ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 1983, p. 91-97
0066-4804/83/010091-07$02.00/0

Vol. 23, No. 1

Characterization of β-Lactamase Induction in *Enterobacter cloacae*

THOMAS D. GOOTZ† AND CHRISTINE C. SANDERS*

Department of Medical Microbiology, Creighton University School of Medicine, Omaha, Nebraska 68178

Received 19 July 1982/Accepted 3 November 1982

The induction of β-lactamase was studied in a strain of *Enterobacter cloacae*. A wide variety of β-lactam compounds were found to induce β-lactamase in this organism, and the degree of induction was directly related to the stability of the inducer to degradation by the enzyme. The kinetics of the induction process were consistent with a system normally under repressor control, suggesting a direct interaction of the β-lactam compound with a repressor protein in the *E. cloacae* cells. Although these characteristics are common to many inducible systems in gram-negative organisms, the induction of β-lactamase in this strain was not subject to catabolite repression with glucose and remained unaffected by exogenous cyclic AMP in the culture medium. This suggests that the organization and function of the β-lactamase regulatory genes in *E. cloacae* are unlike those of other inducible gene systems, such as those composing the well-characterized lactose operon in *Escherichia coli*.

Studies in this and other laboratories have shown that the level of β-lactamase expression in bacteria possessing inducible cephalosporinases plays an important role in determining susceptibility to the newer cephalosporins. Strains reversibly derepressed by cefoxitin or stably derepressed via mutation become resistant not only to the newer cephalosporins but also to expanded-spectrum penicillins (5, 10, 17, 27, 29, 30, 32; C. C. Sanders and W. E. Sanders, Jr., Rev. Infect. Dis., in press). The derepressed enzymes appear to confer resistance to this broad array of β-lactam antibiotics by either (i) hydrolysis of the drug or (ii) creation of a permeability barrier to nonsubstrate drugs that limits access to target proteins (29, 30, 32; Sanders and Sanders, in press).

Strains within the genus *Enterobacter* characteristically produce inducible cephalosporinases (31). Derepression of these enzymes has been associated with therapeutic failures for cefamandole, moxalactam, and ceftriaxone (3, 25, 27; Sanders and Sanders, in press). Also, the ability of cefoxitin to antagonize the activity of various β-lactam antibiotics against this genus both in vitro and in vivo is due to the derepression of β-lactamase (4, 7, 9, 12, 16, 30, 36). Despite the clinical significance of this inducible *Enterobacter* enzyme, very little is known about the process of its production. Therefore, the purpose of this study was to characterize this process through (i) an examination of induction kinetics, (ii) determination of the structural specificity for inducer molecules, and (iii) identification of physiological factors that influence induction.

† Present address: Department of Microbiology and Immunology, University of Washington, Seattle, WA 98195.

MATERIALS AND METHODS

Bacterial strain. A clinical isolate of *Enterobacter cloacae* 55 was employed in these studies. This strain has been characterized previously (10) and possesses an inducible, chromosomally mediated cephalosporinase of Richmond and Sykes type I. This enzyme after induction is capable of hydrolyzing cephalothin, cephaloridine, cefamandole, penicillin G, and, to a small extent, cefoxitin. In standard disk diffusion (2) and broth dilution assays (10), *E. cloacae* 55 (without enzyme induction) is resistant to cephalothin, cephaloridine, and cefoxitin, but susceptible to a number of other β-lactam antibiotics (Table 1). It appears to be susceptible to cefamandole in disk diffusion tests but resistant in broth dilution tests. Stable, derepressed mutants phenotypically identical to *E. cloacae* 55 after cefoxitin induction occur at a rate of 1 in 10^6 to 10^7 cells. These mutants differ from the wild type in their resistance to a number of β-lactam antibiotics (Table 1).

Media. Mueller-Hinton broth (MHB; Difco Laboratories, Detroit, Mich.) was used for all initial induction experiments. For catabolite suppression experiments the minimal salts medium of Makman and Sutherland was employed (20). This contained (per liter of distilled water): 14 g of K_2HPO_4, 6 g of KH_2PO_4, 2 g of $(NH_4)_2SO_4$, and 0.2 g of $MgSO_4$ (Mallinckrodt Inc., St. Louis, Mo.). Minimal salts medium was supplemented with 0.5% (wt/vol) glucose, fructose, lactose, or vitamin-free Casamino Acids (Difco), or one of the complex media MHB or Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.), both used at the manufacturer's suggested concentrations.

