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Lysis of *Escherichia coli* by β-Lactams Which Bind Penicillin-Binding Proteins 1a and 1b: Inhibition by Heat Shock Proteins

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The heat shock proteins (HSPs) of *Escherichia coli* were artificially induced in cells containing the wild-type *rpoH*$^+$ gene under control of a *tac* promoter. At 30°C, expression of HSPs produced cells that were resistant to lysis by cephaloridine and cefsulodin, antibiotics that bind penicillin-binding proteins (PBPs) 1a and 1b. This resistance could be reversed by the simultaneous addition of mecillinam, a β-lactam that binds PBP 2. However, even in the presence of mecillinam, cells induced to produce HSPs were resistant to lysis by ampicillin, which binds all the major PBPs. Lysis of cells induced to produce HSPs could also be effected by imipenem, a β-lactam known to lyse nongrowing cells. These effects suggest the existence of at least two pathways for β-lactam-dependent lysis, one inhibited by HSPs and one not. HSP-mediated lysis resistance was abolished by a mutation in any one of five heat shock genes (*dnaK*, *dnaJ*, *grpE*, *groES*, or *groEL*). Thus, resistance appeared to depend on the expression of the complete heat shock response rather than on any single HSP. Resistance to lysis was significant in the absence of the RelA protein, implying that resistance could not be explained by activation of the stringent response. Since many environmental stresses promote the expression of HSPs, it is possible that their presence contributes an additional mechanism toward development in bacteria of phenotypic tolerance to β-lactam antibiotics.

Penicillin and other β-lactam derivatives bind covalently to a specific set of bacterial proteins, the penicillin-binding proteins (PBPs), which are involved in synthesizing and shaping the cell wall (reviewed in reference 32). In *Escherichia coli*, PBPs 1a and 1b are important for cell wall elongation, PBP 2 functions to maintain the cell's rod shape, and PBP 3 is required for proper septation (24, 32). Inactivation of PBPs 1a and 1b by penicillin derivatives is especially effective in promoting lysis of *E. coli* (24, 32). The mechanism by which lysis is triggered is not entirely understood but is believed to involve a set of cellular enzymes, the autolysins, which hydrolyze the peptidoglycan (26).

Certain growth conditions can protect sensitive bacteria from lysis by β-lactam antibiotics. For example, slowly growing or nongrowing cells are resistant to most β-lactams (for a review, see reference 28). This reversible phenomenon has been termed phenotypic tolerance, to distinguish it from genetic alterations that produce a permanent resistance to one or more of these agents (28). One way phenotypic tolerance can be induced is by starvation for a required amino acid, which produces the stringent response in *E. coli* (3, 6, 11, 14). The mechanism appears to include rapid synthesis of autolysin-resistant peptidoglycan (6, 30).

We have shown previously that artificial induction of heat shock proteins (HSPs) protects *E. coli* against the lytic, but not the lethal, effects of the E lysis protein of φX174 (33). Since the E protein and penicillin share lysis characteristics, we tested whether HSP production would protect *E. coli* against agents which bind and inactivate PBPs 1a and 1b. Such treatment did protect cells from lysis and suggests that induction of the heat shock response plays a role in phenotypic tolerance to some β-lactam antibiotics.

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MATERIALS AND METHODS

Strains and growth conditions. *E. coli* strains and plasmids are described in Table 1. For lysis tests, *E. coli* strains containing the plasmid pDS1 (*rpoH*$^+$ under *tac* promoter control) were diluted from overnight cultures to give an A_{550} of 0.05 and were incubated at 30°C in Luria broth (19) until the A_{550} reached 0.2. Isopropyl-β-D-thiogalactopyranoside (IPTG) was added to portions of the culture (1 mM final concentration) to induce expression of the σ^{32} RNA polymerase subunit, which is known to induce the expression of the HSPs (1a, 7). The MICs of the antibiotics for all strains were between 5 and 10 μg/ml for cephaloridine and between 25 and 50 μg/ml for cefsulodin. The cefsulodin challenges were performed in over 15 independent experiments; challenge with cephaloridine was made in 9 independent experiments; mecillinam and ampicillin challenges were performed in 5 to 7 independent cases. Tests with imipenem and cephaloridine were performed at least twice.

