JOURNAL OF BACTERIOLOGY, Nov. 1979, p. 369-376
0021-9193/79/11-0369/08$02.00/0

Vol. 140, No. 2

Cyclic Adenosine 3',5'-Monophosphate Regulation of the Bacteriophage T6/Colicin K Receptor in *Escherichia coli*†

EDWARD M. ALDERMAN,‡ STEVENS S. DILLS,§ THOYD MELTON, AND WALTER J. DOBROGOSZ*

Department of Microbiology, The North Carolina State University, Raleigh, North Carolina 27650

Received for publication 9 August 1979

Mutant strains of *Escherichia coli* unable to synthesize cyclic adenosine 3',5'-monophosphate (cAMP) or the cyclic adenosine monophosphate receptor protein (CRP) were more resistant than wild-type cells to infection by bacteriophage T6. This resistance was found to be associated with the decreased production of specific T6 receptor protein (also the colicin K receptor) located in the outer membrane protein fraction of these cells. Transcription of this particular outer membrane protein was regulated by the cAMP-CRP complex. A novel affinity technique coupled with sodium dodecyl sulfate-polyacrylamide gel electrophoresis was used in these investigations.

Strains of *Escherichia coli* bearing mutations in the *cya* (adenylate cyclase) or *crp* (cAMP receptor protein) loci are more resistant than wild-type cells to infection by bacteriophage T6 (13). This resistance is attributed to poor adsorption of the phage to the bacterial host (2), which in turn is believed to be caused by an alteration or absence of a specific receptor protein on the cell surface. Although it is well established that the cAMP-cAMP receptor protein (cAMP-CRP) complex is involved in regulating various functions of the inner, cytoplasmic membrane (6–8, 11, 12) and production of at least two periplasmic enzymes (unpublished data), there is no evidence concerning its capacity to regulate synthesis of outer membrane proteins (OMP). The present study was undertaken with the following propositions in mind: (i) resistance to phage T6 infection is caused by a decreased adsorption of phage to host cells; (ii) this decrease is the result of a loss of a specific T6 receptor protein on the cell surface; and (iii) this T6 receptor protein, which is also the colicin K receptor site (17), is under transcriptional control by the cAMP-CRP complex.

Several isogenic bacterial mutant strains were used in these studies. A battery of classical phage methodologies was applied to determine their response to phage T6 and T1 infections. In addition, the OMP of these various strains were analyzed by polyacrylamide gel electrophoresis (PAGE) after solubilization by a standard procedure using the strong ionic detergent sodium dodecyl sulfate (SDS) and after solubilization by a second method which did not destroy biological activity. OMP solubilized by the latter procedure were used for bacteriophage neutralization assays and adapted to a novel affinity electrophoresis technique which takes advantage of the specificity involved in affinity techniques and the sensitivity involved in PAGE analyses.

MATERIALS AND METHODS

Strains. The bacterial strains used in this study are listed in Table 1. All are derived from *E. coli* K-12 (701) (now designated strain NCR-701), originally obtained from R. J. White (30). Strains NCR-C57 and NCR-C51 are *cya* and *crp* mutants derived in our laboratory. They have been analyzed genetically and used extensively in previous studies (7, 8, 11, 12). Strain NCR-C57SM is a mutant which arises spontaneously with high frequency from cultures of our strain NCR-C57. It yields a wild-type phenotype and was thought at one time to be a revertant of the *cya* mutation. Genetic analyses, however, have shown this not to be the case. Adenylate cyclase assay and transductional analyses with P1 phage show that the *cya* genotype is maintained. The mutation is therefore a secondary suppressor mutation which suppresses the cAMP requirement observed in its *cya* parent strain (strain NCR-C57). The suppressor mutation has been shown by P1 transductional analysis to map in or near the *crp* locus (unpublished data). It is believed, therefore, to be a mutation coding for production of an altered CRP which no longer requires modification by cAMP to function as a positive genetic signal. Its tentative genotype is *cya csm-1*, with the *csm* designating a cyclic AMP suppressor mutation. *E. coli*

† Paper no. 5890 of the journal series of the North Carolina State University, Agricultural Research Service, Raleigh, NC 27650.

‡ Present address: Biophysics Research Laboratory, Peter Bent Brigham Hospital, Harvard Medical School, Boston, MA 02115.

