MW 6,000)
CSH7	63	102	98	94	106	138	105
CSH7($\lambda R^- S^-$)	42	93	102	81	107	100	108
CSH7(λR^-)	32	49	30	32	51	76	98

^a The harvested cells were washed with 0.01 M Tris (pH 8.0) and then resuspended in 0.01 M Tris (pH 8.0) containing the indicated compound to give an OD_{600} of about 5.0. Two aliquots (0.2 ml) of each sample were taken, and 2 μ l of 0.5 M EDTA (pH 8.0) was added to each. Egg white lysozyme (2 μ l of 10 mg/ml) was added to one pair from each sample, and the samples were incubated at room temperature for 30 min. Each sample was brought to 1 ml by addition of 0.8 ml of the solution in which they had been suspended, and the OD_{600} was measured. The OD of each lysozyme-treated sample was divided by the OD of the control sample and multiplied by 100 to give the values recorded in the table. MW, Molecular weight.

TABLE 3. Effect of the *S* protein on respiration^a

Cells	O ₂ consumption (μmol/min per mg) with following substrate:						Succinate
	Succinate	Lactate	Glucose	Fructose	Glycerol	<i>K_m</i> (M)	<i>V_{max}</i> (μmol/min per mg)
λ <i>R</i> ⁻ <i>S</i> ⁻ (induced)	3.8	4.3	3.8	6.1	6.1	1.7×10^{-14}	3.1
λ <i>R</i> ⁻ (uninduced)	2.8	3.1	3.5	3.3	3.0	1.2×10^{-4}	3.4
λ <i>R</i> ⁻ (induced)	1.6	1.4	0.1	0	0	2.3×10^{-3}	4.1

^a Cells grown in tryptone broth containing 0.2% fructose and 0.2% succinate were induced and harvested as described in the text. The cells were washed by centrifugation with tanaka salts (28), suspended in 1/10 volume of the same medium, shaken for 15 min at 37°C, and stored at room temperature. Cells (0.2 ml) were added to 1.3 ml of this medium at 26°C, and O₂ consumption was measured with an oxygen electrode for 3 min. The indicated carbon source was then added to give 0.2% final concentration, and the rate of O₂ consumption was measured for another 5 min. The same procedure was used to measure the kinetics of succinate respiration, except that the concentration of added succinate was varied from 2×10^{-5} to 2×10^{-3} for control cells and from 2×10^{-4} to 2×10^{-2} for induced λ *R*⁻ cells.

ies on membrane vesicles. The results of assaying several activities in everted vesicles and of the studies on succinate respiration showed that the activity of the proteins known to be required for energy production, the respiratory chain, and the F1 ATPase were not inhibited by the *S* protein (Table 4).

A product of the *S* gene was identified by two-dimensional polyacrylamide gel electrophoresis run on cell fractions from cells infected with either λ *R*⁻ phage (*S*⁺) or λ *R*⁻ *S*⁻ phage (Fig. 4). A 15,000-molecular-weight protein was identified in the inner membrane fraction (arrow) which was not present in membranes from λ *R*⁻ *S*⁻-infected cells. No reproducible differences were observed between the outer membrane or cytoplasmic fractions from λ *R*⁻- and λ *R*⁻ *S*⁻-infected cells. The 42,000-molecular-weight protein that was present in the inner membrane fraction from λ *R*⁻-infected but not from λ *R*⁻ *S*⁻-infected cells was identified as the bacterial protein EFTu (A. Okabe and D. Wilson, unpublished data).

Evidence that the action of the *S* protein on the *E. coli* membrane is reversible came from experiments with a λ *R*⁻ *S*(Ts) phage. The *S*(Ts) mutation inactivated the *S* protein at 42°C so that lysis did not occur; however, when the temperature was lowered, lysis occurred immediately. The effect of the temperature-sensitive *S* protein on the transport activity of all three classes of *E. coli* transport systems was determined in whole cells both at room temperature and at 42°C. The results (Table 5) showed that although the temperature-sensitive *S* protein does not cause as complete an inhibition of transport as does the wild-type *S* protein, the inhibition it causes is reversed when transport is assayed at 42°C.

