

# Effects of Temperature and of Heat Shock on the Expression and Action of the Colicin A Lysis Protein

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**At low temperature, the synthesis of the colicin A lysis protein in *Escherichia coli* was slowed down, and consequently its functioning was retarded. The rates were restored when the bacteria were shifted for 10 min to 42°C, except in an *rpoH* mutant, suggesting that one or more proteins regulated by  $\sigma^{32}$  is necessary for expression of colicin A lysis protein.**

The colicin A lysis protein, called Cal, is a lipopeptide responsible for the export of colicin A by *Escherichia coli* carrying plasmid pColA. Its synthesis is performed in various steps, like that of all bacterial lipoproteins (11). However, the rate of its synthesis is particularly slow, allowing the detection of all intermediate forms at any given time in the producing cells (6). A similar slow rate of synthesis has been observed for another lysis protein, the cloacin DF13 release protein (16). This rate of synthesis is unique among exported proteins, and its cause is unknown. The simultaneous presence of precursor and mature forms of exported proteins is usually obtained by drastic means such as gene mutations or use of drugs, which affect significantly the life of the cell. It provokes the induction of the heat shock response (20). Previous experiments have suggested that Cal mimics the consequences of growth at elevated temperatures and elicits the same responses (7). The fact that the synthesis of Cal occurs similarly at 42°C as at 37°C (3) might indicate that Cal induces the heat shock regulon at the normal temperature. To verify this, the functioning and synthesis of Cal were successively studied at both low and high temperatures. Surprisingly, they were modified at low but not at high temperature.

**Quasi-lysis is delayed at low temperature.** Quasi-lysis corresponds to a gentle decline in the turbidity of the culture occurring 1 to 2 h after induction of every colicin lysis protein (reviewed in reference 8). The effect of temperature was checked on quasi-lysis provoked by Cal on cells carrying either plasmid pColA9, which contains the wild-type colicin A operon (14), or plasmid pCK4, which contains the *cal* gene under the *tac* promoter (6) (Fig. 1). W3110/pColA9 cells grown at 30°C were induced for colicin A and Cal synthesis and incubated at either 30, 37, or 42°C. At each temperature, the turbidity first increased with time along with the control cells and decreased some time afterward. Both the timing and the rate of the decline in the culture turbidity differed according to the temperature of incubation. The decrease began after 1 h of induction at either 42 or 37°C but only after 3 h at 30°C. The rate of decline in the culture turbidity was fast at 42°C, a little less rapid at 37°C, and particularly slow at 30°C. It seemed that Cal did not stop the growth of the cells at 30°C as efficiently as it did at either 37 or 42°C. Similar, although less significant, results were obtained for induced W3110/pCK4 cells.

The experiments were repeated with cells with a missense mutation in the *pldA* gene, which encodes the phospholipase A of the outer membrane (2), as quasi-lysis provoked by either colicin lysis protein is not obtained in this mutant (17). In W3110 *pldA* cells (5) carrying pColA9, the turbidity of the culture reached a plateau at 37°C, declined gently at 42°C, but was like that of the uninduced cells at 30°C. In *pldA* cells carrying pCK4, the effects of temperature were less significant, as the turbidity of the cells reached a plateau at 30°C but much later than at 37°C (Fig. 1). Thus, quasi-lysis provoked by Cal in the *pldA* mutant was affected by temperature as in the wild type, although in a more discrete way, indicating no role of the phospholipase A in that process.

**Release of proteins is slowed down at low temperature.** The release of proteins promoted by Cal was checked by centrifugation of the culture samples after 4 h of induction and analysis by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis of the cell pellets and the supernatants (Fig. 2). Colicin A released with various proteins by cells carrying pColA9 was assayed in order to quantify the release. Its amount was smaller in the supernatant of cells incubated at 30°C than in the supernatant of cells incubated at high temperature. In contrast, the amount of colicin A present in the cell pellets incubated at 30°C was about twice the amount found in cells at 37°C and three times the amount in cells at 42°C. Thus, at 30°C, the release of proteins was incomplete in 4 h and cell growth occurred before or during production of colicin A. The amounts of the cellular proteins measured by densitometry were higher at 30°C than at other temperatures. The release of proteins by induced cells carrying pCK4 was not as easy to measure, as no specific protein is released. Densitometry of the major protein EF-Tu (not shown) indicated that its amounts were quite similar in the supernatants of cells induced at each temperature. Its amount in the cell pellet, like the amounts of other proteins, was higher than that found in the pellets of cells incubated at either 37 or 42°C, indicating that the cellular content had increased at 30°C.