Antibiotics and reagents. Working antibiotic stan-

TABLE 1. β-Lactam susceptibility of *E. cloacae* 55 and its spontaneous mutant stably derepressed for β-lactamase production

β-Lactam	Minimal inhibitory concn (μg/ml)[a] for:		Zone size (mm)[b]	
	Strain 55	Mutant	Strain 55	Mutant
Carbenicillin	8	128	27 (S)	12 (R)
Piperacillin	8	>128	30 (S)	10 (R)
Mezlocillin	16	>128	27 (S)	11 (R)
Azlocillin	32	>128	22	7
Cephalothin	>128	>128	6 (R)	6 (R)
Cefoxitin	>128	>128	6 (R)	6 (R)
Cefamandole	64	>128	24 (S)	7 (R)
Cefotaxime	16	>128	33 (S)	7 (R)
Ceftriaxone	1	>128	26	8
Moxalactam	4	32	32 (S)	22 (I)
Mecillinam	0.25	0.25	24	26
N-Formimidoyl thienamycin	0.5	0.25	20	23
Sch 29482	2.0	2.0	20	22

[a] Determined with an inoculum of 10^5 CFU/ml in 3 ml of MHB.

[b] Interpretation: S, susceptible; I, intermediate; R, resistant. No interpretation was applied to drugs for which interpretive standards have not been established (22).

dards were prepared in 0.1 M phosphate buffer from the following diagnostic powders: cefamandole, cephalothin, cephaloridine, and moxalactam (Eli Lilly & Co., Indianapolis, Ind.); cephamycin C, cefoxitin, and *N*-formimidoyl thienamycin (Merck Sharp & Dohme, West Point, Pa.); nitrocefin (Glaxo Ltd., Greenford, Middlesex, England); rifampin (Pittman-Moore, Indianapolis, Ind.); chloramphenicol (Sigma Chemical Co., St. Louis, Mo.); and Sch 29482 (Schering Corp., Bloomfield, N.J.). The following reagents were also used: isopropylthio-β-galactoside (IPTG), cyclic AMP (cAMP), *o*-nitrophenyl-β-D-galactopyranoside (ONPG), and EDTA (all from Sigma).

Induction of β-lactamase. The kinetics of induction were studied with *E. cloacae* 55 cells grown to log phase in MHB. An induction culture was prepared by making a 1:20 dilution of an overnight MHB culture in fresh medium and incubating it at 35°C with shaking (150 rpm). In 2 to 3 h, the culture reached a standard optical density of 0.3 at 570 nm (Spectronic 20), which corresponded to a viable count of 1×10^8 to 3×10^8 CFU/ml. β-Lactam compounds were then added as sterile solutions in 0.1 M phosphate buffer, and incubation was continued. Cells were harvested at various time intervals in 75-ml portions, and sodium azide was added (1 mM final concentration) to arrest cell metabolism. The cells were then centrifuged at 7,000 × *g* for 15 min (Sorval RC2-B) and washed once with 0.1 M phosphate buffer. The cell pellet was resuspended in 5 ml of the same buffer and disrupted with a Bronwill ultrasonic disintegrator (Biosonik IV; VWR Scientific, San Francisco, Calif.). Cell debris was removed by centrifugation at 30,000 × *g* for 45 min at 4°C (International Centrifuge). The cleared sonic extracts were filter sterilized and frozen at −20°C. The protein content of all cleared sonic extracts was determined by the method of Lowry et al. (19), with bovine serum albumin used as the standard. The amount of bioactive inducer present at each sampling time was determined by a bioassay described previously (11). In one induction experiment, cells were grown to the standard optical density in MHB, centrifuged as before, and suspended in an equal volume of 0.01 M Tris-hydrochloride buffer (pH 8). The cells were immediately centrifuged at 10,000 × *g* for 5 min (RC2-B), and the resulting pellet was resuspended in the same volume of Tris containing 0.25 mM EDTA to increase outer membrane permeability (18). The cell suspension was manually mixed for 60 s, and the cells were pelleted as before. The pellet was resuspended in the original volume of prewarmed MHB, and the culture was induced for 1 h with a given β-lactam at 20 μg/ml final concentration. At the end of incubation, sodium azide was added and cells were sonicated as described above. For catabolite repression studies, *E. cloacae* 55 was grown with shaking in minimal salts medium containing 0.5% Casamino Acids. When the culture reached an optical density of 0.3 at 570 nm, glucose was added to final concentrations of 0.1, 0.5, or 1%. Incubation was continued for an additional 10 min, and then cefoxitin (20 μg/ml) and IPTG (0.5 mM) were added to induce β-lactamase and β-galactosidase, respectively. Incubation was continued as before, and harvested cells were sonicated. The effect on enzyme induction of exogenously supplied cAMP (5 mM) was studied in a similar manner with cells grown in minimal salts medium with 1% glucose as sole carbon source.