The change in permeability caused by the *S* protein could cause cell lysis in two different ways. Either it could allow internal lysozyme to

pass through the inner membrane to the peptidoglycan layer, or it could inhibit cell wall repair which could prevent external lysozyme from lysing the cell. These models differ in the location of lysozyme in induced λ *S*⁻ lysogens. The results (Table 6) showed that lysozyme is probably present inside the cell, since λ lysozyme was not released by osmotic shock whereas the periplasmic enzyme, acid hexose phosphatase, was released. This result supports the proposed mechanism for cell lysis in which the role of the *S* protein is to allow lysozyme to pass from the cytoplasm through the inner membrane to the peptidoglycan layer of the cell. Furthermore, inhibitors of energy metabolism do not cause lysis of an induced λ *S*⁻ lysogen, whereas freezing and thawing and toluene, which damage the membrane, do cause lysis (24; unpublished data).

DISCUSSION

The present paper provides strong evidence that the active *S* protein greatly increases the permeability of the *E. coli* inner membrane. The results (Fig. 3) showed that the *S* protein allows nucleoside triphosphates to pass through the inner membrane into the medium and that the *S* protein makes the inner membrane permeable to

TABLE 4. Activities of everted vesicles

Source of vesicles ^a	Activity (μmol/min per mg)	
	NADH oxidase	ATPase
Induced λ <i>R</i> ⁻ <i>S</i> ⁻ lysogen	0.32	0.71
Induced λ <i>R</i> ⁻ lysogen	0.33	0.72
λ <i>R</i> ⁻ <i>S</i> ⁻ -infected CSH7	0.17	0.96
λ <i>R</i> ⁻ -infected CSH7	0.18	0.94

^a Vesicles were prepared from 1-liter cultures grown as described in the text.

FIG. 4. Cultures (600 ml) of strain UV159 were grown, infected with either λR^- or $\lambda R^- S^-$ phage, and harvested 60 min after infection, and the inner membrane, outer membrane, and cytoplasmic fractions were prepared as described in the text. Portions of each sample were fractionated by two-dimensional polyacrylamide gel electrophoresis and silver stained as described in the text. Samples (50 μ g) of protein were run for each membrane sample and 30 μ g for each cytoplasmic sample. Panels: (a) λR^- outer membrane; (b) $\lambda R^- S^-$ inner membrane; (c) $\lambda R^- S^-$ cytoplasm; (d) λR^- outer membrane; (e) λR^- inner membrane; (f) λR^- cytoplasm.

glycine, sucrose, and NaCl and even to 6,000-molecular-weight PEG. The protection of spheroplasts containing the *S* protein by 20% PEG (6,000) may be caused by higher-molecular-weight components in this material or by a slow

rate of PEG diffusion through the channel. The lack of protection by 10% PEG (6,000) and protection by 10% PEG (20,000 demonstrate that PEG (6,000) can pass through the inner membrane when the *S* protein is present. The

TABLE 5. Inhibition of transport by the *S*(Ts) protein^a

Cells	Uptake temp (°C)	Transport rate (nmol/min per mg of protein) with following substrate:				
		Glutamine	Proline	Serine	Fructose	α -Methyl glucoside
Induced	22	1.6	0.9	0.7	17	0.8
Induced	42	2.8	1.9	1.2	30	1.7
Uninduced	22	3.4	1.1	1.6	25	1.6
Uninduced	42	3.1	1.3	1.2	23	1.7

^a CSH7 ($\lambda R^- S$ (Ts)) cells were grown, prepared, and assayed for their transport activity as described in the text, except that after induction they were grown at 42°C. After the cells were harvested and washed in the cold as usual, preincubation and transport assays were done at the indicated temperatures.

TABLE 6. Osmotic shock of λ *S*⁻ cells^a

Sample	Total act		
	Lysozyme	Glucose-6-phosphate dehydrogenase	Acid hexose phosphatase
Cell extract	27	0.11	14
Shock fluid	1.2	0	33
Shocked cell extract	34	0.12	5.0
% of activity in shock fluid	3.0	0	87

^a CSH7 lysogenic for λ *S*⁻ was grown in 100 ml of tryptone broth and induced as described in the text. The induced cells were washed in 0.033 M Tris (pH 7.3) containing 0.033 M NaCl and resuspended in 2 ml of 0.033 M Tris (pH 7.3). Cells (0.5 ml) were brought to 2 ml with 0.033 M Tris (pH 7.5) and broken in a small French pressure cell at 10,000 lb/in². The extract was centrifuged at 10,000 $\times$ *g* for 10 min, and the supernatant was assayed for enzyme activity. The other 1.5 ml of cell suspension was added to 1.5 ml of 40% sucrose containing 0.033 M Tris and 0.5 mM EDTA (pH 7.3); all solutions were at room temperature. The cells were then removed by centrifugation at 4°C for 15 min at 9,000 $\times$ *g*. The pellet was quickly suspended in 3 ml of ice-cold distilled water, allowed to stand for 10 min, and then centrifuged at 10,000 $\times$ *g* for 10 min. The supernatant (shock fluid) was then assayed for enzyme activity. The pellet (shock cells) was suspended in 2 ml of 0.33 M Tris (pH 7.3), and an extract was prepared as described above for the untreated cells. The values for the whole cell extract were multiplied by 3 to correct for the difference in the amount of cell suspension used in making the extract and in the osmotic shock procedure.