§ Present address: Warner-Lambert Co., Morris Plains, NJ 07950.

NCR-T6r1 is a spontaneous T6 phage-resistant mutant derived from a tryptophan auxotroph of NCR-701 which has been designated as strain D-392. Phage P1 transductional analysis showed the T6 resistance marker to be cotransducible with the *proA* gene, indicating that it is a mutation in the *tsx* locus (17).

The phage strains used were coliphages T1 and T6, maintained as stocks in this laboratory.

Chemicals. cAMP was obtained from Schwarz/Mann (Orangeburg, N.Y.). The chemicals used in the PAGE assays were from Bio-Rad Laboratories (Richmond, Calif). All other chemicals were of reagent grade and available though commercial sources.

Media and growth conditions. All cultures were grown in a mineral salts medium containing (in grams per liter): K_2HPO_4, 28; KH_2PO_4, 8; $MgSO_4 \cdot 7H_2O$, 0.10; and $(NH_4)_2SO_4$, 1.0. The pH was 7.2, and 20 mM glucose (final concentration) was added as the sole carbon and energy source just before inoculation. cAMP (filter sterilized) was added as indicated just before inoculation at a concentration of 5 mM. All cultures were grown at 37°C under aerobic conditions supplied by vigorous rotary shaking. Growth was monitored turbidimetrically at 420 nm, using a Bausch & Lomb Spectronic 70 colorimeter.

Bacteriophage were grown at 35°C on the standard 1.5% tryptone agar plates containing a 0.5% agar overlay. Their concentration was determined as plaque-forming units (PFU) per milliliter, using freshly harvested, log-phase *E. coli* NCR-701 as the indicator strain.

Bacteriophage purification. Ten to 15 double-layered tryptone agar plates were inoculated with single-plaque phage isolates and after incubation were harvested by the method of Adams (1). The phage-rich fraction obtained was concentrated by the method of Yamamoto and Alberts (31), using polyethylene glycol (PEG) precipitation of the phage. This precipitate was made 10% with regard to sucrose and applied as the upper layer of discontinuous sucrose gradients made up of 5.0 ml each of 55%, 45%, and 25% sucrose and 15.0 ml of 15% sucrose. An additional layer of 1.0 ml of 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*-2'-ethanesulfonic acid) buffer (pH 7.2) was added, and the samples were centrifuged at 4°C in an SW25.1 rotor at 70,000 × *g* for 45 min on a Beckman model D ultracentrifuge. The phage-rich region was seen as a blue-tinted, opalescent layer at the 25/45% sucrose interface and was harvested by gentle aspiration. This concentrated phage fraction was stored at 4°C as high-titer phage stock containing approximately 10^{14} PFU/ml.

TABLE 1. *Bacterial strains used*

Strain[a]	Genetic markers	Source
NCR-701	Wild type	Dallas et al. (7)
NCR-C51	*crp*	Dallas et al. (7)
NCR-C57	*cya*	Dallas et al. (7)
NCR-C57SM	*cya csm-1*	This laboratory
NCR-T6r1	*tsx* Trp^-	This laboratory

[a] All strains are *E. coli* K-12 derivatives. Strains NCR-701, NCR-C51, and NCR-C57 were designated as strains K-12, C-51, and C-57 in previous reports from this laboratory.

Preparation of outer membranes. Crude outer membrane fractions were obtained by the method described by Schnaitman (22), which uses Triton solubilization of the cytoplasmic membrane. The protein concentration of each sample was determined by the procedure of Lowry et al. (15), and the samples were stored at a concentration of 5 mg/ml in 10 mM HEPES buffer (pH 7.4) containing 0.1% sodium azide at 4°C.

Solubilization of OMP. The membrane preparations were solubilized by one of two methods. The first method was used for maximal denaturation and electrophoretic separation of the OMP; the second was used to obtain maximum activity but minimal denaturation of the proteins.

In method I, the samples were solubilized with SDS, urea, and 2-mercaptoethanol, according to the procedure described by Schnaitman (24) for preparing samples for SDS-PAGE. Two drops of bromophenol blue tracking dye was added to each of the samples obtained, and then the samples were heated to 100°C for 5 min in a boiling water bath. The samples were then stored at −20°C for later SDS-PAGE.

