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Heat shock inactivates a supernatant factor(s) specifically required for efficient expression of the *amp* gene in *Escherichia coli*

Yoshitaka Kuriki

Institute for Protein Research, Osaka University, Suita, Osaka 565, Japan

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A 160 000 × *g* supernatant of *E. coli* extract prepared from cells grown at 30°C stimulated specifically the expression of the *amp* gene on pBR322 and pBR328 in an in vitro gene expression system from *E. coli*. This activity of the supernatant was markedly reduced when the cells were exposed to 42°C for 30 min prior to preparing the supernatant. These results are consistent with the view that heat shock-induced repression of the *amp* gene expression is due to inactivation of a supernatant factor(s) required for effective expression of the *amp* gene.

amp gene expression; Heat shock response; (*E. coli*)

1. INTRODUCTION

The ampicillin-resistant gene (*amp* gene) of the plasmid pBR322 [1] encodes β-lactamase, a periplasmic enzyme, that catalyzes the hydrolysis of penicillin to penicilloic acid. Expression of the *amp* gene both in vivo and in vitro has been extensively studied [2]. I have recently reported that the increase in β-lactamase in *E. coli* carrying pBR322 ceases almost completely upon a temperature shift-up from 30 to 42°C, even though this leads to acceleration of cell growth [3]. Since this temperature shift-up does not affect the level of β-lactamase mRNA, it has been suggested that the repression of β-lactamase synthesis involves a translational control [3]. Moreover, I have also shown that expression of the *amp* gene in vitro is stimulated at the translation level by a protein factor purified from a 160 000 × *g* supernatant of *E. coli* extract [4]. The possibility, therefore, arises that the heat shock-induced repression of β-lactamase synthesis is caused by inactivation of the supernatant factor(s) required for efficient translation of β-lactamase mRNA. This communication reports that the 160 000 × *g* supernatant prepared from *E. coli* cells that had been exposed to heat shock was much less effective than that prepared from cells grown at 30°C in stimulating the expression of the *amp* gene in an in vitro gene expression system of Shibuya and Kaziro [5]. It is concluded that the heat-shock treatment inactivates the factor(s) required for efficient expression of the *amp* gene.

Correspondence address: Y. Kuriki, Institute for Protein Research, Osaka University, 3-2 Yamadaoka, Suita, Osaka 565, Japan

2. MATERIALS AND METHODS

2.1. *Materials*

E. coli Q13 (Hfr *met tyr rns pnp*) was used. pBR322 [1] was prepared by the method of Birnboim and Doly [6] and purified essentially as described by Radloff et al. [7]. pBR328 [8] was purchased from Boehringer-Mannheim. Trans ^{35}S label (1100 Ci/mmol) was obtained from ICN Radiochemicals, and Enhance from New England Nuclear.

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2.2. *Preparation of 160 000 × g supernatants*

E. coli Q13 was grown at 30°C in 2.5 l LB medium with vigorous aeration. At the early mid-logarithmic phase, a 1 l portion was transferred to 42°C and incubated at this temperature for 30 min, whereas another 1 l portion was further incubated at 30°C for 30 min. From the cells in these two portions 160 000 × *g* supernatants were prepared as in [4].

2.3. *DNA-directed protein synthesis* in vitro

E. coli Q13 was grown at 37°C in 10 l LB medium with vigorous aeration and harvested at the mid-logarithmic phase after chilling the culture in an ice bath. An in vitro coupled transcription-translation system, which consists of unwashed ribosomes and a polyethylene glycol-treated S30 extract, was prepared from the harvested cells by the methods of Shibuya and Kaziro [5]. Protein synthesis directed by pBR322 or pBR328 was conducted in this system at 30°C in the presence of trans ^{35}S label as described in [4]. The polypeptides thus synthesized were analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) [9] and subsequent fluorography [10].

2.4. *Other methods*

β-Lactamase synthesized in vitro was immunoprecipitated with anti-TEM β-lactamase antiserum essentially as described in [3]. Protein was determined by using a Bio-Rad protein assay kit; bovine serum albumin was used as a standard.