**Killing of cells is reduced at low temperature.** At 30°C, the induction of Cal seemed to neither shut off protein synthesis nor stop the growth of cells as it did at either 37 or 42°C. A measure of colony-forming ability indicated that 33% of W3110/pColA9 cells present at time zero were alive after 4 h of induction at 30°C, whereas 0.002% survived after 3 h of induction at 42°C. In induced cells carrying pCK4, killing was more significant, as 0.6% were surviving at 30°C and 0.006% were surviving at 42°C.

A critical concentration of Cal is thought to be needed for

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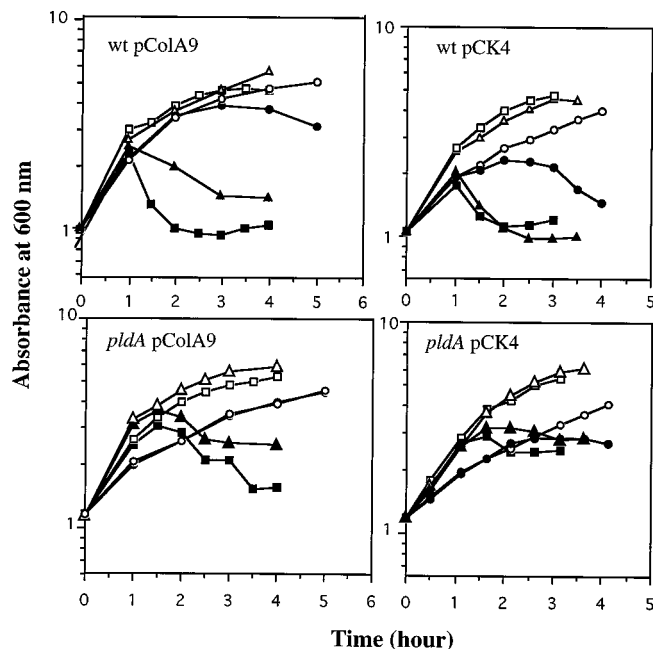


FIG. 1. Effect of temperature on quasi-lysis provoked by Cal. W3110 (wild-type [wt]) cells grown in LB at 30°C were induced (closed symbols) with mitomycin C (300 ng/ml) for cells carrying pColA9 and with IPTG (1 mM) for cells carrying pCK4 or left uninduced (open symbols) and incubated at either 30°C (○, ●), or 37°C (△, ▲), or 42°C (□, ■). The  $A_{600}$  of the cultures was measured as a function of time. The same experiments were performed with W3110 *pldA* cells.

Cal functioning (6). The amount of Cal necessary for killing may not be reached in time at low temperature to prevent cell growth. The cellular content of Cal may be diluting in the multiplying cells in such a way that the critical concentration of Cal was slowly built up and the protein completed its functions much later. It would be reached earlier in cells carrying pCK4 than in cells carrying pColA9, as these cells overproduce Cal (6). Alternatively, a signal needed for the synthesis of Cal may be either missing, inactive, or present in insufficient amounts at 30°C at the time of induction but not later.

**The level of the various forms of Cal varies as a function of temperature.** The amount of Cal synthesized was checked by probing a Western blot (immunoblot) with rabbit polyclonal antibodies (12) (Fig. 3). In the induced W3110 cells carrying

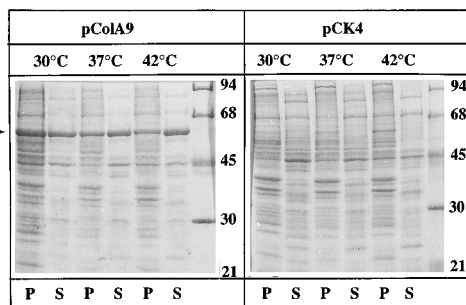


FIG. 2. Influence of temperature on the release of proteins promoted by Cal. W3110 cells carrying either pColA9 or pCK4 grown in LB at 30°C were induced for Cal synthesis and incubated at various temperatures as indicated. After 4 h of induction, samples were centrifuged, and pellets (P) and supernatants (S) were analyzed on SDS-gels. The gels stained with Coomassie blue are presented. The position of the colicin A is indicated by an arrow. Numbers at the right represent the molecular masses in kilodaltons of the standard proteins.