β-Lactamase and β-galactosidase assays. The β-lactamase activity of cleared sonic extracts was determined by a spectrophotometric assay (10) measuring the amount of cephalosporin inactivated in a 100 μM solution in phosphate buffer (pH 7.1) at 37°C with a Beckman DBGT dual beam spectrophotometer. β-Galactosidase activity in the sonic extracts was assayed by the spectrophotometric method of Wallenfels (34). Cleavage of ONPG substrate dissolved in 0.05 M Tris buffer (pH 7.6) was followed at 25°C after addition of an appropriate dilution of the sonic extract. The change in optical density of the solution at 405 nm was monitored as the *o*-nitrophenol cleavage product was

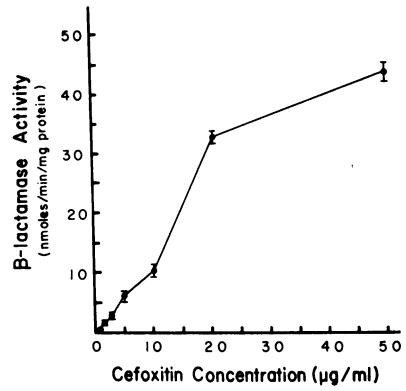

FIG. 1. Influence of cefoxitin concentration on induction of β-lactamase in *E. cloacae* 55. Induction was performed for 3 h, and β-lactamase was assayed with cefamandole as the substrate. Bars indicate 2 standard deviations of the mean of triplicate assays.

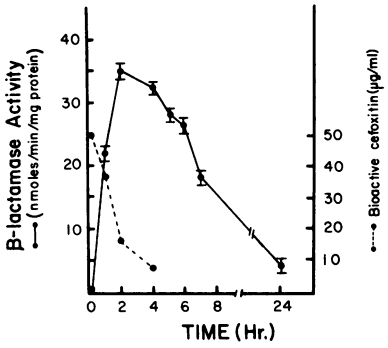

FIG. 2. Kinetics of β-lactamase production (solid line) in *E. cloacae* 55 cells induced with 50 μg of cefoxitin per ml. Sonic extracts prepared from induced cells were used in the spectrophotometric assay with cefamandole as substrate. The concentration of cefoxitin remaining in the induction medium is shown by the dashed line. Bars indicate 2 standard deviations for the mean of triplicate assays.

released. Specific enzyme activities for both assays were calculated from the protein content of the cells.

RESULTS

Kinetics of β-lactamase induction. The induction of β-lactamase in *E. cloacae* 55 was studied initially in log-phase cells induced with cefoxitin. Cells were grown with shaking in MHB to an optical density of 0.3 at 570 nm. The culture was divided into equal portions, and cefoxitin was added to give final concentrations of 1.3 to 50 μg/ml. Incubation was allowed to proceed for 3 h, after which cleared sonic extracts were prepared and evaluated for β-lactamase activity with cefamandole used as a substrate. The amount of β-lactamase induced increased across the entire concentration range of cefoxitin tested (Fig. 1). A decrease in cell viability of no more than 1 log was observed under these conditions. When 50 μg of chloramphenicol or rifampin per ml was added to log-phase cells 20 min before the addition of 20 μg of cefoxitin per ml, β-lactamase induction was completely inhibited. These results suggested that the induction of β-lactamase depended on active protein synthesis. The kinetics of β-lactamase production in *E. cloacae* 55 cells induced in MHB containing 50 μg of cefoxitin per ml are shown in Fig. 2. The induction of cefamandole-inactivating β-lactamase reached a peak at 2 h and then declined as the amount of bioactive cefoxitin remaining in the induction medium declined (Fig. 2). The β-lactamase induction process was rapidly reversible by washing the cefoxitin-induced cells once in 0.1 M phosphate buffer and then reincubating them in drug-free MHB. Induction was uninterrupted, however, when the washed cells were resuspended in MHB containing the original concentration of cefoxitin (Fig. 3).

