Effect of Heat Shock on Protein Synthesis in the Cyanobacterium *Synechococcus* sp. Strain PCC 6301

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The response to heat shock at 47°C was examined in the cyanobacterium (blue-green alga) *Synechococcus* sp. strain PCC 6301. On heat shock, the growth of the cells decreased and they preferentially synthesized a limited number of polypeptides. The rate of synthesis of these proteins increased markedly in the early period of temperature shift up and gradually decreased afterwards. Among the proteins greatly affected by temperature shift up were those with apparent molecular weights of 91,000 (91K), 79K, 78K, 74K, 65K, 64K, 61K, 49K, 45K, 24K, 22K, 18K, 16K, 14K, 12K, and 11.4K, based on their mobilities in sodium dodecyl sulfate-polyacrylamide gels. From these initial studies on *Synechococcus* sp. strain PCC 6301 we conclude that in cyanobacteria a heat shock response similar to that known to occur in other eucaryotes and procaryotes might exist.

The heat shock phenomenon is known to occur in a wide variety of organisms, including eucaryotes (animal cells and plants) and procaryotes (2, 3, 24). Generally, a short exposure of cells to elevated temperatures reduces the synthesis of normal cellular proteins and at the same time induces the synthesis of a new set of proteins, the heat shock proteins (HSPs).

Cyanobacteria (blue-green algae) are oxygen-evolving, photosynthetic procaryotes and are claimed to be phylegetically and physiologically related to the chloroplasts of photosynthetic eucaryotes. Most organisms which belong to this group are obligate phototrophs and are therefore dependent on light as the only energy source for growth. Cyanobacteria require CO2 for carbon skeleton synthesis; they fix CO2 through the reactions of the reductive pentose phosphate (Calvin) cycle (for a review, see Stanier and Cohen-Bazire [20]).

Most of the authors dealing with the regulation of gene expression in cyanobacteria have emphasized the pleiotropic nature of cyanobacterial regulatory responses (7, 9, 19, 20). Therefore, the ability to specifically manipulate one or several genes could simplify attempts to understand the regulation of gene expression in cyanobacteria.

In this paper we report investigations on the response of exponentially growing *Synechococcus* sp. strain PCC 6301, an obligate phototrophic cyanobacterium, to an elevated temperature. We found that the transfer of cells from normal growth temperature (39°C) to an elevated temperature (47°C) dramatically altered the pattern of cellular protein synthesis and induced a new set of proteins. As a first step, the kinetics of induction of HSPs in *Synechococcus* spp. were studied. The results obtained indicate that the “induction” of HSPs occurs at the level of transcription. Therefore, the cyanobacterial heat shock response may be a good system for studying cyanobacterial gene expression.

**MATERIALS AND METHODS**

**Growth conditions.** *Synechococcus* sp. strain PCC 6301 (*Anacystis nidulans* ATCC 27144) was grown as described previously (17), with minor modification (6). Cells were grown in the liquid medium described by Allen (1). Jacketed culturing glass vessels (20 ml) were thermostatically maintained at 39°C by circulating water and illumination with warm white fluorescent light (3.6 × 10^4 mW m^-2), except where otherwise stated. Agitation and CO2 were provided continuously by bubbling sterile 5% CO2 in air (vol/vol) through the cultures.

Growth of the cultures was monitored either spectrophotometrically at 800 nm or by measurement of their chlorophyll a content in 90% acetone extracts, or both. The concentration of chlorophyll a is given in micrograms per milliliter (10). Occasionally the growth of *Synechococcus* cultures was followed by determination of the number of CFU per milliliter. Samples (0.1 ml) were diluted aseptically into sterile Allen medium and plated on Allen medium solidified with 1.5% agar and incubated under light for 1 to 1.5 weeks at 28°C. To check chance bacterial contaminations, samples were plated on Luria broth solidified with agar (12), and portions of cultures were carefully examined under a phase contrast microscope. Occasionally, the oxygen-evolving capacity of the cells was measured by a Clark-type oxygen electrode (18).

**Stress conditions.** Logarithmically growing *Synechococcus* cultures (0.120 to 0.230 optical density [800 nm] units) were heat shocked by a change to a circulating-water bath thermostated to the elevated temperature. In recovery studies, the temperature was returned to the normal 39°C after the stress period.