Genetic manipulations. Plasmids were prepared by the alkaline lysis procedure, and plasmid transformations were performed by the standard $CaCl_2$ technique (23). P1 transductions were done by the method of Miller (19). *E. coli* CSQ recipients of the *relA*::Kan null allele were selected by plating for resistance to kanamycin (50 μg/ml) and by screening candidates for the acquisition of sensitivity to 3-amino-1,2,4,-triazole (Sigma Chemical Co., St. Louis, Mo.). Screening was performed on M9 minimal medium (19) supplemented with adenine and with the 19 essential amino acids, minus histidine, as described by Rudd et al. (22).

Autolysis test. Autolysis of *E. coli* CSQ(pDS1) was artificially induced as described by Leduc et al. (16). A log-phase culture in LB medium was grown at 30°C to an A_{550} of 0.2. HSPs were induced in one-half of the culture by adding IPTG to 1 mM. At various times after induction, 6 ml was removed and the cells were pelleted in an Eppendorf microcentrifuge at 12,000 × *g* for 1 min. All medium was removed, and the pellets were resuspended with agitation into 3 ml of

TABLE 1. Bacteria and plasmids

Strain or plasmid	Genotype or characteristic[a]	Source or reference
E. coli		
JX10	W1485 F⁻ *thi supE lacI*^{q} *lacZ*::Tn*5*	33
CSQ	JX10 *lacI*^{q} *lacZ*$^+$ (P1 transduction from LB104 [2])	This report
JX10K	JX10 *dnaK756 thr*::Tn*10*	33
JX10J	JX10 *dnaJ259 thr*::Tn*10*	33
JX10E	JX10 *grpE280 tyrA*::Tn*10*	33
JX10EL	JX10 *groEL140* Tn*10*-linked	33
JX10ES	JX10 *groES30* Tn*10*-linked	33
CF1651	MG1655 (prototroph) *relA251*::Kan	M. Cashel
KDY104	CSQ *relA251*::Kan (P1 transductant from CF1651)	This report
NQ	*hsr rpsL lacI*^{q} *lacZ*::Tn*5* (*E. coli* B/r strain)	33
CAG2041	*supC*(Ts) Δ(*pro lac*) *galK trp*(Am) F′ *pro*$^+$ *lacI*^{q} *lacZ*::Tn*5*	C. Gross
CQ4	*ara leu lacI*^{q} *lacZ*::Tn*5 purE gal his argG rpsL xyl mtl ilv met thi*	33
Plasmid		
pDS1	*rpoH*$^+$ gene under control of *tac* promoter (pSC101 replicon); Cm^r	H. Bahl, 1[a]
pTJ3	pDS1 with inactivated *rpoH* gene	33
pGroESL	*groES*$^+$ and *groEL*$^+$ under control of λ*plac* promoter (p15A replicon); Cm^r	A. Gatenby, 5
pGroES	*groES*$^+$ under control of λ*plac* promoter (p15A replicon); Cm^r	A. Gatenby, 5
pGroEL	*groEL*$^+$ under control of λ*plac* promoter (p15A replicon); Cm^r	A. Gatenby, 5

[a] Cm, chloramphenicol.

1 mM EDTA in sterile distilled water. After the absorbance was measured at zero time, the tubes were incubated at 37°C without shaking and absorbance was monitored over time.

Expression of lysis rate. The equation for the generation time of a bacterial culture is $t_d = [1/\ln (x_t/x_0)](0.693)(\Delta t)$, where t_d is the doubling time of the culture, x_t and x_0 are the absorbances at times t and 0, respectively, and Δt is the elapsed time in minutes between times t and 0 (12). To express the rate of lysis, we replaced t_d with the term t_h to designate the time required for the absorbance to decrease by one-half its value. We refer to this number as the degeneration time of the culture. Smaller absolute values of t_h indicate a more rapid decrease in absorbance (lysis is faster). Thus, a culture with a t_h of −20 min is lysing twice as rapidly as one with a t_h of −40 min.