Stability of inducers to β-lactamase. To test the relationship between β-lactamase stability and inducer structure in determining optimal induction in this strain, we compared a number of β-lactams for their ability to induce enzyme at a concentration of 20 μg/ml. In these tests, one half of the cells were pretreated with EDTA before induction to minimize permeability differences between the β-lactam compounds employed. Induction was conducted for 1 h, after which cell-free sonic extracts were prepared and tested for β-lactamase activity against cephaloridine. This compound was selected as the substrate in the spectrophotometric assay in this

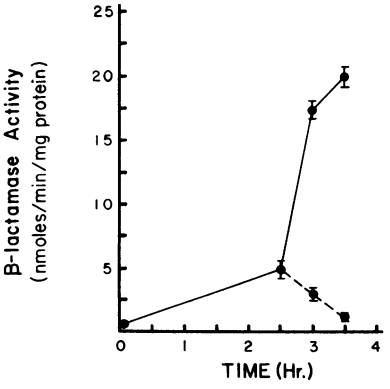

FIG. 3. Effect of washing on induction of β-lactamase in *E. cloacae* 55. Cells were induced with cefoxitin (10 μg/ml) for 2.5 h and then washed in phosphate buffer. Cells were then resuspended in either MHB (dashed line) or MHB containing cefoxitin (10 μg/ml) (solid line). The substrate was cefamandole. Bars indicate 2 standard deviations of the mean of triplicate assays.

TABLE 2. Induction of β-lactamase by various β-lactam antibiotics in cells with and without EDTA treatment

β-Lactam	β-Lactamase activity[a] (nmol inactivated/min per mg ± 2 SD) in:		EDTA-treated/untreated activity ratio
	Untreated cells	EDTA-treated cells	
Cephalothin	11 ± 1	12 ± 1	1.1
Cephamycin C	126 ± 15	312 ± 4	2.5
Cefamandole	186 ± 23	406 ± 28	2.2
N-Formimidoyl thienamycin	826 ± 20	2,245 ± 160	2.7
Cefoxitin	429 ± 13	1,951 ± 28	4.5
Moxalactam	522 ± 15	1,639 ± 77	3.1
Sch 29482	642 ± 29	2,089 ± 68	3.3

[a] Substrate was cephaloridine.

and all further studies since it was found to be significantly more sensitive than cefamandole to degradation by the induced β-lactamase (10). The ability of seven different β-lactam antibiotics to induce β-lactamase in *E. cloacae* 55 cells with and without EDTA treatment is shown in Table 2. EDTA treatment of the cells increased the amount of enzyme induced by these agents. This effect was greatest for cefoxitin, moxalactam, and Sch 29482. However, the relative order of inducer activity (compared with cephalothin) was not influenced greatly by EDTA treatment (Table 3). In general, the better inducers were those antibiotics that were not susceptible to hydrolysis by the β-lactamase (Table 3). No clear structural specificity was found which determined the degree of inducer activity. Moxalactam, like cefoxitin, contains a 7-alpha-methoxy group and was also a good inducer of β-lactamase. However, two new compounds structurally unrelated to cefoxitin, *N*-formimidoyl thienamycin, a carbapenem, and Schering 29482, a penem, were the best inducers of β-lactamase in this strain with or without EDTA treatment (Table 2).

Physiological studies. The expression of most inducible operons in gram-negative bacteria has been shown to be influenced by the composition of the growth medium. Therefore, the effect of carbon source on the induction of β-lactamase in *E. cloacae* 55 cells was studied. Cells were grown to log phase (optical density of 0.3 at 570 nm) in minimal salts medium supplemented with either a single or complex carbon source. Cefoxitin (50 μg/ml) and IPTG (0.5 mM) were then added, and cells were induced for 1 h. Sonic extracts produced from such induced cells were used to measure the levels of β-lactamase and β-galactosidase. Induction of β-galactosidase was

TABLE 3. Inducer activity and β-lactamase stability of various β-lactam antibiotics

β-Lactam	Relative inducer activity[a] for:		Relative rate[b] (% control) of hydrolysis by the induced enzyme
	Untreated cells	EDTA-treated cells	
Cephalothin	1	1	100
Cephamycin C	12	27	15
Cefamandole	18	35	1
N-Formimidoyl thienamycin	78	195	<0.5
Cefoxitin	40	170	<0.5
Moxalactam	49	143	<0.5
Sch 29482	61	182	<0.5

[a] Amount of each enzyme induced was determined with cephaloridine used as the substrate. The amount of enzyme induced by cephalothin was assigned a value of 1.