Method II is a modification of the procedure described by Yu et al. (32) for the selective solubilization of human erythrocyte membrane proteins. The protein concentrations of the membrane preparations were determined by the procedure of Lowry et al. (15). The purified OMP were then suspended at a concentration of 5 mg/ml in a solution containing 1.0% Triton X-100, 0.5% sodium deoxycholate, and 5 mM EDTA in 0.066 M phosphate buffer (pH 7.2). The solubilized samples were then stored at −20°C for later use.

Electrophoresis. SDS-PAGE was carried out on the samples solubilized by method I according to the procedure described by Schnaitman (24). The electrode buffers used in all electrophoretic procedures are those described by Bragg and Hou (4). Electrophoresis was carried out at a constant current of 5 mA/gel for a period of 7 to 8 h, at which time the tracking dye front approached the bottom of the tubes. The gels were removed from the tubes and stained for 2 h in an aqueous solution containing 0.25% Coomassie brilliant blue, 45% methanol, and 10% acetic acid (3). Destaining was completed in 24 h after four to five changes of an aqueous solution containing 25% methanol and 7.5% acetic acid (3). After destaining, the gels were scanned at 540 nm and at a rate of 2 cm/min in a model 1200 Gilford spectrophotometer with linear transport attachment, with slit set at 0.5 nm.

OMP preparations solubilized by method II were subjected to affinity electrophoresis on two-phase disc gels. The lower, separatory gel phase consisted of 7.5 g of acrylamide, 200 mg of bisacrylamide, 30 mg of ammonium persulfate, and 40 μl of TEMED (*N,N,N',N'*-tetramethylenediamine) per 100 ml of 0.1 M sodium phosphate buffer (pH 7.2) containing 0.1% Triton X-100. After mixing and degassing, the solution was poured into uncoated, calibrated glass tubes (inner diameter, 5 mm) to a height of 90 mm and overlaid with distilled water. After polymerization, the upper surfaces of the gels were washed with five rinses of 0.1 M sodium phosphate buffer (pH 7.2). The upper, affinity gels consisted of 500 mg of acrylamide, 15 mg of bisacrylamide, 3 mg of ammonium persulfate, and 4 μl of TEMED per 9.0 ml of 0.1 M sodium phosphate buffer. After mixing, 1.0 ml of high-titer phage stock

(approximately 10^{14} PFU/ml) was added to the solution. The phage-laden solution was degassed and pipetted above the separatory gel to a final height of 100 mm in the calibrated tubes and then overlaid with distilled water. After polymerization, the gel tops were washed with five rinses of 0.1 M sodium phosphate buffer, overlaid with the same buffer, and incubated overnight at room temperature. Control affinity gels were prepared as above with the substitution of 1.0 ml of 0.1 M sodium phosphate buffer for the phage solution. The protein samples were then loaded and electrophoresed, using Bragg and Hou (4) buffers containing 0.1% Triton X-100. The electrophoresis was carried out at a constant current of 2 mA/gel for approximately 18 h. The gels were then removed, stained, destained, and scanned as described above.

Where indicated, actual molecular weights were determined by SDS-PAGE according to the procedure described by Shapiro et al. (28).

Phage neutralization. OMP preparations from all strains were tested for the ability to neutralize phages T1 and T6 by the plaque reduction assay and by the release of viral DNA into the reaction mixture (1). Tubes containing 1 ml of high-titer phage stocks (in the range of 10^{14} PFU/ml) and 1 ml (5 mg of protein/ml) of OMP preparation solubilized by method II were mixed and incubated for 10 min at 37°C. Total PFU per milliliter used in each case was determined by plaque assay on control tubes from which the OMP preparations were omitted. After incubation, the phage were precipitated by addition of 0.5 ml of 35% PEG to each tube (31), and the tubes were refrigerated at 4°C for 1 h. They were then centrifuged at 10,000 × *g* for 10 min in a refrigerated RC-2 centrifuge. The supernatant fluid was gently removed from each tube, and the DNA content was determined on 0.2-ml samples by the diphenylamine assay (5). Total DNA was determined on samples of unprecipitated material. Plaque assays were carried out on dilutions of pellet and supernatant fractions in all cases. Samples of the supernatant fractions from some of the above reaction mixtures were resolubilized by method I and then subjected to SDS-PAGE analysis.