**Isotope labeling conditions.** The temperature of logarithmically growing *Synechococcus* cultures was increased as indicated for each experiment, and 1-ml portions of the culture were removed at appropriate intervals, pulse labeled with 0.5 MBq of 14C-uniformly labeled protein hydrolysate (1.3 GBq mmol^-1; Chemapol, Prague, Czechoslovakia) for 0.5 or 1 h, and prepared for polyacrylamide gel electrophoresis. To determine the rates of total protein synthesis during temperature shifts, 0.5-ml aliquots of cell suspension were pulse labeled as above at each point with 2.4 MBq of l-[35S]methionine (396 GBq mmol^-1; Isocommerz, German Democratic Republic) per ml for 10 min and 0.2-ml aliquots were pipetted into an equal volume of a solution containing 0.4 mg of bovine serum albumin and immediately mixed with
FIG. 1. Growth of *Synechococcus* sp. strain PCC 6301 on temperature shift up. (A) Log-phase culture grown in Allen medium at 39°C was divided into two parts (zero time) and further cultured at 39°C (∆, ○) and 47°C (△, ●), respectively. Growth was monitored by measuring turbidity (optical density at 800 nm; ∆, ○) and chlorophyll a content (∆, ●). (B) Logarithmically growing *Synechococcus* cultures were shifted from 39 to 47°C at zero time and 2 h later back to 39°C. Growth of the culture was monitored by measuring their turbidity (optical density at 800 nm; ○), chlorophyll a content (●), and CFU per milliliter (∆).

0.8 ml of cold 12.5% trichloroacetic acid. The precipitates were collected by centrifugation and washed twice with cold 5% trichloroacetic acid and ethanol. The pellets were dried and dissolved in 0.2 M sodium hydroxide. The radioactivity was determined by scintillation in Bray solution. For measuring radioactivity, a Delta Searle 300 liquid scintillation spectrometer was used.

**Polycrylamide gel electrophoresis of proteins.** Pulse-labeled cyanobacterial cells were chilled on ice, collected by centrifugation, and washed with water. The pellets were dissolved in the sample buffer described by Laemmli (11) or, for the two-dimensional polyacrylamide gels, in the O’Farrell lysis buffer (16), and the electrophoresis was performed on 10 to 18% linear sodium dodecyl sulfate (SDS)-polyacrylamide gradient gels as described previously (11, 16). After electrophoresis, the gels were processed for staining and fluorography (5), dried (gel slab drier model 224; BioRad Laboratories, Richmond, Calif.), and exposed to X-ray films. The low-molecular-weight calibration kit of Pharmacia Fine Chemicals AB was used as molecular weight markers. For one-dimensional SDS gels, the relative rate of HSP synthesis was estimated by the method of Suissa (21) on the basis of the spectrophotometric quantitation of silver grains of autoradiograms. The data are given as percentages of total proteins synthesized as a function of time after the temperature shift up (to 47°C) and normalized to that for the zero time sample (39°C), which was taken as a unit.

**RESULTS**

**Effect of elevated temperature on the growth and protein synthesis of *Synechococcus* sp. strain PCC 6301.** When a log-phase culture of *Synechococcus* sp. was transferred from 39 to 47°C, its growth rate, as determined by optical density measurement, decreased (Fig. 1A). A similar, even more obvious tendency was obtained by measuring the chlorophyll a content (Fig. 1A). Since in *Synechococcus* cells, high temperatures (above 48°C) may provoke photobleaching, we have chosen 47°C for heat shock induction (18). Under these conditions no photobleaching occurred, and after treatment for 2 h at the elevated temperature (47°C) the cells exhibited growth, as determined by optical density measurements. When the cells were shifted back to normal temperature, a 1-h lag period followed by resumption of growth was observed (Fig. 1B). When growth was monitored by measuring the chlorophyll a content, there was a more obvious decrease in the growth rate after temperature shift up, followed by a lag and recovery of the growth rate at normal temperature. To further assess the physiological significance of the elevated temperature in *Synechococcus* spp., alterations of colony-forming ability (CFU per milliliter) were also determined (Fig. 1B). The transfer of cells from 39 to 47°C allowed the cells to divide further for 30 min; cell division then stopped (Fig. 1B). After heat shock and return to normal temperature (39°C), a 40% decrease in CFU was observed; cell division then continued at about the control rate (Fig. 1B). Throughout the experiments the oxygen-evolving capacity of the cells did not change markedly (within 10%; data not shown).

To study the effect of heat shock on protein synthesis in heat-shocked cells at 47°C, cells were pulse labeled with L-[35S]methionine at various times before and after the temperature shift. The rate of protein synthesis in *Synechococcus* cells immediately decreased after heat treatment (Fig. 2). However, within 30 min the rate of protein synthesis increased again and reached a maximum, after which it decreased to a new steady-state level.