RESULTS

Expression of HSPs increases resistance to lysis by cephaloridine and cefsulodin. The plasmid pDS1 carries the wild-type *rpoH* gene under control of the *tac* promoter (1a). Adding IPTG to an *E. coli* strain containing pDS1 induces the production of most HSPs in the absence of a temperature shift (7). Induction of HSPs in this way avoids complicating the results with the additional variable, temperature (33).

Cephaloridine, a β-lactam derivative which binds specifically to PBPs 1a and 1b (27; unpublished observations), was added to *E. coli* CSQ(pDS1) grown at 30°C with or without prior addition of IPTG to induce HSP expression. Rapid lysis began 15 min after exposure to cephaloridine in the absence of IPTG (Fig. 1). Identical lysis also occurred after β-lactam addition to IPTG-induced cells containing the control plasmid, pTJ3, which is pDS1 with an inactive *rpoH* gene (four experiments in the CSQ background, four in the CSH57B background, and one each for NQ and CAG2041; data not shown) (also, see reference 33). Lysis was inhibited when HSPs were expressed by adding IPTG to CSQ(pDS1) 15 min before exposure to cephaloridine. Cells induced to produce HSPs did not lyse at all in the presence of 10 μg of cephaloridine per ml, and the lysis rate was reduced 18-, 9-, and 5-fold at concentrations of 25, 50, and 100 μg/ml,

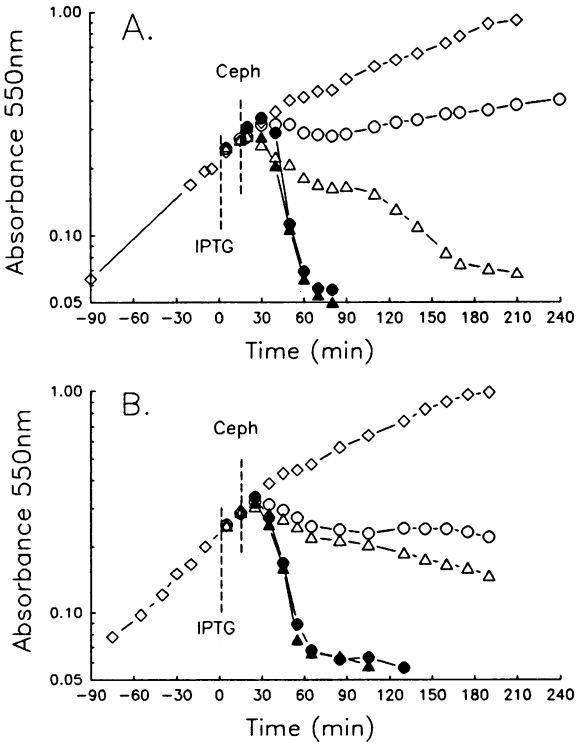

FIG. 1. HSP protection against lysis by cephaloridine. *E. coli* CSQ(pDS1) cells were grown in LB medium at 30°C to an A_{550} of 0.2. At that time (time zero), expression of HSPs was induced in some cultures by adding IPTG to 1 mM final concentration. Fifteen minutes after time zero, the test antibiotic was added to various final concentrations. (A) Symbols: ◇, IPTG only, no antibiotic; ●, cephaloridine (Ceph) only (10 μg/ml); ○, IPTG plus cephaloridine (10 μg/ml); ▲, cephaloridine only (100 μg/ml); △, IPTG plus cephaloridine (100 μg/ml). (B) Symbols: ◇, IPTG only, no antibiotic; ●, cephaloridine only (25 μg/ml); ○, IPTG plus cephaloridine (25 μg/ml); ▲, cephaloridine only (50 μg/ml); △, IPTG plus cephaloridine (50 μg/ml).