Phage neutralization was calculated from these experiments as follows: the number of PFU contained in each reaction mixture was calculated from duplicate plaque assays. The PFU recovered in the supernatant and pellet fractions after PEG addition and centrifugation were also plaque assayed. All calculations were made on the basis of PFU per milliliter of the original phage stock, and the percentage of phage neutralization was determined.

Characterization of phage infection. The rate constants of irreversible adsorption of phages T1 and T6, and the saturation capacity of each bacterial strain for these phages, were measured by procedures originally described by Schlessinger (21), Ellis (9), and Ellis and Delbruck (10). The average generation time and phage yield per infected cell were determined according to the methods described by Miller (18) and Ellis and Delbruck (10).

RESULTS

Analysis of OMP. When examined under maximally denaturing conditions on SDS-PAGE gels, the OMP of the wild-type strain were in accord with the results previously reported by Schnaitman (22–27). The OMP I and III were clearly evident and were used as approximate molecular weight markers in the range of 40,000 (22). Direct comparison of the densitometric tracings obtained after SDS-PAGE of the OMP from strain NCR-701 and its spontaneously T6-resistant mutant (strain NCR-T6r1) showed a single major difference (Fig. 1). In comparison with the parental strain, the latter lacked a single protein peak with a mass of less than 40,000 daltons. This peak was considered likely to represent the phage T6 receptor protein coded for by the *tsx* gene (17). In additional experiments, six SDS-gels were overloaded (1.0 mg of protein/gel) with either NCR-701 or NCR-T6r1 OMP and electrophoresed simultaneously. One gel from each of the samples was stained for approximate location of the area of interest. The remaining gels were sliced to obtain portions from each sample rich in the *tsx* protein. SDS-PAGE of this material according to the procedure described by Shapiro et al. (28) confirmed that the mass of this protein is approximately 32,000 daltons (data not shown).

Neutralization of phage. Neither phage T1 nor phage T6 was neutralized by the OMP preparations from strain NCR-701 which had been solubilized by method I; however, when solubilized by method II, the OMP preparations neutralized both phage strains. Method II was then applied to the OMP from both bacterial strains, and the results of the neutralization studies were monitored by plaque assay (Table 2). These data indicated that the OMP from strain NCR-T6r1 did not possess the T6 neutralization potential displayed by the wild-type strain. Essentially

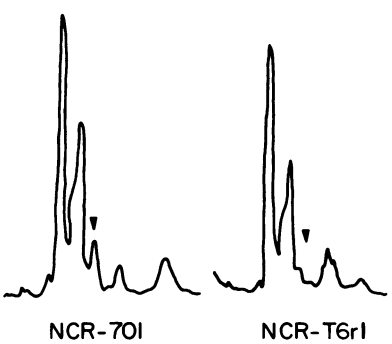

FIG. 1. *Representative scans of OMP from E. coli strains NCR-701 and NCR-T6r1. The preparations were solubilized by method I and subjected to SDS-PAGE. Gel tops are to the left and anodes are to the right of each scan. In this and all subsequent figures, the arrow identifies the migration position of the protein under study.*

identical results were obtained when these neutralization studies were monitored by viral DNA release (data not shown). Both strains displayed similar capacities for phage T1 neutralization.

Detection of phage T6 receptor. Method II-solubilized OMP preparations from both of these bacterial strains were applied to affinity gels which had been pre-electrophoresed to remove free phage proteins and unreacted free radicals (Fig. 2). The first member of each gel set contained high-titer phage T6 in the affinity layer. The second gel of each set contained phage T1 in the affinity layer, whereas the final gel contained no phage and served as a control. OMP preparations from wild-type but not strain NCR-T6r1 cells showed an isolated peak present in the control and T1 affinity gels, but not in the T6 affinity gels.

Additional electrophoretic procedures were carried out to determine if the protein represented by this missing peak truly displayed affinity toward phage T6. In these experiments, samples of the OMP preparations from both bacterial strains were examined electrophoretically, both before and after neutralization of phage T6 (Fig. 3). These experiments indicated that after neutralization of phage T6, a single protein peak was lost from the OMP preparation of strain NCR-701. This band was not evident in identical OMP preparations from strain NCR-T6r1 either pre- or post-neutralization (see Fig. 1 and 2).