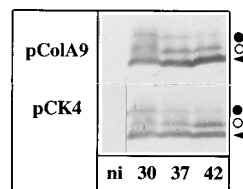


FIG. 3. Effect of temperature on the synthesis of the various forms of Cal. W3110 cells carrying either pColA9 or pCK4 grown in LB at 30°C were either noninduced (ni) or induced for Cal synthesis and incubated at various temperatures as indicated. After 4 h of induction, samples (15  $\mu$ l) were taken and analyzed on a urea-SDS-gel. The Western blot of the bottom of the gel is presented. The various forms of Cal are indicated: precursor form (○), modified precursor form (●), and mature form (◄).

either pColA9 or pCK4, the three forms of Cal were detected at all temperatures: the unmodified precursor form, pCal, the modified precursor form, pCal<sup>m</sup>, and the mature form, Cal. The signal peptide of Cal which is stable was not detected, as the serum used did not contain antibodies against it. The proportions of each form of Cal varied with the temperature. Mainly, the level of pCal was weak at 30°C and increased with the temperature, being significant at 42°C. The mature form of Cal was the most abundant form, but its level was lower at 30°C than at 37 or 42°C.

**A heat shock increases quasi-lysis.** When cells are shifted from low to high temperature, a set of heat shock proteins is induced (13, 21). To test whether these proteins influence the synthesis and functioning of Cal, a shift-up in temperature was performed during Cal induction at 30°C. Quasi-lysis was obtained earlier and was more pronounced on cells shifted during 10 min to 42°C than on nonshifted cells (Fig. 4). The earlier the shift in temperature, the greater the effect on quasi-lysis obtained. Its effect was less significant on cells carrying pCK4 than on cells carrying pColA9. Almost similar results were obtained after a shift-up to 37°C (not shown).

The experiment was performed on cells mutated in the *rpoH* gene, encoding the RNA polymerase sigma factor  $\sigma^{32}$ , which specifically recognizes promoters of the two-thirds of the heat shock genes (10). A shift-up in the temperature did not provoke any change in the timing of quasi-lysis on *E. coli* 1320 *rpoH* cells (19) carrying either pColA9 or pCK4 (Fig. 4). Thus, one or more proteins among the set of proteins under  $\sigma^{32}$  control plays a role in either Cal expression or action.

**A heat shock increases the synthesis of Cal.** To determine whether the observed change in quasi-lysis following a heat shock was due to an altered rate of the synthesis of Cal, pulse-chase experiments with [<sup>35</sup>S]methionine were performed as described previously (3) (Fig. 5). The effect of temperature on the kinetics of Cal synthesis was drastic in cells carrying pColA9. At 42°C, the synthesis was as reported previously (3): pCal was the major protein labeled in 1 min and was chased into pCal<sup>m</sup>; next, pCal<sup>m</sup> was chased into Cal and the signal peptide, which both accumulated after 30 min of chase. At 30°C, pCal was labeled in 1 min and slowly chased; both pCal<sup>m</sup> and Cal were slightly detected. A heat shock provoked a significant increase of both the rate and the level of Cal synthesis. In cells carrying pCK4, every intermediate of the synthesis of Cal was detected at each temperature (the signal peptide is not shown on Fig. 5). Labeling of cells was stronger and chase of pCal was faster at 42°C than at 30°C. At 30°C, a heat shock provoked an increase of the labeling of Cal. In cells carrying either plasmid, the later the heat shock was performed, the less significant was the increase in the level of Cal synthesis (not shown).