TABLE 2. HSP-mediated lysis protection and growth inhibition

Strain[a]	Lysis protection factor[b]	HSP growth inhibition[c]
CSQ (100 μg/ml)	5 (18 min → 94 min)	0.3 (55 min → 140 min)
CSQ (50 μg/ml)	9 (18 min → 158 min)	0.3
CSQ (25 μg/ml)	18 (17 min → 308 min)	0.3
CSQ (10 μg/ml)	No lysis[d]	0.3
NQ	5 (19 min → 95 min)	0.7 (53 min → 75 min)
CAG2041(FK)	3 (37 min → 110 min)	0.7 (53 min → 77 min)
CQ4	2 (34 min → 66 min)	None (53 min → 56 min)

[a] Each strain contained the plasmid pDS1, and each was induced with 1 mM IPTG to express HSPs 15 min before exposure to cephaloridine. The antibiotic concentration was 10 μg/ml except as noted in parentheses. The MIC of cephaloridine for each strain was approximately equal (data not shown). All experiments were performed at 30°C.

[b] The lysis protection factor is the degeneration time of the culture in the presence of HSPs divided by the degeneration time of the culture in the absence of HSPs. Larger numbers reflect greater protection. The figures in parentheses are the degeneration times in the absence of and in the presence of HSPs, respectively.

[c] The generation time of the culture when HSPs were expressed in the absence of antibiotic, expressed as a fraction of the growth rate before induction with IPTG. The figures in parentheses are the generation times before and after HSP induction, respectively.

[d] No lysis occurred; absorbance remained stationary.

respectively (Fig. 1 and Table 2). In all cases, growth was arrested at the time of normal lysis onset (Fig. 1).

We repeated the experiment using cefsulodin, another β-lactam which binds PBPs 1a and 1b (27) but which we found to bind avidly to PBPs 7 and 8 as well (data not shown). Induction of HSPs 15 min before the addition of cefsulodin (100 μg/ml) gave complete protection against lysis. Cells grown at 37°C lysed in the presence of either antibiotic regardless of whether HSPs were expressed. We had previously found this to be true for HSP protection against lysis by the E protein of φX174 (33). At present, we can only speculate that temperature-dependent differences in membrane composition or basal levels of HSP production contribute to the effect. All subsequent experiments were performed at 30°C.

When IPTG was added to *E. coli* CSQ(pDS1) in the absence of cephaloridine, the culture showed a decline in growth rate to about one-half or one-third that of normal, beginning 30 to 40 min after IPTG induction of HSPs (Fig. 1 to 4). This inhibition of growth rate and HSP-mediated resistance to lysis was strain dependent (Table 2). Three

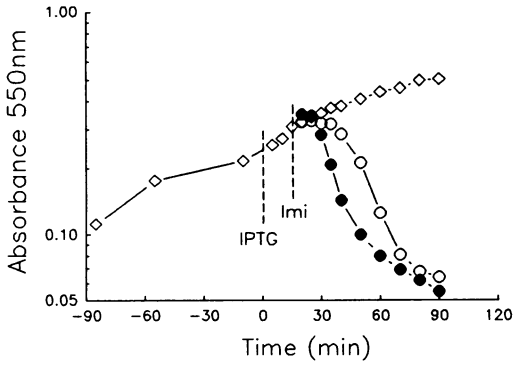

FIG. 2. Absence of HSP protection against lysis by imipenem (Imi). Cells were grown and treated as described in the legend to Fig. 1. Symbols: ◇, IPTG only, no antibiotics; ●, imipenem only (10 μg/ml); ○, IPTG plus imipenem (10 μg/ml).