Role of cAMP-CRP in phage T6 infection. Mutant strains altered in the cAMP-CRP functions were analyzed with respect to various parameters associated with the phage infection process, including adsorption constant, saturation capacity, burst size, and latent period. These parameters were measured for both phage T6 and phage T1, with the latter serving as a control for comparison. The data for T1 infection are not shown, since the values showed no significant variations from strain to strain and were unaffected by inclusion of cAMP in the culture medium. The average values for the absorption constant, saturation capacity, burst size, and latent period, respectively, for T1 infection were 2.8, 151, 93, and 19 (see Table 3 for units).

TABLE 2. *Phage T1 and T6 neutralization by viral plaque assay*

E. coli strain	Phage	PFU (10^{13}/ml)		% Neutralized
		Total	Recovered	
NCR-701	T6	8.8	5.2	41
NCR-701	T1	10.4	4.9	53
NCR-T6r1	T6	8.6	8.1	6
NCR-T6r1	T1	10.2	6.0	41

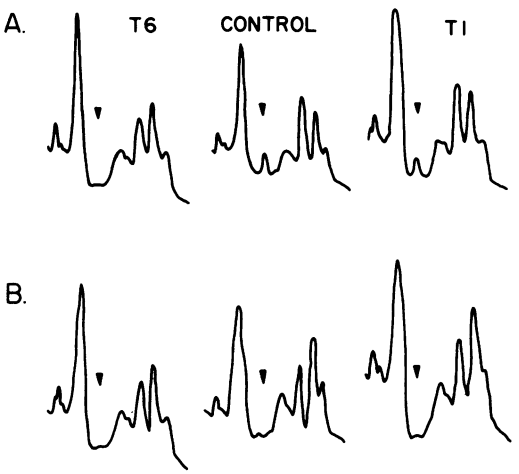

FIG. 2. *Representative scans of OMP from E. coli strains NCR-701 (A) and NCR-T6r1 (B), solubilized by method II and subjected to affinity electrophoresis. The center column represents control gels containing no bacteriophage, the left column shows scans from gels containing phage T6 in the affinity layer, and the right column shows scans from gels containing phage T1 in the affinity layer. Gel tops are to the left and anodes are to the right of each scan.*

Significant differences among the strains were noted when T6 infection was analyzed (Table 3). In comparison with strain NCR-701, the *cya* culture grown without cAMP and the *crp* culture grown with or without cAMP had a significantly lower adsorption constant and saturation capacity, smaller burst size, and slightly longer latent period. The suppressor mutant, on the other hand, exhibited a greater capacity for binding and reproducing T6 particles than did the wild-type culture. The addition of 5 mM cAMP to these cultures had little or no effect on these functions except for the *cya* mutant strain, in which case the normal wild-type functions were completely restored.

Analyses of OMP in cAMP-CRP mutant strains. Representative scans of OMP isolated from these strains and solubilized by method I followed by SDS-PAGE are shown in Fig. 4. The T6 receptor protein was produced by the wild-type and suppressor mutant cells, but was considerably repressed in the *crp* and *cya* strains. The latter strain produced the full complement of this polypeptide only when the culture was grown in the presence of 5 mM cAMP.

Affinity gels containing high concentrations of phage T1 or T6 (and control gels containing no phage) were prepared as described earlier. Samples of OMP preparations from strains NCR-701, NCR-C57, NCR-C51, and NCR-C57SM

