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Heat Shock Proteins in Bacilli

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Five strains of bacilli, including a nonsporulating strain, when heat shocked, accelerated the synthesis of a specific subset of proteins. The major heat shock protein in all bacilli had a molecular weight of 66,000. The response persisted for at least 40 min and could be eliminated upon a shift down to 37°C.

The induction of a specific subset of proteins (heat shock proteins) after heat or stress shock has been extensively described in both eucaryotes and procaryotes (1, 3, 8, 13, 18, 20). The procaryotic heat shock systems are of particular interest since mutants affecting various aspects of the response can be generated. In *Escherichia coli*, the synthesis of a set of 17 proteins is accelerated after heat shock (14, 15). The synthesis of these proteins is controlled by the *htpR* gene (13), which codes for a sigma factor (5, 7) and is modulated by *rpoD* (15,16), and by the *dnaK* gene, which codes for the major heat shock protein (molecular weight, 66,000 [66K]) and limits the response (2, 19).

The bacilli represent an excellent class of microorganisms to examine for a heat shock response. This genus is gram positive, can exist over a wide range of optimal growth temperatures, features sporulation, a unique morphogenetic event, and contains strains of medical and industrial importance, and in the case of several species, especially *Bacillus subtilis*, there are well-developed genetic systems to study the control and expression of the response. Wachlin and Hecker have described the induction of several proteins after the shift of vegetative *B. subtilis* cells from 30 to 44°C (21). In this report we provide the initial characterization of the heat shock response in bacilli.

We grew *B. subtilis* YB886, (*trpC2 metB10* SPβ^s), *B. subtilis* SR22 (*trpC2 spo0A12*), *B. anthracis* 141851, *B. megaterium* 12114, and *B. cereus* 8534 at 37°C in 20 ml of minimal growth medium (18) or in antibiotic assay medium no. 3 (BBL Microbiology Systems, Cockeysville, Md.) to a cell concentration of 10^8 cells per ml. All bacilli were then washed and suspended in fresh minimal medium without methionine or in fresh antibiotic assay broth and incubated for 5 min at 37°C. At this time, one sample was pulse-labeled (controls) while the other samples were incubated at a heat shock temperature (48°C) for 10 min and then pulse-labeled (Fig. 1). It is apparent that all bacilli tested produced a heat shock response. It is interesting to note that the major heat shock protein in all of these strains was 66K. The 66K protein correlates in size closely to other major heat shock proteins in *E. coli* and *Drosphila* spp. (2, 19).

The heat shock response in *B. subtilis* featured at least 12 proteins (14K to 97K), not all of which appeared immediately and which persisted for at least 40 min (Fig. 2). The *E. coli* response, once induced within the physiological range, diminished after 15 min and was maintained at a new, lower steady-state level (9, 22). This was apparently also the case in *B. subtilis* when tested at 48°C, a temperature at which this organism can grow. The 66K protein was maximally synthesized at 5 and 10 min after heat shock. At later times (20 and 40 min), the level of synthesis of this protein was noticeably lower. Other experiments (not shown) revealed that the 66K protein level seen at 20 min was maintained for at least 120 min. It is also interesting to note that certain proteins appeared to be induced at different times during the heat shock regimen. The reason for this differential expression is not obvious at this time but has been noted in other systems (17). Densitometer tracings of several gels (not shown) demonstrated that the 66K protein comprises maximally 30% of all the cell protein produced 10 min after heat shock and 67% of the heat shock-related proteins. We are presently examining the maintenance of the heat shock response at 52°C, a nonphysiological temperature for *B. subtilis*, to determine whether the heat shock proteins persist at this temperature, as they do in *E. coli* at nonphysiological temperatures (9, 15).

To determine whether the heat shock response can be abrogated, we heat shocked *B. subtilis* SR22 at 48°C for 10 minutes, pulse-labeled at that time, and then returned the cell cultures to 37°C and examined for heat shock proteins after 10, 20, and 40 min. Within 10 min, the cells resumed normal protein synthesis and the heat shock response, as judged by the 66K protein, was diminished and rapidly decreasing. It should be noted, however, that some proteins (i.e., 97K) seen during heat shock but not in controls persisted through the temperature shift-down. Similar results were obtained when Chinese hamster ovary cells were released from oxygen deprivation (17). There may well be several different functional classes of proteins represented among the various types we have observed during initial heat shock, during maintenance of heat shock, and after down-shift of temperature.

Another important aspect to consider is whether a mutant which is unable to sporulate (strain SR22, blocked at stage 0) produces a heat shock response. When compared with strain YB866 (Fig. 2), the sporulation-deficient mutant demonstrated a heat shock response and apparently produced most of the proteins which are induced in sporulation-proficient strains (Fig. 1, lanes A and B; Fig. 3).