3. RESULTS AND DISCUSSION

As mentioned earlier, there is the possibility that the heat shock-induced repression of β-lactamase synthesis in *E. coli* carrying pBR322 [3] is due to inactivation of the factor or factors that are present in the 160 000 × *g* supernatant of *E. coli* extract and stimulate the expression of the *amp* gene selectively [4]. To test this possibility, two 160 000 × *g* supernatants were prepared; one from *E. coli* cells grown at 30°C (S160) and the other from cells that had been exposed to 42°C for 30 min (h-S160). The effects of these two supernatants on β-lactamase synthesis directed by pBR322 and pBR328 were then examined in the in vitro gene expression system described by Shibuya and Kaziro [5]. pBR328 is a derivative of pBR322 and contains a gene encoding chloramphenicol acetyltransferase in addition to the *amp* gene [8]. In the absence of the supernatants, protein synthesis at 30°C in the in vitro system with pBR322 and pBR328 proceeded linearly for about 40 min. As reported previously [4], the rate of pBR322-directed protein synthesis was only about one-tenth that observed in the pBR328-containing system. As shown in fig.1, pBR322-directed protein synthesis was stimulated by the addition of S160 and h-S160, although the stimulation by S160 was much more pronounced than by h-S160. At 28 μg protein per incubation vessel, S160 stimulated the synthesis about 4-fold, whereas that by h-S160 was only 1.5-fold. S160 and h-S160 also enhanced pBR328-directed protein synthesis. S160 (causing about 2-fold stimulation) was again more effective than h-S160 (causing about 1.5-fold stimulation).

It was clear from these results that the heat shock treatment of *E. coli* cells results in a reduc-

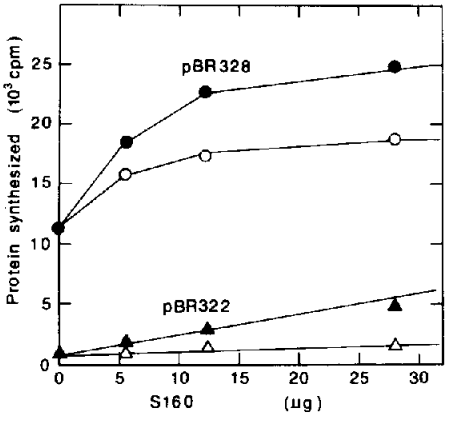

Fig.1. Effects of S160 and h-S160 on pBR322- and pBR328-directed protein synthesis in vitro. The reaction mixture (final volume, 35 μl) was the same as used in [4], except that trans ^{32}S label (15 μCi) was used instead of [^{35}S]methionine and the indicated amount (in terms of protein) of S160 (●,▲) of h-S160 (○,△) was added. After preincubation at 30°C for 2 min, the reaction was started by adding pBR322 or pBR328 (5 μg) and incubation was continued to 30°C for 20 min. The reaction was terminated by adding 65 μl of 77 mM Tris-HCl (pH 6.8) containing 1.5% SDS, 15% glycerol, 3.8% 2-mercaptoethanol and 5×10^{-3}% bromophenol blue. The mixture was boiled for 10 min. 2 μl of the boiled reaction mixture was spotted onto a paper disc. The paper disc was treated with hot trichloroacetic acid [4] and its radioactivity was measured in a Beckman liquid scintillation counter.

tion of the activity of their S160 fraction to stimulate protein synthesis both directed by pBR322 and pBR328. It was, however, still uncertain whether h-S160 from the heat-treated cells was specifically deprived of the activity to stimulate the *amp* gene expression. To examine this point, the protein products synthesized with pBR328 as template in the absence and presence of S160 or h-S160 (28 μg protein per vessel) were analyzed by SDS-PAGE and subsequent fluorography. As shown in fig.2A, the protein products from the three incubations displayed bands corresponding to pre-β-lactamase and chloramphenicol acetyltransferase together with many bands having lower molecular masses. The band of chloramphenicol acetyltransferase, which was the major protein product, did not seem to be appreciably affected by the presence of S160 and h-S160 in the reaction mixture. On the other hand, the band of pre-β-lactamase was intensified by the addition of S160, but not appreciably by h-S160. To confirm further the effects of S160 and h-S160 on pre-β-lactamase synthesis, the preenzyme synthesized was immunoprecipitated with antiserum raised against TEM β-lactamase and the immunoprecipitate was analyzed. As shown in fig.2B, the addition of both S160 and h-S160 to the in vitro gene expression system increased the amount of immunoprecipitable protein, but the effect of S160 was clearly more pronounced than that of h-S160.