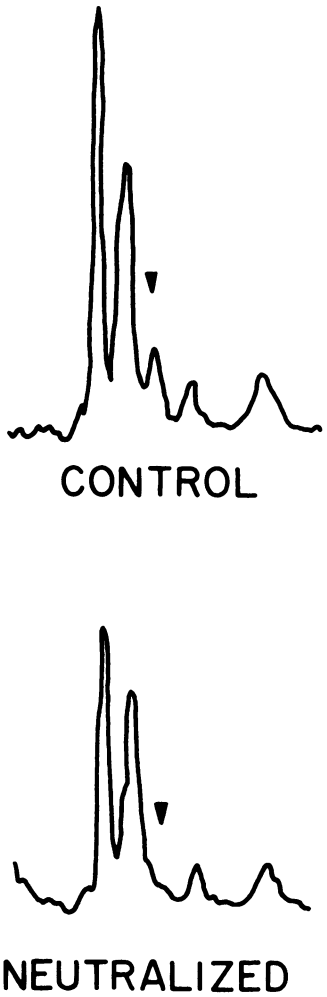

FIG. 3. *SDS-PAGE analysis of phage-neutralized OMP from E. coli NCR-701. Phage neutralization analyses were carried out as described in Materials and Methods. After addition of PEG to precipitate the phage, samples of the remaining supernatant fractions from phage-treated (neutralized) and untreated (control) preparations were solubilized by method I and subjected to SDS-PAGE analyses. Gel tops are to the left and anodes are to the right of each scan.*

were solubilized by method II and analyzed (Fig. 5). It was again clearly seen from these data that a functional cAMP-CRP complex was required for full production of the particular polypeptide which was specifically absorbed to affinity gels containing T6 phage.

This requirement was also seen when OMP preparations obtained from these cultures were solubilized by method II and then subjected to phage neutralization analyses. These tests were carried out by the plaque reduction assay and (in data not shown) by measure of the release of viral DNA into the reaction mixture as described in Materials and Methods. These tests were conducted with both phage T6 and phage T1. On the average, the OMP obtained from *crp* cells or *cya* cells grown without cAMP had only 10 to 20% of the wild-type capacity for T6 phage neutralization (Table 4). Data obtained for phage T1 neutralization are not shown, since no differences in neutralization were detected from strain to strain under these same conditions.

DISCUSSION

Since some of the OMP are known to be translated from long-lived, stable mRNA, Lee and Inouye (14) hypothesized that these proteins were under some unique regulatory mechanism. Whether this is the case or not, we have established that the cAMP-CRP complex is a major factor in regulating synthesis of one of these proteins. Previously we had shown that this complex plays a key role in the synthesis and function of inner, cytoplasmic membrane systems. Mutants deficient in either of these regulatory components are unable to transport hexoses (11) or hexose phosphates (12) by the normal wild-type processes; they show decreased capacity for synthesis of ATP via chemiosmotic processes owing to impairments in synthesis of key membrane dehydrogenases, cytochrome components, and flavins (6, 8). The membrane fatty acid composition of these mutants is also different from that of wild-type cells (7).

The resistance to infection by phage T6 displayed by *cya* and *crp* mutants was shown in this study to be related to a decrease in the rate of adsorption of this phage by the mutants. The fact that adsorption rates approaching the wild-

TABLE 3. *Phage T6 adsorption studies*

E. coli strain	AMP[a]	Adsorption constant ($\times 10^{-9}$ phage/bacterium per s)	Saturation capacity (phage/bacterium)	Burst size (phage/bacterium)	Latent period (min)
NCR-701	+	1.9	146	146	19
	−	2.4	137	158	19
NCR-C57	+	2.6	148	144	21
	−	0.3	92	126	27
NCR-C51	+	0.3	90	135	26
	−	0.2	87	129	28
NCR-C57SM	+	15.9	163	167	19
	−	19.3	196	174	14

[a] Grown in the presence (+) or absence (−) of 5 mM cAMP.