Of course, it would be very interesting to determine whether any of the heat shock proteins correspond to a sigma factor in the *B. subtilis* system (4, 10, 11, 16) or if the controlling gene for this heat shock response is a sigma factor (5, 7). Isolation and studies of mutants in the control of the heat shock response have been initiated in our laboratory.

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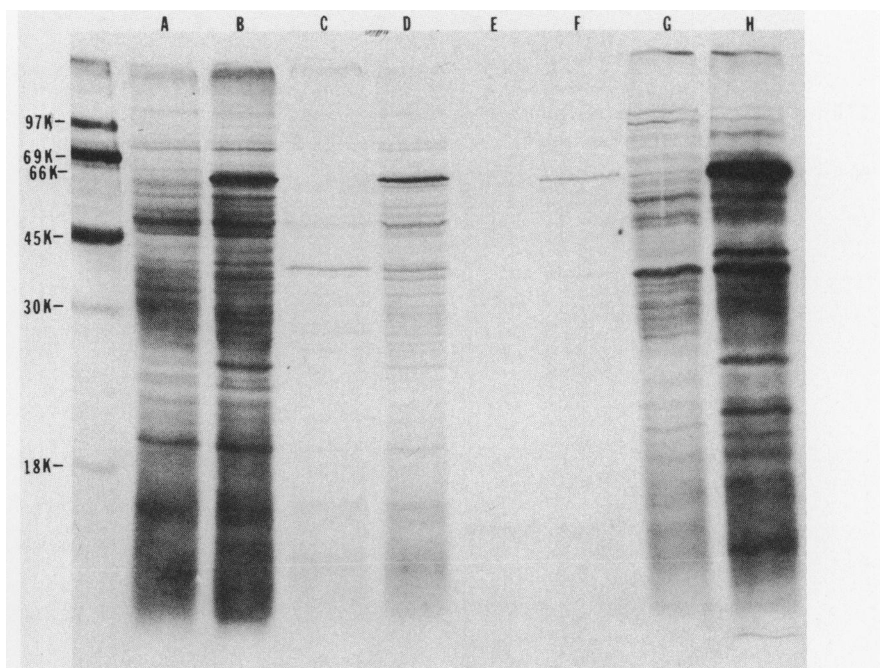

FIG. 1. Heat shock proteins in bacilli. Strains of bacilli, *B. subtilis* SR22 (lanes A and B), *B. megaterium* 12114 (lanes C and D), *B. anthracis* 141851 (lanes E and F), and *B. cereus* 8534 (lanes G and H), were grown in Spizizen (18) minimal medium with 0.5% glucose, supplemented with required amino acids (*B. subtilis*) or antibiotic assay broth (other bacilli) to 60 Klett units at 37°C, washed, suspended in 2 ml of fresh medium with no methionine, and incubated at 37°C for 5 min with 48 μl of 0.01-μg/ml unlabeled L-methionine. One sample (1 ml) of each bacillus was pulse-labeled with 2 μl of L-[^{35}S]methionine (1,002 Ci/mmol; 1.0 Ci/0.11 μl; New England Nuclear Corp., Boston, Mass.) at 37°C for 3 min, the label was chased with 10 μl of 2-mg/ml unlabeled L-methionine, and the sample was cooled rapidly. These samples constituted the controls and are represented in lanes A, C, E, and G. Another sample (1.0 ml) was shifted to 48°C for 10 min and then pulse-labeled and chased as described above. These test samples are represented in lanes B, D, F, and H. All samples were washed and suspended in 75 μl of lysozyme buffer containing 50 μg of lysozyme and 10 μg of DNase per ml. Next, 40 μl of Laemmli sample buffer was added (6), and the samples were boiled for 3 min. A part of each control and test sample was assayed for protein content (12) and radioactivity. The same amount of radioactivity was applied to the gel for control and test samples. The remaining samples were applied to 12% polyacrylamide gels in a sodium dodecyl sulfate running buffer (6), and the gels were dried and exposed to X-ray film.