inhibition of all three classes of transport systems, each of which uses a different mechanism and energy source, is readily explained by the increased permeability of the inner membrane but is difficult to explain by any other mechanism. This is especially true for α -methyl glucoside uptake, which is resistant to most metabolic inhibitors.

The inhibition of active transport provides a simple and sensitive assay for the presence of the active λ *S* gene product in whole cells or membranes. It should be stressed that it is only the concentration of substrates by active transport, not the movement of substrates through the membrane, which is prevented by the *S* protein.

The other observed effects of the *S* protein (inhibition of protein synthesis, DNA synthesis, and respiration) could all be caused by the increased permeability of the inner membrane, especially the loss of the nucleotide pool.

The *S* protein did not inhibit the activity of either the respiratory chain or the F1 ATPase

(Tables 3 and 4), and the action of the *S*(Ts) protein could be reversed when it was inactivated (Table 5). These results rule out some earlier proposals that the *S* protein functions as a specific inhibitor of a protein required for energy production or as a degradative enzyme (phospholipase or protease).

The formation of a channel through the inner membrane would cause lysis if the channel were large enough to allow λ lysozyme, which has a molecular weight of 18,000, to pass through the inner membrane to its substrate, the peptidoglycan layer. This model for *S* function requires that λ lysozyme be synthesized as a cytoplasmic protein rather than as a secreted protein. The results (Table 6) suggested that λ lysozyme is present in the cytoplasm in *S* mutants. Additional evidence that λ lysozyme is not a secreted protein is that no signal peptide is encoded in the λ *R* gene DNA sequence (D. Daniels and F. Blattner, personal communication; F. Sanger, personal communication).

Reader and Siminovitch (24) have previously shown that the presence of the *S* protein in *E. coli* cells increases their permeability to sucrose. In addition, they showed that inhibitors of energy metabolism do not cause lysis of cells infected with λ *S* mutant phage, but the addition of chloroform does cause lysis. These results led them to conclude that the *S* protein altered the cell membrane, allowing lysozyme to act. The work in this paper confirms and extends their results and demonstrates that the *S* protein is present in the inner membrane. The proposed mechanism for lysis in which the *S* protein allows internal lysozyme to pass through the inner membrane is more complex than the one in which λ lysozyme is secreted directly as it is synthesized. However, it does allow a large pool of lysozyme to accumulate in the cell and, as shown in Fig. 3, lysis is essentially complete within a few minutes after the *S* gene acts. With direct lysozyme secretion, partial lysis might occur, trapping the phage in a dead cell.

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LITERATURE CITED

- Adhaya, S., A. Sen, and S. Mitra. 1971. The role of gene *S*, p. 743-746. In A. R. Hershey (ed.), *The bacteriophage lambda*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Ames, G. F.-L., and K. Nikaido. 1976. Two-dimensional gel electrophoresis of membrane proteins. *Biochemistry* 15:616-623.
- Baker, R., and I. Tessman. 1967. The circular genetic map