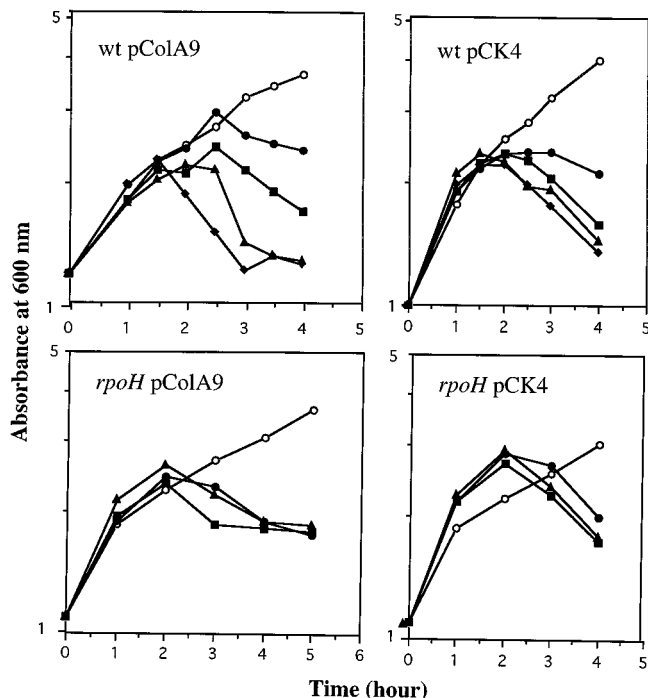


FIG. 4. Effect of a shift-up of temperature on quasi-lysis promoted by Cal. W3110 (wild-type [wt]) cells carrying either pColA9 or pCK4 grown in LB at 30°C were induced (closed symbols) for Cal synthesis or left uninduced (open symbols) and incubated at 30°C. At various times of induction, samples were incubated at 42°C for 10 min. The same experiment was performed with 1320 *rpoH* cells. Absorbance of the cultures was measured as a function of time for cells incubated at 30°C without a shift (○, ●) and with a shift at 0 min (◆), at 30 min (▲), and at 60 min (■). Absorbance of the shifted control cells is not indicated for the sake of clarity but does not differ greatly from that of unshifted cells.

The kinetics of Cal synthesis at 30°C differed according to the plasmid encoding Cal, in contrast to what is observed at either 37°C (6) or 42°C (3). They were changed greatly in cells carrying pColA9 but not so much in cells carrying pCK4, suggesting a role of colicin A in the expression of Cal despite the fact that colicin A is not required for Cal functioning (1).

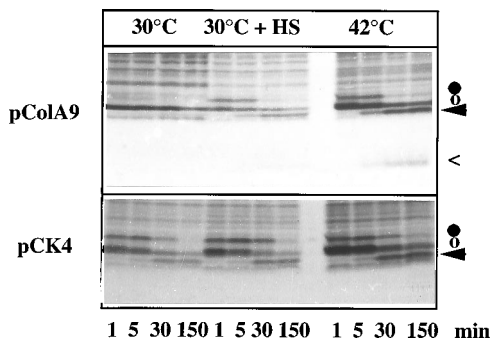


FIG. 5. Effect of a heat shock on the synthesis of Cal. W3110 cells carrying either pColA9 or pCK4 grown at 30°C in M9 medium with lactate as a carbon source were incubated at either 30 or 42°C after induction for Cal synthesis. At time zero of induction, a sample of cells was incubated at 42°C for 10 min and returned to 30°C (30°C + HS). Labeling was done with [<sup>35</sup>S]methionine for 1 min. After various times of chase as indicated, samples were taken and analyzed on a urea-SDS-gel. The relevant part of the fluorogram is shown. The various forms of Cal are indicated: precursor form (○), modified precursor form (●), mature form (◄), and signal sequence (<).