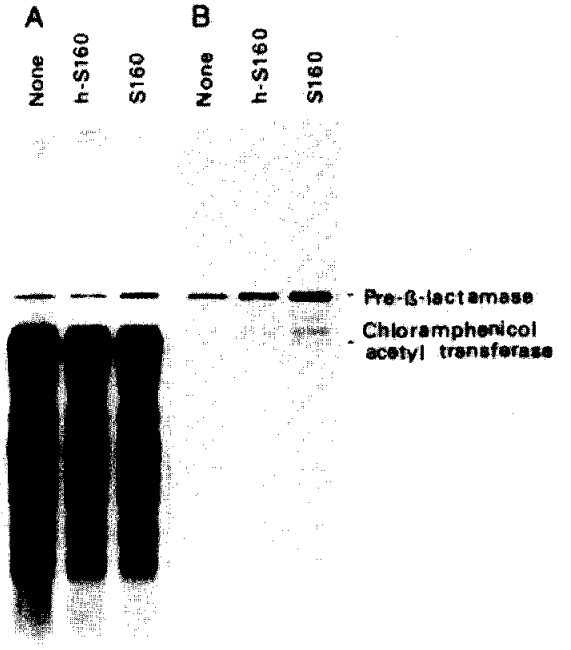

Fig.2. SDS-PAGE analysis of the protein products synthesized in vitro with pBR328 as template. The genes on pBR328 were expressed in the absence and presence of S160 or h-S160 (28 μg protein) as described in fig.1. (A) A portion of the reaction product (4.28×10^3 cpm) was subjected to SDS-PAGE (15% gel) [9] and protein bands were visualized by fluorography [10]. (B) Another portion of the reaction product (3.45×10^5 cpm) was treated with anti-TEM β-lactamase antiserum as described in [3]. A 20 μl aliquot of the immunoprecipitate dissolved in 100 μl of 50 mM Tris-HCl (pH 6.8) containing 1% SDS, 10% glycerol, 2.5% 2-mercaptoethanol and 3×10^{-3}% bromophenol blue was analyzed by SDS-PAGE (15% gel) [9] and subsequent fluorography [10].

To obtain more quantitative data, the protein products synthesized in the experiments shown in

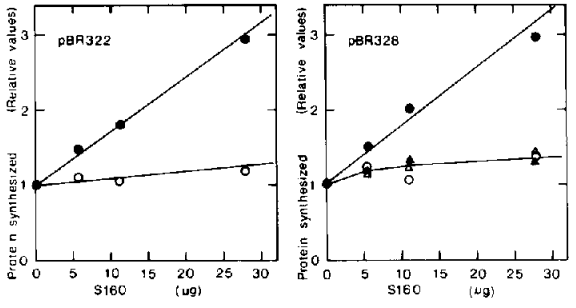

Fig.3. Effects of S160 and h-S160 on the in vitro synthesis of pre-β-lactamase and chloramphenicol acetyltransferase with pBR322 as templates. A 3 μl aliquot of the reaction product obtained in the experiments shown in fig.1 was subjected to SDS-PAGE (15% gel) [9] and subsequent fluorography [10]. The dried up gel was exposed at −70°C to an X-ray film for 2 days to estimate chloramphenicol acetyltransferase synthesized, and then to another X-ray film for 8 days to estimate pre-β-lactamase synthesized. The intensities of pre-β-lactamase and chloramphenicol acetyltransferase bands were measured at 500 nm in a Beckman DU8 spectrophotometer [4]. Pre-β-lactamase and chloramphenicol acetyltransferase synthesized are expressed as relative values. (●,○) Pre-β-lactamase synthesized in the presence of S160 and h-S160, respectively. (▲,△) Chloramphenicol acetyltransferase synthesized in the presence of S160 and h-S160, respectively.

fig.1 were subjected to SDS-PAGE. For estimation of chloramphenicol acetyltransferase, the SDS-PAGE gel that had been treated with Enhance and then dried up on a Whatman no. 3 paper was exposed to a X-ray film for a shorter period of time than for pre-β-lactamase to avoid interference due to the other protein bands. The intensities of the bands corresponding to pre-β-lactamase and chloramphenicol acetyltransferase were estimated by tracing the fluorographs [4] and plotted against the amount of S160 (and h-S160) added to the incubation mixture. As can be seen in fig.3, pre-β-lactamase synthesized in the pBR322- and pBR328-directed reactions increased linearly as the amount of S160 added was increased. The addition of h-S160, on the other hand, stimulated pre-β-lactamase synthesis only very weakly (about one-tenth that caused by S160). The stimulation of pBR328-directed synthesis of chloramphenicol acetyltransferase by both S160 and h-S160 was also very weak and the extent of stimulation was practically the same as that attained in pre-β-lactamase synthesis by h-S160. It is likely that such slight stimulation was due to supplementation of the system with factors and enzymes required for protein synthesis in general. In any case, it is reasonable to assume that a factor(s) in S160 stimulates the synthesis of pre-β-lactamase specifically and this factor(s) is inactivated by the heat-shock treatment of cells. In a previous work [4], two fractions were isolated from S160; one stimulated pBR322-directed RNA synthesis and the other enhanced the translation of mRNA encoded by the *amp* gene. Since there is evidence that the response of the *amp* gene expression in cells to heat shock is regulated at the translation level [3], it is likely that the factor(s) that is inactivated by heat shock is the latter one, though further work is still needed to confirm this conclusion.

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