[b] Hydrolysis of each substrate was measured with a single preparation of β-lactamase induced by cefoxitin. Hydrolysis of cephalothin was assigned a value of 100%.

TABLE 4. Influence of carbon source on induction[a] of β-lactamase and β-galactosidase in *E. cloacae* 55

Carbon source	β-Lactamase activity (nmol inactivated/min per mg ± 2 SD)	β-Galactosidase activity (nmol cleaved/min per mg ± 2 SD)
Group A[b]		
Glucose	2,027 ± 52	864 ± 48
Fructose	2,011 ± 94	1,140 ± 84
Trypticase soy	7,235 ± 248	674 ± 26
Group B[c]		
Lactose	3,806 ± 292	1,457 ± 58
Casamino Acids	2,692 ± 197	2,190 ± 58
MHB	1,979 ± 52	2,790 ± 70

[a] β-Lactamase was induced with cefoxitin and tested with cephaloridine as the substrate. β-Galactosidase was induced with IPTG and tested with ONPG as the substrate.

[b] Contained glucose or fructose as carbon source.

[c] Contained carbon sources other than glucose or fructose.

significantly decreased in media containing glucose or fructose (Table 4). In contrast, the glucose content of the medium did not suppress β-lactamase production. The highest specific activity of this enzyme was obtained in cells grown in Trypticase soy broth containing glucose. The effect of glucose on the induction of β-lactamase was more directly determined by growing cells of *E. cloacae* 55 to log phase in minimal salts medium containing 0.5% Casamino Acids as the sole carbon and energy source. When the cells reached the standard optical density, glucose was added at various concentrations. Incubation was continued for 10 min, and then cefoxitin and IPTG were added as before. Induced cells were harvested at timed intervals, and enzyme activity was evaluated from the sonic extracts produced. The induction of β-galactosidase was suppressed by the addition of glucose, whereas the induction of β-lactamase was not (Fig. 4). Similarly, the addition of cAMP (5 mM final concentration) to cells grown in minimal salts medium containing 1% glucose as sole carbon source had no effect on cefoxitin-induced β-lactamase production (Fig. 5).

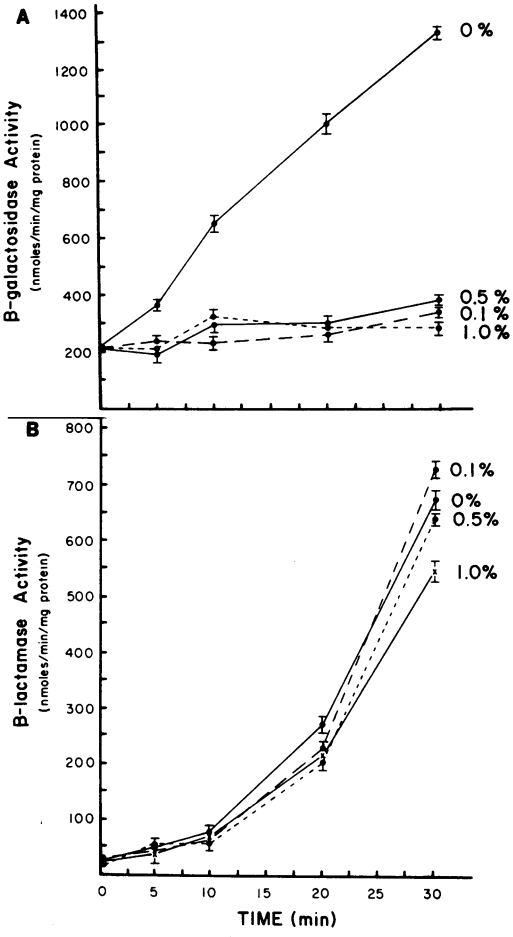

FIG. 4. Effect of glucose on induction of β-galactosidase (A) and β-lactamase (B) in *E. cloacae* 55 cells. Numbers at the end of each line indicate the glucose concentration of the medium. Cells were grown to log phase in minimal salts medium with Casamino Acids as the sole carbon source and induced with IPTG (0.5 mM) and cefoxitin (50 μg/ml). Glucose was added 10 min before the addition of inducers. Sonicated cell extracts prepared from induced cells were used to measure the degradation of cephaloridine and ONPG. Bars indicate 2 standard deviations of the mean of triplicate assays.