- of phage S13. *Proc. Natl. Acad. Sci. U.S.A.* **58**:1438-1445.
4. Black, L. W., and D. S. Hogness. 1969. The lysozyme of bacteriophage λ . I. Purification and molecular weight. *J. Biol. Chem.* **244**:1968-1975.
 5. Britton, J. R., and R. Haselkorn. 1975. Permeability lesions in male *E. coli* infected with bacteriophage T7. *Proc. Natl. Acad. Sci. U.S.A.* **72**:2222-2226.
 6. Campbell, J. H., and B. G. Rolfe. 1975. Evidence for a dual control of the initiation of host-cell lysis caused by phage lambda. *Mol. Gen. Genet.* **139**:1-8.
 7. Futai, M. 1974. Reconstitution of transport dependent on D-lactate or glycerol-3-phosphate in membrane vesicles of *Escherichia coli* deficient in the corresponding dehydrogenases. *Biochemistry* **13**:2327-2333.
 8. Greenblatt, J. 1972. Positive control of endolysin synthesis *in vitro* by the gene N protein of phage λ . *Proc. Natl. Acad. Sci. U.S.A.* **69**:3606-3610.
 9. Groman, N. V., and G. Suzuki. 1963. Quantitative study of endolysin synthesis during reproduction of lambda phages. *J. Bacteriol.* **86**:187-194.
 10. Hertzberg, E. L., and P. C. Hinkle. 1974. Oxidative phosphorylation and proton translocation in membrane vesicles prepared from *Escherichia coli*. *Biochem. Biophys. Res. Commun.* **58**:178-184.
 11. Imada, M., and A. Tugita. 1971. Amino acid sequence of λ phage endolysin. *Nat. New Biol.* **233**:230-231.
 12. Josslin, R. 1970. The lysis mechanism of phage T₄: mutants affecting lysis. *Virology* **40**:719-726.
 13. Kaback, H. R. 1972. Transport across isolated bacterial cytoplasmic membranes. *Biochim. Biophys. Acta* **265**:367-416.
 14. Kornberg, A., and B. L. Horecker. 1955. Glucose-6-phosphate dehydrogenase. *Methods Enzymol.* **1**:323.
 15. Lengyel, J. A., and R. Calendar. 1974. Control of bacteriophage P₂ protein and DNA synthesis. *Virology* **57**:305-313.
 16. Mukai, F., G. Streisinger, and B. Miller. 1967. The mechanism of lysis in phage T₄ infected cells. *Virology* **33**:398-404.
 17. Mukherjee, P. K., and R. K. Mandal. 1976. Role of S gene of bacteriophage lambda in host lysis. *Biochem. Biophys. Res. Commun.* **70**:302-309.
 18. Nelson, E. T., and C. S. Butler. 1974. Phospholipase activity in bacteriophage-infected *Escherichia coli*. I. Demonstration of a T₄ bacteriophage-associated phospholipase. *J. Virol.* **33**:398-404.
 19. Neu, H. C., and L. A. Heppel. 1965. The release of enzymes from *E. coli* by osmotic shock and during the formation of spheroplasts. *J. Biol. Chem.* **240**:3685-3692.
 20. Oakley, B. R., D. R. Kirsch, and N. R. Morris. 1980. A simplified ultrasensitive silver stain for detecting proteins in polyacrylamide gels. *Anal. Biochem.* **105**:361-363.
 21. O'Farrell, P. Z., H. M. Goodman, and P. H. O'Farrell. 1977. High resolution two-dimensional electrophoresis of basic as well as acidic proteins. *Cell* **12**:1133-1142.
 22. Reader, R. W., and L. Siminovitch. 1971. Lysis defective mutants of bacteriophage lambda: genetics and physiology of S cistron mutants. *Virology* **43**:607-622.
 23. Reader, R. W., and L. Siminovitch. 1971. Lysis defective mutants of bacteriophage lambda: on the role of the S function in lysis. *Virology* **43**:623-637.
 24. Rolfe, B. G., and J. H. Campbell. 1974. A relationship between tolerance to colicin K and the mechanism of phage-induced host-cell lysis. *Mol. Gen. Genet.* **133**:293-297.
 25. Sato, K. 1970. Genetic map of bacteriophage ϕ 80: genes on the right arm. *Virology* **40**:1067-1069.
 26. Schnaitman, C. A. 1970. Protein composition of cell wall and cytoplasmic membrane of *Escherichia coli*. *J. Bacteriol.* **104**:890-901.
 27. Tanaka, S. S., A. Lerner, and E. C. C. Lin. 1967. Replacement of a phosphoenolpyruvate-dependent phosphotransferase by a nicotinamide adenine dinucleotide dehydrogenase for the utilization of mannitol. *J. Bacteriol.* **93**:642-648.
 28. Wilson, D. B. 1974. Source of energy for the *Escherichia coli* galactose transport system induced by galactose. *J. Bacteriol.* **120**:866-871.
 29. Wilson, D. B. 1978. Cellular transport mechanisms. *Annu. Rev. Biochem.* **47**:937-938.
 30. Young, R., J. Way, S. Way, J. Yin, and M. Syvanen. 1979. Transposition mutagenesis of bacteriophage λ : a new gene affecting cell lysis. *J. Mol. Biol.* **132**:307-322.