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The heat shock response in meso- and thermoacidophilic chemolithotrophic bacteria

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1. SUMMARY

The heat shock response was studied in a chemolithotrophic thermoacidophilic archaebacterium *Sulfolobus acidocaldarius* (shifted from 70° to 85°C) and a mesoacidophilic microorganism *Thiobacillus ferrooxidans* (from 30° to 41°C). When transferred from their normal growth temperature to the stress temperature, cells showed a decrease in the incorporation of $Na_2{}^{14}CO_3$ into proteins, and at the same time, the synthesis of a specific subset of heat shock proteins was observed. Ethanol (4%) at 30°C, also caused a response similar to the heat shock upon *T. ferrooxidans* cells, whereas *Sulfolobus* cells at 70°C did not incorporate radioactive CO_2 in the presence of ethanol, apparently being damaged by the organic solvent.

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2. INTRODUCTION

The defense mechanism which cells utilize when confronted with high temperatures in their local environment is known as the heat shock response [1,2]. This response has been described extensively in both eucaryotes and procaryotes [1,3,4–8]. In general, a short exposure of cells to elevated temperatures or other agents reduces the synthesis of normal cellular proteins and at the same time induces a transient overproduction of a specific group of proteins, the so-called heat shock proteins (HSPs) [1,2].

Many kinds of cells acquire a transient thermotolerance when subjected to a heat shock [1,2]. The HSPs appeared to be required for thermotolerance in *Escherichia coli*, and it has been suggested that these cells grown at 45°C have a permanently increased thermal resistance compared to those grown at 30°C [9]. This effect might be related to the extraordinary cellular concentrations reached by HSPs at high temperatures [2].

Thermophilic microorganisms on the other hand, normally grow at high temperatures, and therefore should have a higher thermotolerance. Thus, it was of interest to find out whether these

thermophilic bacteria also responded to abrupt temperature changes with a heat shock-like response. For this, a comparison was made of the proteins which are synthesized in response to heat by a thermoacidophilic archaebacteria *Sulfolobus acidocaldarius* with those made by a mesoacidophilic microorganism *Thiobacillus ferrooxidans*. Both chemolithotrophic bacteria are industrially important since they participate in the bioleaching of minerals and may normally be subjected to temperature changes that take place during bioleaching operations [10,11].

3. MATERIALS AND METHODS

3.1. Bacterial strains and growth conditions

T. ferrooxidans strain DSM 583 and *S. acidocaldarius* were a kind gift of Dr. P. Norris, University of Warwick, U.K. The latter bacteria were grown at pH 1.7 and 70 °C in the presence of $K_2S_4O_6$, as described previously [13]. *T. ferrooxidans* was grown at 30 °C in a modified 9K liquid medium at pH 1.5–1.6 [12,14].

3.2. Shock and labeling conditions

Exponentially growing *T. ferrooxidans* cells were harvested by centrifugation, washed three times in 0.01 N H_2SO_4 and were resuspended at a density of about 5×10^9 cells/ml in fresh medium (0.5 ml). About the same number of *S. acidoaldarius* cells were employed, except they were not centrifuged prior to use. The bacterial suspensions were always preincubated for 30 min at their growth temperature. After this time, the control samples were incubated for 60 more min at the same temperature and the experimental samples were shifted to 41 °C (*T. ferrooxidans*) or 85 °C (*Sulfolobus*) for 60 min (heat shock). After this point, between 4 to 8 μCi of $Na_2{}^{14}CO_3$ (55 mC/mMol, Amersham International) were added to each sample and incubation continued in the presence of the isotope for 30 min in sealed vessels. To test for the effect of ethanol, after the 30 min preincubation ethanol was added (4% final) to the cells which were then incubated at their normal growth temperature for a total of 90 min before the addition of the radioactive carbonate.