**Effect of heat shock on the protein pattern of *Synechococcus* sp. strain PCC 6301.** Aliquots (1 ml) of *Synechococcus* cultures were labeled for 1 h with uniformly labeled [14C]-protein hydrolysate at different temperatures ranging from 39 to 51°C. Cell extracts were prepared from the labeled cells and analyzed by SDS-polyacrylamide gel electrophoresis (Fig. 3). A number of newly synthesized polypeptide bands may be seen in the extracts obtained from heat-treated samples.
FIG. 2. Effect of temperature shift up on the rate of protein synthesis in Synechococcus sp. strain PCC 6301 cultures. Cells were grown in Allen medium and pulse labeled with L-[35S]methionine (2.4 MBq ml\(^{-1}\)) for 10 min at different times. After each pulse the acid-insoluble radioactivity was determined in aliquots of the culture as described in the text. Horizontal bars represent 10-min pulses.

FIG. 3. Temperature dependence of heat shock response in Synechococcus sp. strain PCC 6301. Aliquots of a log-phase culture were labeled with \(^{35}\)S-protein hydrolysate (0.5 MBq ml\(^{-1}\)) for 1 h at the indicated temperatures. Cellular proteins were extracted from equal volumes of cultures and analyzed by SDS-polyacrylamide gel electrophoresis (10 to 18% linear gradient gel) as described in the text. The positions of molecular weight markers are indicated in kilodaltons (K).

FIG. 4. Effect of temperature shift up on polypeptide synthesis in Synechococcus sp. strain PCC 6301 cells. A log-phase culture was shifted from normal (39°C) to elevated temperature (47°C). Portions (1 ml) were removed before (zero time; a) or after temperature shift up and pulse labeled (30 min) with \(^{35}\)S-protein hydrolysate (0.5 MBq ml\(^{-1}\)) at different times during a 2-h experimental period (b, 0 to 30 min; c, 30 to 60 min; d, 60 to 90 min; e, 90 to 120 min). The samples (equal volumes, 1 ml) were analyzed for labeled proteins on a 10 to 18% linear gradient SDS-polyacrylamide gel and processed for fluorography as described in the text. The positions of molecular weight markers, as well as of polypeptides affected by temperature shift (HSPs), are indicated in kilodaltons (K).

(45 to 49°C) when compared with the proteins synthesized at 39°C. These bands, the HSPs, can already be detected after the exposure of cells to 43°C. In samples exposed to 45°C, however, their preferential synthesis can be clearly seen. Maximum induction of HSPs occurred at 47°C. If extracts from equal volumes of cyanobacterial cultures rather than equal radioactivities (counts per minute) were loaded in each lane of gel, little label was seen in the heat shock bands obtained from cells exposed to 51°C compared with those exposed to 47°C. Above 45°C, the synthesis of proteins normally present in cells was drastically reduced (Fig. 3).

When aliquots of heat-treated Synechococcus cultures were pulse labeled for 30 min during a 2-h period, the relative amounts of most HSPs increased during the first hour of incubation (Fig. 4). Between 1 and 2 h after the exposure to heat shock, the synthesis of heat shock bands progressively decreased. The apparent molecular weights of the polypeptides synthesized under heat shock conditions are indicated in Fig. 4. The molecular weights of these
inducible polypeptide chains have been estimated to be 91,000 (91K), 78K, 74K, 65K, 64K, 61K, 49K, 45K, 24K, 22K, 18K, 16K, 14K, 12K, and 11.4K on the basis of their mobilities in SDS-polyacrylamide gels.

We analyzed the kinetics of polypeptide synthesis under heat shock by the method of Suisa (21). The relative proportions of several heat-shock-induced proteins in the total polypeptides synthesized was estimated from the autoradiogram shown in Fig. 4. It is clear that the synthesis of these proteins follows two types of kinetics (Fig. 5). Several HSPs transiently reached a maximum at about 30 min after the temperature shift up and decreased afterwards (see the 91.0K, 78.0K, 18.0K, etc., polypeptides in Fig. 5A). Some, like the 45.0K HSP band, reached a transient maximum later (Fig. 5A). It seems noteworthy that the level of a second set of "induced" proteins increased during the whole period of temperature shift up (see the 65.0K, 64.0K, 14.0K, etc., polypeptides in Fig. 5B). The total amount of heat-inducible polypeptides measured by spectrophotometric quantitation of silver grains of autoradiograms (data not shown) and the kinetics of protein synthesis, as measured by radioactive methionine pulse labeling, are comparable (Fig. 2, 4, and 5).

The specific inhibitors of transcription (100 μg of rifampin ml⁻¹; Sigma Chemical Co., St. Louis, Mo.) and translation (100 μg of chloramphenicol ml⁻¹; Serva Co.) inhibited the synthesis of heat-inducible polypeptides (data not shown).