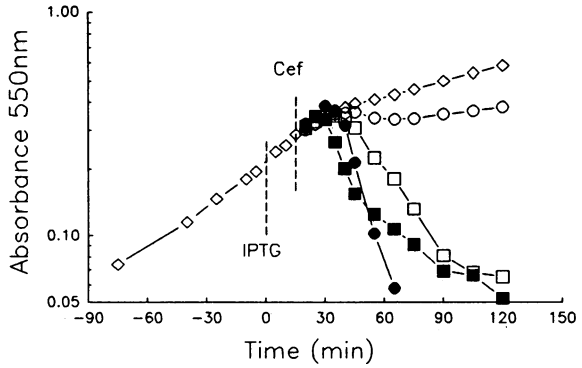

FIG. 3. Reversal by mecillinam of heat shock protection against cefsulodin-induced lysis. Cells were grown and treated as described in the legend to Fig. 1. Cefsulodin (100 μg/ml) (Cef) or the PBP 2-binding antibiotic, mecillinam (10 μg/ml), were added at the indicated times, alone or simultaneously. Symbols: ◇, IPTG only, no antibiotics; ●, cefsulodin only; ○, IPTG plus cefsulodin; ■, cefsulodin and mecillinam; □, IPTG plus cefsulodin and mecillinam.

strains (CAG2041FK, NQ, and CQ4) showed less HSP-dependent growth inhibition in the absence of antibiotic but continued to be measurably protected against lysis by cephaloridine (Table 2). These results illustrate that lysis protection was not due solely to growth reduction. The strain variations themselves could derive from differences in the relative expression of HSPs (which we did not measure) or from mutations in any of several heat shock genes (discussed below).

In *E. coli* CSQ(pDS1), the level of lysis resistance depended on the relative times of cephaloridine addition and HSP induction. Resistance increased with longer HSP expression (Table 3). Complete lysis protection developed if the antibiotic was added as soon as 5 min after HSP induction. The rate of lysis decreased by one-half even when cephaloridine was added 10 min before HSP induction (Table 3). For *E. coli* NQ, the lysis rate was reduced about 30% with prolonged expression of HSPs (Table 3).

HSP expression did not completely inhibit the killing of

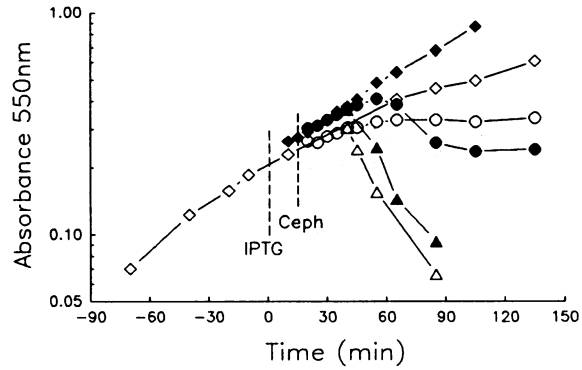

FIG. 4. Effect of *relA*::Kan deletion on HSP protection against cephaloridine-induced lysis. *E. coli* CSQ(pDS1) (*relA*$^+$) and *E. coli* KDY104(pDS1) (CSQ Δ*relA*) were grown and treated as described in the legend to Fig. 1. Symbols: ◆, Δ*relA*, IPTG only, no antibiotic; ◇, *relA*$^+$, IPTG only, no antibiotic; ▲, Δ*relA*, cephaloridine only (10 μg/ml); △, *relA*$^+$, cephaloridine only (10 μg/ml); ●, Δ*relA*, IPTG plus cephaloridine; ○, *relA*$^+$, IPTG plus cephaloridine. The figure represents one of three equivalent experiments.

TABLE 3. Dependence of lysis protection on time of antibiotic addition

Strain	Cephaloridine treatment[a]	Degeneration rate (min)[b]
CSQ	0, no IPTG	28
	−10	48
	0	106
	+5	No lysis
NQ	0, no IPTG	19
	0	95
	15	102
	25	104
	35	126

[a] Time of addition (in minutes) of cephaloridine (10 μg/ml) relative to the time of IPTG induction of HSP expression. IPTG was added at time zero (0) except where noted.

[b] Time required for the absorbance to decrease by one-half its value at the time of lysis onset. Smaller numbers represent a faster rate.

E. coli CSQ(pDS1) after exposure to cefsulodin. However, the rate of killing was noticeably slower, requiring 2 to 3 h to reduce viable cell counts to a level of 1 to 0.1% of the original value (data not shown).