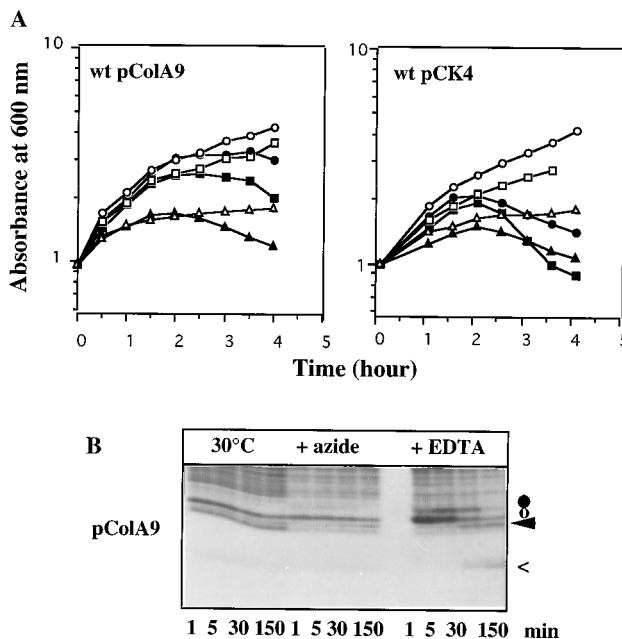


FIG. 6. Effect of azide and EDTA on quasi-lysis and synthesis of Cal at low temperature. (A) W3110 (wild-type [wt]) cells carrying either pColA9 or pCK4 grown in LB at 30°C were induced (closed symbols) for Cal synthesis or left uninduced (open symbols) and incubated at 30°C in the presence or absence of either EDTA (3 mM) or sodium azide (3 mM). Absorbance of the cultures was measured as a function of time for cells incubated at 30°C without treatment (○, ●) and treated with EDTA (□, ■) and sodium azide (△, ▲). (B) W3110/pColA9 cells grown in M9 medium at 30°C were incubated at 30°C after induction by mitomycin C in the presence or absence of either sodium azide or EDTA. Labeling was done with [<sup>35</sup>S]methionine for 1 min. After various times of chase as indicated, samples were taken and analyzed on urea-SDS-gel. The relevant part of the fluorogram is shown. The various forms of Cal are indicated: precursor form (○), modified precursor form (●), mature form (◄), and signal sequence (<).

Furthermore, a delay, increased at low temperature and decreased by a heat shock, may be involved in the expression of the colicin A operon. A lag in the expression of the colicin E1 operon has been shown to be affected by growth conditions (9, 18).

**EDTA and azide increase Cal synthesis and functioning at low temperature.** The effects of low temperature on Cal functioning were somewhat similar as those of divalent cations: in the presence of divalent cations, Cal provokes no quasi-lysis, reduced killing, and slow release of proteins; EDTA has the opposite effect (5). Both agents were tested on cultures induced at 30°C. In the presence of MgSO<sub>4</sub> (20 mM), the turbidity of induced cells was identical to that of control cells (not shown); in the presence of EDTA (3 mM), it decreased earlier and more significantly than the turbidity of untreated cells (Fig. 6A).

Sodium azide was tested, as it is a specific and potent inhibitor of the SecA translocase (19) from which Cal has been shown to be independent (3). Azide added to induced cells at 30°C increased quasi-lysis (Fig. 6A).

The rate of Cal synthesis at 30°C in the presence of either EDTA or azide was measured by pulse-chase labeling on cells carrying pColA9. Both the level and the rate of Cal synthesis increased in the presence of azide and, more significantly, in the presence of EDTA compared with untreated cells (Fig. 6B).

Thus, the retarded functioning of Cal observed at low temperature was caused by a slowdown of its synthesis. This re-

duced synthesis may be due to either an altered stability of one form of Cal or a stable association at 30°C of pCal with a protein. A stable interaction with a chaperone has been suggested for the precursor of truncated derivatives of Cal (4). A heat shock would either inactivate the protease(s) or dissociate the chaperone interaction, as would treatment with either EDTA or azide.