DISCUSSION

The inducibility of β-lactamase in *Enterobacter* was first described by Hennessey (14). Other investigators have since shown that enzyme expression in this genus is normally under repressor control (10, 17). Both spontaneous and temperature-sensitive mutants that have a derepressed phenotype have been characterized. The rapid reversal of enzyme induction after removal of the inducer and the prevention of induction by chloramphenicol and rifampin described in the current study further support a repressor control mechanism for the expression of this β-lactamase. The induction of β-lactamase in *E. cloacae* appears to resemble the induction mechanism for the chromosomally mediated β-lactamase in *Pseudomonas aeruginosa* (24). The expression of this enzyme has also been shown to be under repressor control, and recent data indicate that β-lactam molecules may interact with a repressor protein in the cell (6, 24, 26).

Initial induction studies with *E. cloacae* 55 showed that cefoxitin was a better β-lactamase inducer than cephalothin or cefamandole (28). This suggested that some molecular specificity may exist for the inducer molecule. The results of this study showed that a variety of structurally diverse β-lactam antibiotics were excellent inducers of the enzyme. Stability to the β-lactamase correlated with inducer activity. The best inducers in this study were two new compounds, *N*-formimidoyl thienamycin and Sch 29482. These differ markedly in overall structure from cefoxitin. However, like cefoxitin, they are not good substrates for the induced enzymes (1, 8, 23, 29). Thus, an intact β-lactam ring was the only factor shared by the antibiotics with good inducer activity in this study. An association between enzyme stability and inducer activity has also been shown by Minami et al. (21). These authors obtained similar results in tests with 10-μg/ml concentrations of the inducers used in our study. However, they found that several enzyme-stable compounds are not good

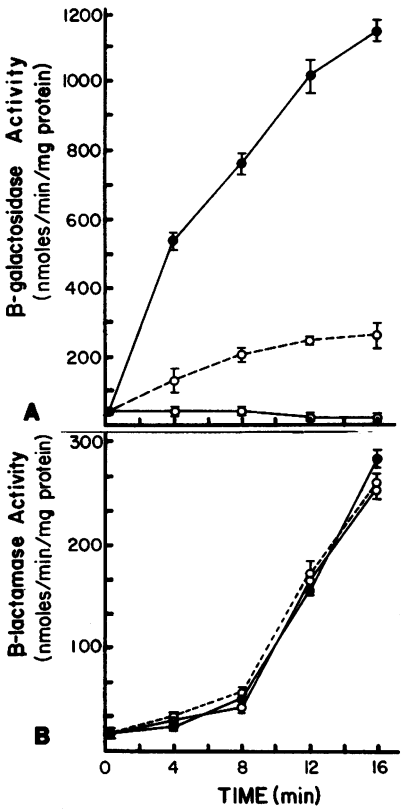

FIG. 5. Effect of cAMP on induction of β-galactosidase (A) and β-lactamase (B) in *E. cloacae* 55 cells. The medium was minimal salts with 1% glucose. Cells were induced with 50 μg of cefoxitin per ml (O——O), cefoxitin plus 0.5 mM IPTG (O------O), or cefoxitin plus IPTG plus 5 mM cAMP (●——●). The substrates were cephaloridine and ONPG. Bars indicate 2 standard deviations of the mean of triplicate assays.

inducers. Since we have not evaluated these agents, it is unclear why they were poor inducers. Also, the different methodologies employed in the two studies prevent any detailed comparison of results. Thus, a complete delineation of the association between enzyme stability and inducer activity will require further study.

Many characteristics of the β-lactamase system in *E. cloacae* were similar to the classical β-galactosidase operon of *Escherichia coli* (35). Thus, studies were performed to determine whether β-lactamase induction was affected by the cAMP level of the cell. Results indicated that β-lactamase induction in *E. cloacae* cells was not suppressed by growth in media containing glucose and was independent of regulation by cAMP. In fact, the expression of β-lactamase was highest in minimal medium which had been supplemented with Trypticase soy broth containing glucose (Table 4). This higher expression of β-lactamase may be due to the increased growth rate of the organism in media containing complex supplements. Such a growth rate effect has been observed for the constitutive production of β-lactamase in *Escherichia coli* (15). Thus, it appears that the expression of β-lactamase in *E. cloacae* differs greatly from the β-galactosidase operon of *Escherichia coli*. It differs also from the expression of other genes for noncatabolic enzymes, such as those coding for the constitutive production of chloramphenicol acetyltransferase and inducible cloacin DF13 production, which have been shown to depend on cellular levels of cAMP (13, 15, 33).