Artificial induction of HSPs does not protect against lysis by all agents. Induction of HSPs might inhibit lysis by suppressing the activity of autolysins or by altering the cell wall to an autolysin-resistant form. In either case, cells in which HSPs are induced would be expected to resist other treatments which promote or require autolysis. To test this, we examined the protective effect of HSPs against three other treatments known to induce autolysis of *E. coli*.

Imipenem is one of several antibiotics which, unlike classical β-lactams, lyse nongrowing cells (31). Induction of HSPs failed to protect cells against lysis by imipenem (Fig. 2), indicating that some autolysins remained functional in the presence of HSPs.

The β-lactam mecillinam binds specifically to PBP 2 (27). Mecillinam-treated cells do not lyse but instead lose their rod shape to grow as enlarged spheres. However, when combined with several other antibiotics, mecillinam lyses cells in a synergistic manner (9, 21). We confirmed that mecillinam did not lyse cells in the presence or absence of HSPs and that sphere formation was unaffected by HSP induction (data not shown). When mecillinam and cefsulodin were added to cells simultaneously, lysis began slightly earlier than with cefsulodin alone (Fig. 3). While HSP induction protected cells against lysis by cefsulodin, simultaneous addition of cefsulodin and mecillinam erased this lysis protection (Fig. 3). In a similar manner, simultaneous addition of cefsulodin and ampicillin decreased the protective effect of HSP expression (data not shown). On the other hand, simultaneous addition of cefsulodin and aztreonam (which binds specifically to PBP 3) did not remove lysis protection (data not shown). These results indicated that autolysins could be activated even during the reduced growth rates after HSP induction and that the pattern of their activation was PBP dependent.

Autolysis of *E. coli* can be induced artifically by resuspending growing cells in 1 mM EDTA (16, 17). *E. coli* CSQ(pDS1) was induced to autolyse with or without prior IPTG induction of HSPs. When HSPs were induced in cells 30 min prior to harvest, the EDTA-provoked lysis rate for the first 15 min was equal to that of cells without HSPs (data not shown). After that time, cells containing HSPs became more resistant to further lysis, whereas cells without HSPs continued to lyse. The final difference, 50 and 60% lysis of cells with and without HSPs, respectively, was small (data not shown).

Binding of PBP 2 is not sufficient to eliminate HSP-dependent protection from lysis. The results with different combinations of antibiotics suggested a simple model in which binding of PBPs 1a, 1b, and 2 by any combination of antibiotics would result in cell lysis regardless of the expression of the HSPs. To test this prediction, we challenged *E. coli* CSQ(pDS1) with ampicillin, which alone can bind all the major PBPs. No lysis occurred in cells previously induced to produce HSPs (data not shown). This contradicted the idea that β-lactam-dependent lysis in the presence of HSPs was simply a matter of binding PBPs 1a, 1b, and 2.

The model was undermined further by the observation that mecillinam, which binds PBP 2 and which eliminated HSP-dependent lysis protection against cephaloridine and cefsulodin, did not eliminate HSP-dependent lysis protection against ampicillin (data not shown). Since ampicillin also binds PBP 2, the possibility existed that no PBP 2 had bound mecillinam. However, the same results were obtained when mecillinam was added simultaneously with ampicillin or when mecillinam was added prior to ampicillin. In no case did mecillinam eliminate HSP-dependent protection from ampicillin as it had done for antibiotics specific for PBPs 1a and 1b.

On the other hand, induction of HSPs gave only slight protection against lysis induced by exposure to penicillin G, which can also bind all the major PBPs (data not shown). These results suggest that the nature or effective concentration of the antibiotic bound to PBPs 1a, 1b, and 2 is important in determining whether or not protection occurs.