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#### REFERENCES

1. **Baty, D., R. Llobès, V. Geli, C. Lazdunski, and S. P. Howard.** 1987. Extracellular release of colicin A is non-specific. *EMBO J.* **6**:2463–2468.
2. **Brock, R. G. P. M., E. Brinkman, R. van Boxel, A. C. A. P. A. Bekkers, H. M. Verheij, and J. Tommassen.** 1994. Molecular characterization of enterobacterial *pldA* genes encoding outer membrane phospholipase A. *J. Bacteriol.* **176**:861–870.
3. **Cavard, D.** 1992. Colicin A and colicin E1 lysis proteins differ in their dependence on *secA* and *secY* gene products. *FEBS Lett.* **298**:84–88.
4. **Cavard, D.** 1994. Truncated mutants of the colicin A lysis protein are acylated and processed when overproduced. *FEMS Microbiol. Lett.* **117**:169–174.
5. **Cavard, D., S. P. Howard, and C. Lazdunski.** 1989. Functioning of the colicin A lysis protein is affected by Triton X-100, divalent cations and EDTA. *J. Gen. Microbiol.* **135**:1715–1726.
6. **Cavard, D., S. P. Howard, R. Llobès, and C. Lazdunski.** 1989. High-level expression of the colicin A lysis protein. *Mol. Gen. Genet.* **217**:511–519.
7. **Cavard, D., C. Lazdunski, and S. P. Howard.** 1989. The acylated precursor form of the colicin A lysis protein is a natural substrate of the DegP protease. *J. Bacteriol.* **171**:6316–6322.
8. **Cavard, D., and B. Oudega.** 1992. General introduction to the secretion of bacteriocins, p. 297–305. *In* R. James, C. Lazdunski, and F. Pattus (ed.), *Bacteriocins, microcins and lantibiotics*. NATO ASI Series, vol. 65. Springer-Verlag, Heidelberg.
9. **Eraso, J. M., and G. M. Weinstock.** 1992. Anaerobic control of colicin E1 production. *J. Bacteriol.* **174**:5101–5109.
10. **Grossman, A. D., J. W. Erickson, and C. A. Gross.** 1984. The *htpR* gene product of *E. coli* is a sigma factor for heat-shock promoters. *Cell* **38**:383–390.
11. **Hayashi, S., and H. C. Wu.** 1990. Lipoproteins in bacteria. *J. Bioenerg. Biomembr.* **22**:451–471.
12. **Howard, S. P., and L. Lindsay.** 1992. Structure/function relationships in the signal sequence of the colicin A lysis protein, p. 317–329. *In* R. James, C. Lazdunski, and F. Pattus (ed.), *Bacteriocins, microcins and lantibiotics*. NATO ASI Series, vol. 65. Springer-Verlag, Heidelberg.
13. **Lemaux, P. G., S. L. Herendeen, P. L. Bloch, and F. C. Neidhardt.** 1978. Transient rates of synthesis of individual polypeptides in *E. coli* following temperature shifts. *Cell* **13**:427–434.
14. **Llobès, R., D. Baty, and C. Lazdunski.** 1986. The promoters of the genes for colicin production, release and immunity in the ColA plasmid: effects of convergent transcription and LexA protein. *Nucleic Acids Res.* **14**:2621–2636.
15. **Oliver, D. B., R. J. Cabelli, K. M. Dolan, and G. P. Jarosik.** 1990. Azide-resistant mutants of *Escherichia coli* alter the SecA protein, an azide-sensitive component of the protein export machinery. *Proc. Natl. Acad. Sci. USA* **87**:8227–8231.
16. **Oudega, B., O. Mol, P. van Ulsen, F. Stegehuis, F. J. van der Wal, and J. Luirink.** 1993. *Escherichia coli* SecB, SecA, and SecY proteins are required for expression and membrane insertion of the bacteriocin release protein, a small lipoprotein. *J. Bacteriol.* **175**:1543–1547.
17. **Pugsley, A. P., and M. Schwartz.** 1984. Colicin E2 release: lysis, leakage or secretion? Possible role of a phospholipase. *EMBO J.* **3**:2393–2397.
18. **Salles, B., J. M. Weisemann, and G. M. Weinstock.** 1987. Temporal control of colicin E1 induction. *J. Bacteriol.* **169**:5028–5034.
19. **Tobe, T., K. Ito, and T. Yura.** 1984. Isolation and physical mapping of temperature-sensitive mutants defective in heat-shock induction of proteins in *Escherichia coli*. *Mol. Gen. Genet.* **195**:10–16.
20. **Wild, J., W. A. Walter, C. A. Gross, and E. Altman.** 1993. Accumulation of secretory protein precursors in *Escherichia coli* induces the heat shock response. *J. Bacteriol.* **175**:3992–3997.
21. **Yamamori, T., and T. Yura.** 1978. Transient regulation of protein synthesis in *Escherichia coli* upon shift-up of growth temperature. *J. Bacteriol.* **134**:1133–1140.