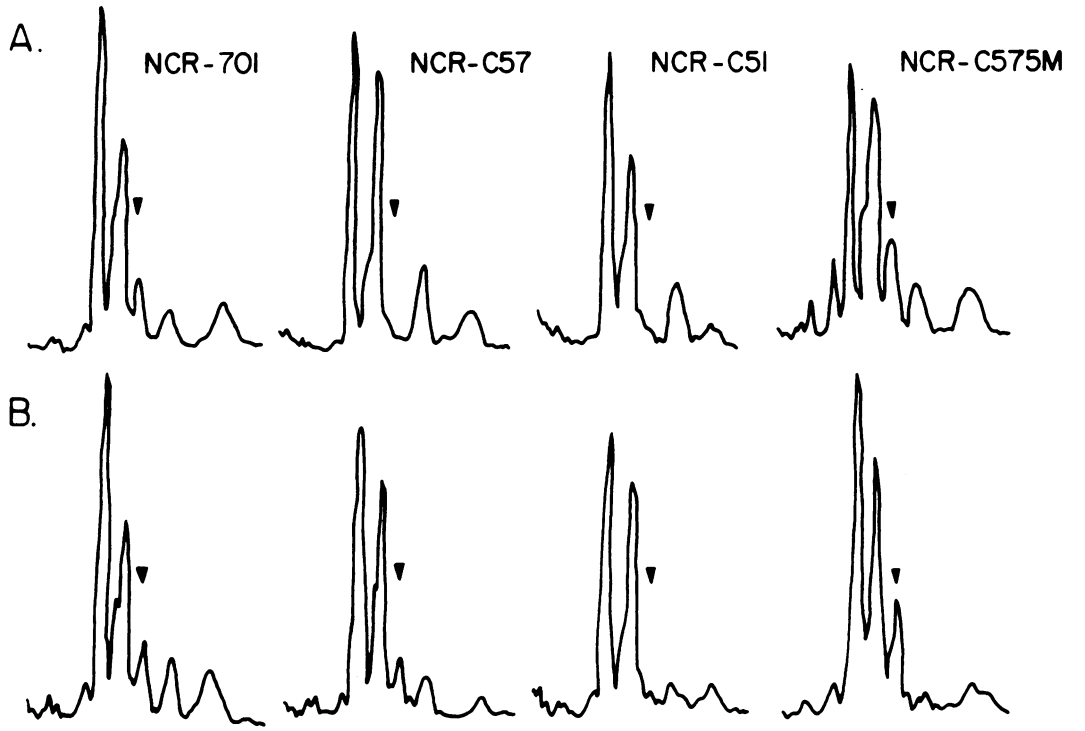

FIG. 4. *Representative scans of OMP from strains NCR-701, NCR-C57, NCR-C51, and NCR-C57SM. All strains were grown in the absence (A) or presence (B) of exogenous cAMP. The OMP were solubilized by method I and subjected to SDS-PAGE. Gel tops are to the left and anodes are to the right on each scan.*

type level can be restored to *cya* but not to *crp* mutants by the addition of 5 mM cAMP to the growth medium suggests that the T6 receptor protein is the product of a system subject to regulation by the cAMP-CRP complex and therefore subject to classical catabolite repression.

Studies on T6-resistant mutants (*tsx*) of *E. coli* by Manning and Reeves (17) have shown that these mutants lack an OMP with an approximate mass of 32,000 daltons; this protein also serves as the receptor for colicin K. The SDS-PAGE analysis undertaken in the present study demonstrates that such a protein is present in barely detectable quantities in the outer membrane of *cya* and *crp* mutants but reappears in the mutant strain (NCR-C57SM) which carries a suppressor for the cAMP requirement. This protein is virtually absent from the *tsx* mutant membrane preparations. It accounts for approximately 7% of the total OMP of wild-type cells and *cya* mutants grown in the presence of 5 mM cAMP (17, 26, 27). The presence of this protein in the OMP preparations of the various strains tested in this study correlates with the ability of the OMP preparation to neutralize phage T6. OMP preparations containing barely detectable or nondetectable amounts of this protein showed no neutralizing activity.

Additional evidence that this particular protein is the phage T6/colicin K receptor, i.e., the product of the *tsx* genes, was provided by the affinity-PAGE technique developed during the course of this study. In this procedure, a high concentration of phage was immobilized in a polyacrylamide matrix of rather large pore size. OMP preparations solubilized by method II were electrophoresed gently through this layer and onto a separatory gel. The data indicated that the T6 receptor molecules either were bound tightly to the T6 phage tail fibers and retained in this affinity layer or were retarded in passage through this layer and "smeared" in the upper portion of the separatory gel. It should be noted that appropriate concentrations of the detergents (Triton X-100 and sodium deoxycholate) used in solubilizing the OMP and appropriate ratios of phage to OMP were obtained by trial and error. In this connection, the procedure will undoubtedly require modification for each system being studied. In the isolation of receptors for extremely large moieties such as bacteriophage, care must be taken to select an affinity matrix of sufficient average pore size to allow

the passage of the solubilized membrane proteins, but not of the phage, onto the separatory gel. An extremely high phage concentration in the affinity gel tends to prevent the membrane proteins from passing through the matrix onto the separatory gel, whereas a low phage concentration will not bind enough receptor to make their absence from the separatory gels evident. Smaller molecules may be bound covalently to commercially available dextran beads which are immobilized in the affinity gel for the isolation of receptors for low-molecular-weight substances (20). The isolation of membrane receptor proteins should allow further elucidation of transport phenomena as well as determination of specific functions for individual membrane proteins. It is conceivable that with this methodology, the structure and function of the outer membrane of gram-negative bacteria and the plasma membrane or eukaryotic organisms can be further described and characterized.