3.3. Polyacrylamide gel electrophoresis of proteins

The samples treated as described above were cooled rapidly and washed two times by centrifugation with 200 μl of lysozyme buffer (50 mM Tris-HCl, pH 8, 1 mM EDTA). Finally, the cells were resuspended in 20 μl of the same buffer containing 5 μg of lysozyme and 1 μg of DNAase. After 5 min of incubation at room temperature, 10 μl of Laemmli sample buffer [15] were added and the samples boiled for 5 min. Unless stated otherwise, the same amount of radioactivity was applied to each gel lane. The labeled components separated by SDS–PAGE corresponded to proteins, as determined by proteinase K digestion as previously described [12]. After electrophoresis, all the gels were processed for staining and fluorography using Amplify (Amersham International) [12,16]. After drying, the gels were exposed to Kodak X-Omat film for one to three weeks at −70 °C.

4. RESULTS AND DISCUSSION

4.1. The heat shock response of the mesoacidophilic T. ferrooxidans

Fig. 1 shows that when *T. ferrooxidans* cells are transferred from 30 ° to 41 °C in the presence of radioactive sodium carbonate, and the radioactively labeled cell components analyzed by SDS–PAGE followed by fluorography, there was a decrease in the synthesis of several proteins, indicating an inhibition of protein synthesis due to the abrupt temperature change. There was also an increase in the synthesis of a few specific protein components (arrow heads, Fig. 1b).

Some of the presumptive heat shock proteins have approximate molecular weights of 92, 74, 66, 33, 22, and 16 kDa and possibly one with a molecular weight lower than 14.4 kDa. Employing the high resolution of the two-dimensional gel electrophoresis (Jerez C.A., Abstracts, XIV International Congress of Microbiology, Manchester, 1986), at least ten proteins could be considered as HSPs. The heat shock response was reversible and was observed, at least for the major proteins, as early as 5 or 10 min after heat shock (not shown).

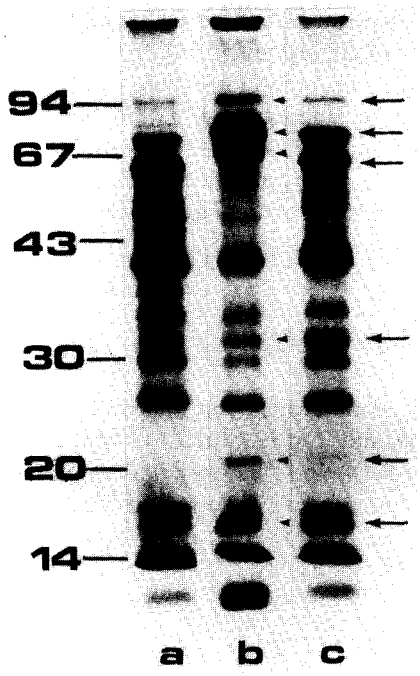

Fig. 1. Heat shock response and effect of ethanol on protein synthesis by *T. ferrooxidans*. Cells of *T. ferrooxidans* grown at 30 °C were incubated at the same temperature in the absence (a) or in the presence of 4% ethanol (c) or were subjected to a heat shock by incubation at 41 °C (b). The microorganisms were incubated in the presence of $Na_2{}^{14}CO_3$ as described in MATERIALS AND METHODS, and the proteins synthesized were analyzed by 10% SDS–PAGE and fluorography. Numbers refer to molecular mass markers in kilodaltons. Arrowheads and arrows indicate the major proteins synthesized after the specific stress.

Ethanol at a 4% concentration, is known to exert a response similar to a heat shock in bacteria and several other organisms [1–3,7]. This solvent also elicited a similar response in *T. ferrooxidans* grown at 30 °C as shown in Fig. 1. The main proteins induced by heat (Fig. 1, b) are also apparently induced by the presence of ethanol (arrows, Fig. 1, c). For example, the two induced proteins indicated by the second arrow or arrowhead gave identical in situ proteolysis patterns with protease V8 (not shown). Nevertheless, these inductions were less pronounced in the presence of the organic solvent. In addition, as seen in other bacteria [2,3], the repression of normal protein synthesis by ethanol (1c) was less pronounced compared with the repression after heat shock (1b).