Lysis resistance is not specified by any one HSP. To determine which HSPs were responsible for lysis resistance, we tested a set of isogenic strains for their resistance to cephaloridine in the presence or absence of HSP induction. Each of the five strains contained a temperature-sensitive mutation in one of five heat shock genes (*dnaK*, *dnaJ*, *grpE*, *groES*, or *groEL*) (33). Cells were tested at the permissive temperature, 30°C. The parent strain, JX10, exhibited complete lysis resistance in the presence of HSPs, as had *E. coli* CSQ (from which JX10 was derived). However, any of the five heat shock mutations restored complete lysis sensitivity and relieved the growth inhibition observed when cells were induced with IPTG (data not shown).

Since replacement of either *groES* or *groEL* with a temperature-sensitive allele eliminated lysis resistance against cephaloridine, we determined whether production of one or both of these proteins would protect against lysis in the absence of the other HSPs. The wild-type genes for *groES*, *groEL*, or both *groES* and *groEL* have been cloned under control of the *lac* promoter in the plasmids pGroES, pGroEL, and pGroESL, respectively (5). Each plasmid was transformed into CSQ and was tested for resistance to cephaloridine (10 μg/ml) with or without IPTG induction of the respective GroE proteins. Each transformant remained sensitive to lysis regardless of whether the GroE proteins were overexpressed singly or in combination with one another.

A *relA* null mutation does not eliminate lysis protection afforded by HSPs. A *relA*::Kan null mutation (a deletion of the *relA* gene) has been constructed and characterized (18). When HSPs were expressed in cells with this *relA* deletion, the onset of cephaloridine-induced lysis was delayed by 15 to 20 min compared with that in Δ*relA* cells without HSPs. The overall protection against lysis in Δ*relA* cells was significant, although slightly less than that afforded by *relA*$^+$ cells (Fig.

4). This implies that the bulk of HSP-determined lysis resistance was independent of RelA expression. Note also that the Δ*relA* allele eliminated the decrease in growth rate which normally occurred after the addition of IPTG (Fig. 4).

DISCUSSION

Expression of a plasmid-encoded RNA polymerase σ^{32} subunit induces almost all of the HSPs of *E. coli* in the absence of a temperature shift (1a, 7). We found that such cells had complete or increased resistance to the lytic effects of several β-lactams, especially to those which bound PBPs 1a and 1b. The mechanism which generates this resistance may shed light on the normal pathway of bacterial cell wall synthesis and dissolution.

Decreased growth rate does not explain protection against antibiotic-induced lysis. The growth rate of *E. coli* CSQ(pDS1) decreased at about 30 to 40 min after artificial induction of HSPs. Thus, it was possible that the protection against lysis was merely a side effect of this growth inhibition. There are several arguments against this interpretation. First, complete lysis protection developed in cells when cephaloridine was added only 5 min after HSP induction, although the growth rate remained unchanged for 25 to 35 min thereafter. Significant resistance to lysis developed even when the antibiotic was added to cells as much as 10 min before HSP expression. Since it is the growth rate before antibiotic addition which appears to govern the lysis rate (29), the time periods we observed should have been long enough for antibiotic lytic effects to occur.

A second argument is that the generation time increased only 2.3- to 3.0-fold after HSP induction, to approximately 120 min. At this growth rate, antibiotic-induced killing and lysis are still rapid (29). Third, since the rates of killing and lysis are directly proportional to decreases in growth rate (29), the rate of lysis should have decreased by approximately the same factor. Instead, lysis either did not occur at all or its rate was much less than that predicted by growth rate effects alone. Fourth, some *E. coli* strains had only small decreases in growth rate but remained resistant to cephaloridine. Finally, a Δ*relA* strain showed no inhibition of growth after HSP induction but did exhibit significant resistance to lysis. We conclude that factors other than growth rate contributed to lysis resistance when HSPs were induced.

Role of RelA protein in protection against lysis. The RelA protein mediates the stringent response, a global reduction in macromolecular synthesis particularly important during amino acid starvation (for a review, see reference 3). One of the effects of this response is that within 5 min of starvation, wild-type cells become resistant to lysis by cephaloridine (and other penicillin derivatives), but *relA* cells remain sensitive for up to 30 min (6, 28). This RelA-dependent lysis protection is believed to be due to a combination of a decrease in peptidoglycan synthesis (10, 13) and the production of a modified, autolysis-resistant peptidoglycan (6, 30).