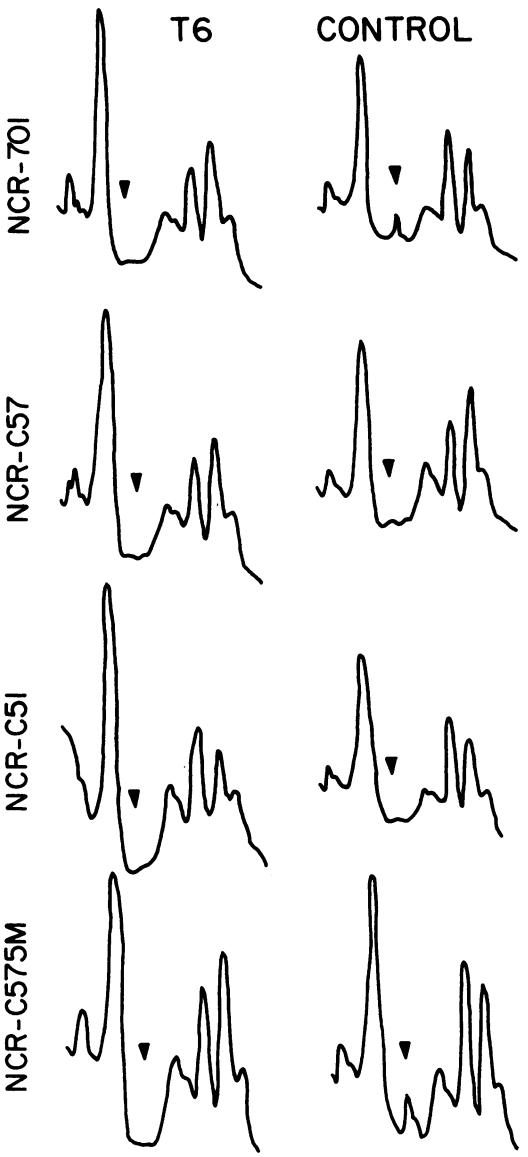

FIG. 5. *Affinity electrophoresis of OMP derived from strains NCR-701, NCR-C57, NCR-C51, and NCR-C57SM. OMP were solubilized by method II and subjected to affinity electrophoresis. The left column shows scans of gels prepared with phage T6 in the affinity layer; the right column shows scans of gels prepared in an identical manner but without phage T6. Gel tops are to the left and anodes are to the right in each scan. All cultures used in this experiment were grown without exogenous cAMP.*

TABLE 4. *Phage T6 neutralization studies*

E. coli strain	cAMP[a]	PFU (10^{13}/ml)		% Neutralized
		Total	Recovered	
NCR-701	+	8.7	4.6	47
	−	8.8	5.2	41
NCR-C57	+	8.6	4.6	47
	−	9.0	8.1	10
NCR-C51	+	8.7	8.1	7
	−	8.9	8.4	6
NCR-C57SM	+	8.9	4.5	49
	−	8.6	4.2	51

[a] Grown in the presence (+) or absence (−) of 5 mM cAMP.

If the product of the *tsx* gene were a phage T6 receptor and played no physiologically functional role, it would have been surprising to find it under cAMP-CRP control and thereby subject to catabolite repression. This would be so because catabolite repression seems to govern synthesis only of those proteins which contribute in some way to the carbon and energy metabolism of growing cultures (16, 19). It has been shown, however, that the product of this gene is bifunctional, serving also as the colicin K binding site (17). Furthermore, it has now been established that binding of colicin K to this receptor protein causes rapid and extensive collapse of the organism's chemiosmotic gradient (29), thereby destroying a major source of its metabolic energy. In this respect then, catabolite repression of *tsx* transcription is not at all incongruous with its established role in regulation of bacterial metabolism.

ACKNOWLEDGMENTS

This research was supported in part by the U.S. Army Research Office (grant RDRD-1P-12368-L).

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