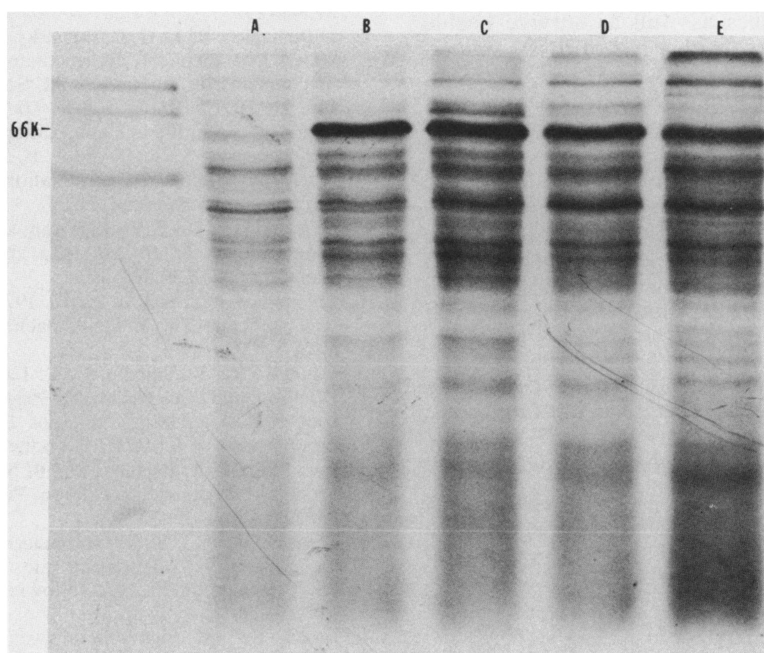

FIG. 2. Maintenance of heat shock. *B. subtilis* YB886 was grown at 37°C in minimal medium, and then the cells were shifted to a heat shock temperature of 48°C and pulse-labeled at 5 min (lane B), 10 min (lane C), 20 min (lane D), or 40 min (lane E). The control (lane A) was pulse-labeled at 37°C. All samples were lysed and assayed. The labeling and lysing procedures are described in more detail in the legend to Fig. 1.

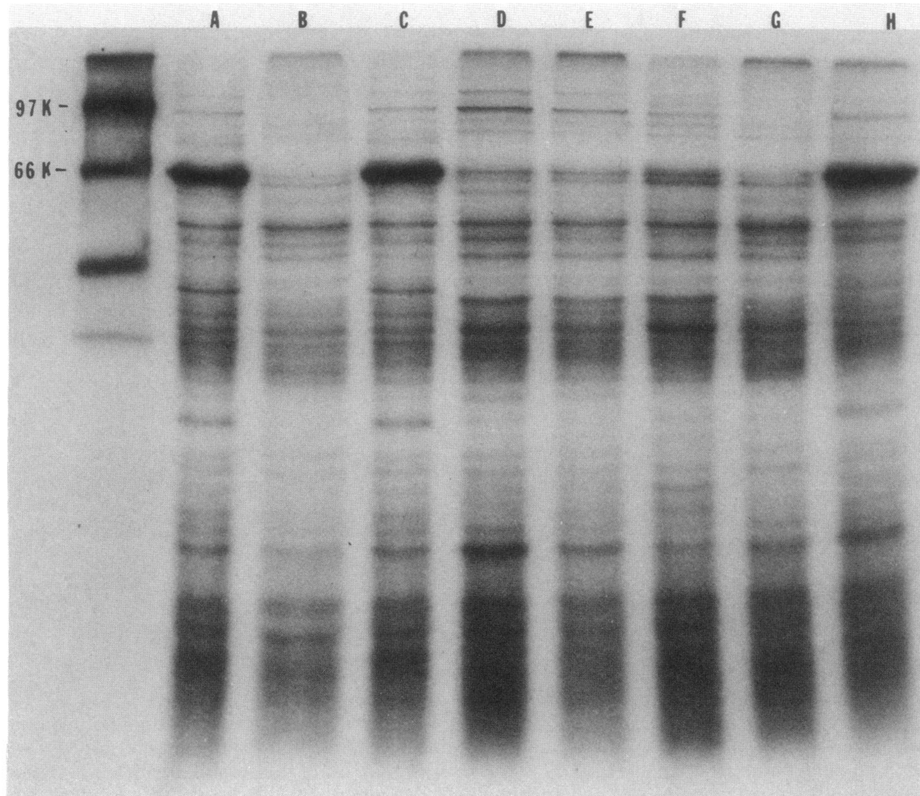

FIG. 3. Reversal of the heat shock response. *B. subtilis* SR22 was grown at 37°C in minimal medium, and then the cells were shifted to a heat shock temperature of 48°C for 10 min and pulse-labeled (lanes A and H). The culture was returned to 37°C and pulse-labeled at 10 min (lane D), 20 min (lane E), or 40 min (lane F). The control (lanes B and G) was pulse-labeled at 37°C. An additional sample was heat shocked for 40 min and then pulse labeled (lane C). All samples were lysed and then assayed as described in the legend to Fig. 1.

Also of great interest is the possiblity that the heat shock response may be instrumental in the ability of bacilli to exist over wide temperature ranges as well as survive hostile environments by sporulation. In this regard,*B. stearothermophilus*, a bacterium which can grow at 37 to 65°C (N. E. Welker, personal communication), exhibits a strong heat shock response, and *B. subtilis* can be heat shocked from 37 to 52°C (manuscript in preparation).

In conclusion, we have demonstrated that bacilli produce a heat shock response over a wide temperature range. This response appears to persist and features a major heat shock protein at 66K. These findings will allow us to probe in greater depth the molecular basis for heat tolerance in bacilli and the factors controlling the heat shock response and heat adaptation.

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