When HSPs were induced in a *relA* deletion mutant of *E. coli*, lysis protection was roughly equivalent, although not equal, to that observed in a *relA*$^+$ isogenic strain (Fig. 4). The heat shock response is reported to be normal in *relA* mutants of *E. coli* (cited in reference 8). Thus, the bulk of HSP-mediated protection against cephaloridine probably results from a mechanism other than simple induction of the stringent response.

Role of HSPs in lysis protection. Replacement of any one of five heat shock genes with a temperature-sensitive allele eliminated resistance to β-lactam lysis at 30°C, the permissive temperature. In addition, overproduction of GroES and/or GroEL was insufficient to produce lysis resistance to the antibiotics. Thus, each of the tested HSPs was necessary but not sufficient for the development of lysis resistance. That lysis resistance depended on expression of several HSPs suggests that they have to act as a unit to produce the resistant phenotype or that a global (secondary) effect of the complete heat shock response is necessary. An example of such a secondary effect is the deficiency in proteolysis exhibited by some heat shock mutants. But, as discussed in relation to HSP protection against lysis by the E protein (33), the reported deficiencies do not parallel the development of lysis resistance. For example, although a *groES* mutant retains full proteolytic activity toward several substrates (25), it nevertheless became sensitive to lysis by the derivatives we tested here.

We reported previously that artificial expression of HSPs produced resistance to lysis by the E protein of φX174 (33). The present report constitutes additional evidence that lysis by E protein and by β-lactams proceeds via comparable mechanisms. However, differences do exist. For example, resistance to E protein is eliminated by mutants of only three of the five tested heat shock alleles (*dnaK*, *dnaJ*, and *groES*) (33), and overproduction of the GroES protein, but not GroEL, is itself sufficient to produce lysis resistance to E protein (1).

Mechanism of lysis by β-lactams. In the two-step model of lysis proposed by Tomasz (26), a β-lactam antibiotic first binds to and inactivates one or more PBPs, after which autolysins are activated to produce lysis. In principle, one should be able to discover conditions in which only the second (lysis) step is inhibited. HSP-mediated lysis resistance is consistent with this possibility: growth of bacteria is halted but lysis does not occur. This is reminiscent of but evidently separate from the RelA-dependent stringent response.

A scheme of multiple pathways leading to lysis has been proposed to explain the lethal activity of imipenem toward nongrowing cells (4). Consistent with the existence of alternate lysis pathways are the results that imipenem or certain antibiotic combinations lysed HSP-induced cells, while other antibiotics did not. PBP 2 may be a potential branch point since its inactivation triggered a lysis pathway not inhibited by HSPs.

Mechanism of lysis protection. No reduction in labeling of PBPs was observed when HSPs were induced (21a), and PBP binding by cefsulodin or cephaloridine had no effect on cell growth until lysis would otherwise have begun, at which time growth stopped. Therefore, the inhibitory mechanism did not interfere with the first step (PBP binding) or with the timing of lysis. Instead, lysis completion was inhibited.

Resistance to lysis could be mediated by inhibiting the activation of relevant autolysins, by inhibiting autolysin activity, or by modifying the substrate (peptidoglycan) to an autolysin-resistant form. Inhibition of the enzymatic activity of autolysins would be consistent with the data presented here and by Kusser et al. (15), but additional work is required to distinguish between these predicted biochemical changes.

Summary. It is clear that expression of HSPs augments lysis resistance toward certain antibiotics. The effect exhibits specificity for particular β-lactams, appears to require the activity of multiple HSPs, and cannot be explained by a simple decrease in growth rate or by induction of the stringent response. Since the heat shock response can be

induced by a variety of stresses (20), it is possible that such expression could reinforce phenotypic tolerance to antibiotics in vitro and perhaps in vivo.

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