To obtain a more accurate pattern of heat-inducible proteins, we analyzed the protein extracts of control and heat-shocked Synechococcus cells by two-dimensional polyacrylamide gel electrophoresis (Fig. 6). Proteins whose synthesis was induced or drastically altered are indicated by squares (Fig. 6). Three classes of proteins can be observed in this organism: (i) proteins synthesized under normal (39°C) conditions only (the bulk of cellular proteins); (ii) polypeptides synthesized under both control (39°C) and heat shock (47°C) conditions (not necessarily at the same rate); (iii) proteins induced specifically under heat shock conditions. Occasionally we pulse labeled the heat-shocked cells with NaH¹⁴CO₃, ¹⁴C-labeled protein hydrolysate, or ³⁵S-methionine. The protein pattern in the case of NaH¹⁴CO₃ labeling was identical with that obtained by using the radioactive amino acid labeling, but in the extracts of cells labeled with ³⁵S-methionine several bands were missing (most obviously the 14K protein), indicating the presence of polypeptides having no or a low level of sulfur-containing amino acids among the HSPs (Fig. 7).

DISCUSSION

Procaryotic and eucaryotic cells respond to temperatures higher than their normal growing temperature by altering the pattern of growth and protein synthesis (2). Indeed, the data presented above indicate that the growth rate of Synechococcus sp. strain PCC 6301 cells incubated at 47°C decreases. The elevation of the temperature from 39 to 47°C in logarithmically growing cultures is rapidly followed by changes in protein synthesis. At 47°C, protein synthesis is considerably reduced compared with that at 39°C. Moreover, Synechococcus sp. responds to a shift up in the growth temperature (47°C) by a transient induction of a specific set of polypeptides, thereby raising their cellular level quickly and markedly to that found in steady-state growth at normal temperature. At 47°C, many cellular proteins are synthesized at decreased rates or not at all, whereas others remain unaffected or their synthesis is increased. Analysis of the detailed results indicates that a temperature of 43°C or higher increases the incorporation of label into the inducible subset of these proteins. The apparent molecular weights of these inducible polypeptides, estimated on the basis of their mobilities in SDS-polyacrylamide gels, falls into the range which is known to be characteristic for the heat shock phenomenon in Escherichia coli (13, 14, 25, 26).

The heat shock response in a cyanobacterial system seems to share many properties in common with the heat shock response of procaryotic systems, both eubacteria and archaea, and eucaryotic cells, both plant and animal, which have been studied so far (8, 13, 14, 25, 26). HSP synthesis may therefore also represent a part of the cellular adaptation mechanism for survival at high temperatures in cyanobacteria. Such a wide occurrence of analogous phenomena suggests that HSP synthesis might be a part of an adaptive response of cells to sudden temperature changes in...
the natural environment (2). Although the biological significance of the heat shock response has not yet been fully elucidated, in the case of *E. coli* the induction of high-temperature regulon is under the control of a gene called *hpR* (4, 13, 15, 22, 23, 27, 28). From these initial studies on *Synechococcus* sp., a cyanobacterium, we can predict the possible existence of a similar regulatory system. The polypeptides found to be inducible may be the product(s), at least partly, of a cyanobacterial high-temperature regulon.

Finally, the results reported herein suggest that the heat shock response of *Synechococcus* spp. seems to be a good alternative system for studying cyanobacterial gene expression (9). Such studies under different light regimes (light-dark) would be of interest from the viewpoint of light-dependent cyanobacterial gene expression and may provide information about its characteristics.

**FIG. 6.** Two-dimensional resolution of total cellular proteins synthesized in *Synechococcus* sp. strain PCC 6301 grown under normal (39°C) and under heat shock (47°C) conditions. Log-phase cells, labeled for 1.5 h with 14C-protein hydrolysate (0.5 MBq ml−1), were separated by the method of O’Farrel (16), and the radioactive polypeptides were detected by fluorography on the second dimension (10 to 18% linear gradient SDS-polyacrylamide gel). Proteins whose synthesis was induced by heat shock at 47°C are indicated by squares, whereas the absence of these proteins in control cells is indicated by squares placed in the appropriate position on the gel of the 39°C sample. Some heat-shock-induced proteins were synthesized to various extents in control cells.

**FIG. 7.** Comparison of heat-shock-induced proteins in *Synechococcus* sp. labeled with NaH14CO3 (0.6 MBq ml−1; a), 14C-protein hydrolysate (0.5 MBq ml−1; b) and l-[14S]methionine (0.5 MBq ml−1; c), respectively, for 1 h and separated on a 10 to 18% linear gradient SDS-polyacrylamide gel. Arrows indicate proteins having either no or a low number of sulphur-containing amino acids.

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**LITERATURE CITED**