4.2. The heat shock response of a thermoacidophile

When the thermophilic archaebacterium *Sulfolobus acidocaldarius* was transferred from 70 ° to 85 °C (Fig. 2B, C), there was a decrease in the synthesis of several proteins, as previously seen with the other bacteria analyzed. In addition, an increase was observed in the synthesis of those proteins indicated by the arrows. The two protein bands with molecular weight of about 64–66 kDa are major proteins at 70 °C, suggesting that these proteins may also have a function under normal or optimum growth conditions. However, there is a clear increase in the amounts of these presumptive HSPs at 85 °C. Fig. 2 also shows the increase

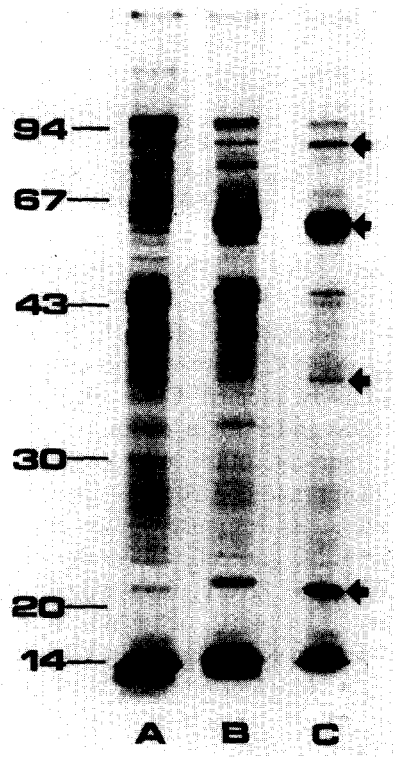

Fig. 2. Effect of heat shock on protein synthesis by *Sulfolobus acidocaldarius*. Cells from *S. acidocaldarius* grown at 70 °C, were incubated in the presence of $Na_2{}^{14}CO_3$ at the same temperature (B), transferred to either 60 ° (A) or 85 °C (C). Numbers indicate molecular mass markers in kilodaltons. Arrows indicate the major heat shock proteins synthesized.

of the synthesis of an 86, 38, and a 22 kDa protein.

Interestingly, when the thermophilic cells grown at 70°C were shifted to 60°C (Fig. 2a), the two major 64–66 kDa bands synthesized were greatly reduced. The 22 kDa band induced at 85°C also showed a decrease in synthesis. This suggests that the synthesis of these two or three proteins may be temperature regulated, as it has been described for some envelope proteins from *Tetrahymena thermophila* [17].

A similar heat shock-like response was also observed when the moderate acidophilic thermophile (LM2) [13] grown at 50°C was shifted to 60°C (not shown). To our knowledge, the only other archaebacteria studied for their heat shock response are those from the genus *Halobacterium*, in which three groups of HSPs, with M_r ranges between 75 000–105 000, 44 000–45 000 and 21 000–28 000 were induced after shifting the cells from 37° to 60°C [6]. From the evolutionary point of view, it will be of interest to know if the highly conserved major HSP 70 protein from eubacteria and eukaryotes is homologous to some of the major heat-induced proteins from *S. acidocaldarius*, especially after the suggestion that 'the early ancestors of eukaryotes probably lacked nuclei, metabolized sulfur and lived at near-boiling temperatures' [18].

When ethanol was added (4% final) to *Sulfolobus* cells growing at 70°C, there was no incorporation of radioactively labeled CO_2, suggesting damage to the cells under these conditions (not shown). In the case of other microorganisms [2,7,8,19], some membrane components appear to be involved in the stress response. However, the lack of outer membrane and cell wall in the iron-oxidizing *Sulfolobus* sp. [20,21] may explain in part the extreme sensitivity of these microorganisms to the presence of ethanol. In addition, one has to consider that thermoacidophiles have to contend with not only high temperature but also the stress of high acidity.

In conclusion, although thermophiles are thermotolerant, they also showed a heat shock response similar to that observed in mesophilic microorganisms. However, the maximum induction temperature correlated with the normal range of environmental exposure of these bacteria, as previously seen for other organisms [1].

Current studies are being undertaken to further characterize the heat shock response in these industrially important microorganisms, with special interest in the role this response may play during adaptation and survival of the bacteria in extreme environments.

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