In summary, the β-lactamase operon of *E. cloacae* appears to be functionally independent of cAMP regulation, although it is subject to derepression by gratuitous inducers. Since derepression of β-lactamase in this and other genera may be important in determining the outcome of therapy with the newer β-lactam antibiotics (3, 25, 27; Sanders and Sanders, in press), further work should be performed to delineate the control mechanisms of these enzymes.

ACKNOWLEDGMENT

We thank John C. Sherris for his helpful comments on this manuscript.

LITERATURE CITED

1. Albers-Schonberg, G., B. H. Arison, O. D. Hensens, J. M. Hirshfield, K. Hoogsteen, E. A. Kaczka, R. R. Rhodes, J. S. Kahan, F. M. Kahan, R. W. Ratcliffe, E. Walton, L. J. Ruswinkle, R. B. Morin, and B. G. Christensen. 1978. Structure and absolute configuration of thienamycin. J. Am. Chem. Soc. 100:6491–6498.
2. Bauer, A. W., W. M. M. Kirby, J. C. Sherris, and M. Turck. 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. 45:493–496.
3. Beckwith, D. G., and J. A. Jahre. 1980. Role of cefoxitin-inducible beta-lactamase in a case of breakthrough bacteremia. J. Clin. Microbiol. 12:517–520.
4. Chattopadhyay, B., and I. Hall. 1979. Antagonism between cefoxitin and cefuroxime. J. Antimicrob. Chemother. 5:490–491.
5. Findell, C. M., and J. C. Sherris. 1976. Susceptibility of *Enterobacter* to cefamandole: evidence for high mutation rate to resistance. Antimicrob. Agents Chemother. 9:970–974.
6. Flett, F., N. A. C. Curtis, and M. H. Richmond. 1976. Mutant of *Pseudomonas aeruginosa* 18S that synthesizes type Id β-lactamase constitutively. J. Bacteriol. 127:1585–1586.
7. Fu, K. P., and H. C. Neu. 1981. The role of inducible β-lactamases in the antagonism seen with certain cephalosporin combinations. J. Antimicrob. Chemother. 7:104–107.
8. Ganguly, A. K., V. M. Girijavallabhan, S. McCombie, P. Pinto, R. Rizvi, P. D. Jeffrey, and S. Lin. 1982. Synthesis of Sch 29482—a novel penem antibiotic. J. Antimicrob. Chemother. 9(Suppl. C):1–5.
9. Goering, R. V., C. C. Sanders, and W. E. Sanders, Jr. 1982. Antagonism of beta-lactam antibiotics by cefoxitin in treatment of experimental infections in mice. Antimicrob. Agents Chemother. 21:961–967.
10. Gootz, T. D., C. C. Sanders, and R. V. Goering. 1982. Resistance to cefamandole: derepression of beta-lacta-

mase by cefoxitin and mutation in *Enterobacter cloacae*. J. Infect. Dis. **146**:34–42.
11. **Gootz, T. D., C. C. Sanders, and W. E. Sanders, Jr.** 1979. In vitro activity of furazlocillin (Bay k 4999) compared with those of mezlocillin, piperacillin, and standard beta-lactam antibiotics. Antimicrob. Agents Chemother. **15**:783–791.
12. **Grimm, H.** 1982. Bacteriological antagonism between acylureidopenicillins and cephalosporins. J. Antimicrob. Chemother. **9**(Suppl. A):31–34.
13. **Harwood, J., and D. H. Smith.** 1971. Catabolite repression of chloramphenicol acetyl transferase synthesis in *E. coli* K-12. Biochem. Biophys. Res. Commun. **42**:57–62.
14. **Hennessey, T. D.** 1967. Inducible beta-lactamase in *Enterobacter*. J. Gen. Microbiol. **49**:277–285.
15. **Jaurin, B., T. Grundström, T. Edlund, and S. Normark.** 1981. The *E. coli* β-lactamase attenuator mediates growth rate-dependent regulation. Nature (London) **290**:221–225.
16. **Kuck, N. A., R. T. Testa, and M. Forbes.** 1981. In vitro and in vivo antibacterial effects of combinations of beta-lactam antibiotics. Antimicrob. Agents Chemother. **19**:634–638.
17. **Lampe, M. F., B. J. Allan, B. H. Minshew, and J. C. Sherris.** 1982. Mutational enzymatic resistance of *Enterobacter* species to beta-lactam antibiotics. Antimicrob. Agents Chemother. **21**:655–660.
18. **Leive, L.** 1965. A non-specific increase in permeability in *Escherichia coli* produced by EDTA. Proc. Natl. Acad. Sci. U.S.A. **53**:745–750.
19. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
20. **Makman, R. S., and E. W. Sutherland.** 1965. Adenosine 3′,5′-phosphate in *Escherichia coli*. J. Biol. Chem. **240**:1309–1314.
21. **Minami, S., A. Yotsuji, M. Inoue, and S. Mitsuhashi.** 1980. Induction of beta-lactamase by various beta-lactam antibiotics in *Enterobacter cloacae*. Antimicrob. Agents Chemother. **18**:382–385.
22. **National Committee for Clinical Laboratory Standards.** 1982. Performance standards for antimicrobic disc susceptibility tests, vol. 2, no. 2. National Committee for Clinical Laboratory Standards, Villanova, Pa.
23. **Neu, H. C., and P. Labthavikul.** 1982. Comparative in vitro activity of *N*-formimidoyl thienamycin against gram-positive and gram-negative aerobic and anaerobic species and its beta-lactamase stability. Antimicrob. Agents Chemother. **21**:180–187.
24. **Nordström, K., and R. B. Sykes.** 1974. Induction kinetics of β-lactamase biosynthesis in *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **6**:734–740.
25. **Preheim, L. C., R. G. Penn, C. C. Sanders, R. V. Goering, and D. K. Giger.** 1982. Emergence of resistance to β-lactam and aminoglycoside antibiotics during moxalactam therapy of *Pseudomonas aeruginosa* infections. Antimicrob. Agents Chemother. **22**:1037–1041.
26. **Rosselet, A., and W. Zimmermann.** 1973. Mutants of *Pseudomonas aeruginosa* with impaired beta-lactamase inducibility and increased sensitivity to beta-lactam antibiotics. J. Gen. Microbiol. **76**:455–457.
27. **Sanders, C. C., R. C. Moellering, R. R. Martin, Jr., R. L. Perkins, D. G. Strike, T. D. Gootz, and W. E. Sanders, Jr.** 1982. Resistance to cefamandole: a collaborative study of emerging clinical problems. J. Infect. Dis. **145**:118–125.
28. **Sanders, C. C., and W. E. Sanders, Jr.** 1979. Emergence of resistance to cefamandole: possible role of cefoxitin-inducible beta-lactamases. Antimicrob. Agents Chemother. **15**:792–797.
29. **Sanders, C. C., and W. E. Sanders, Jr.** 1982. Sch 29482: comparative activity against Enterobacteriacae multiply resistant to β-lactam antibiotics and mycobacteria. J. Antimicrob. Chemother. **9**(Suppl. C):59–69.
30. **Sanders, C. C., W. E. Sanders, Jr., and R. V. Goering.** 1982. In vitro antagonism of beta-lactam antibiotics by cefoxitin. Antimicrob. Agents Chemother. **21**:968–975.
31. **Sykes, R. B., and M. Matthew.** 1976. The beta-lactamases of gram-negative bacteria and their role in resistance to beta-lactam antibiotics. J. Antimicrob. Chemother. **2**:115–157.
32. **Then, R. L., and P. Angehrn.** 1982. Trapping of nonhydrolyzable cephalosporins by cephalosporinases in *Enterobacter cloacae* and *Pseudomonas* as a possible resistance mechanism. Antimicrob. Agents Chemother. **21**:711–717.
33. **Van Tiel-Menkveld, G. J., A. Rezee, and F. K. De Graaf.** 1979. Production and excretion of cloacin DF13 by *Escherichia coli* harboring plasmid CloDF13. J. Bacteriol. **140**:415–425.
34. **Wallenfels, K.** 1962. Beta-galactosidase assays. Methods Enzymol. **5**:212–213.
35. **Wanner, B. L., R. Kodaira, and F. C. Neidhardt.** 1978. Regulation of *lac* operon expression: reappraisal of the theory of catabolite repression. J. Bacteriol. **136**:947–954.
36. **Waterworth, P. M., and A. M. Emmerson.** 1979. Dissociated resistance among cephalosporins. Antimicrob. Agents Chemother. **15**:497–503.