ISOFORM COMPOSITION OF INDUCIBLE HSP 70 IN THE RAT MYOCARDIUM AFTER HEAT SHOCK

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UDC 616.127-008.9-001.16./.17-07

KEY WORDS: heat shock; cellular response.

Organisms ranging from prokaryotes to the higher eukaryotes respond in a remarkably varied manner to environmental stress. Meanwhile a general characteristic feature of the cellular response to stress in many cases is rapid synthesis of what are called heat shock proteins (hsp) [9, 10]. The principal representatives of this family are heat shock proteins with molecular weight of about 70 kilodaltons (hsp 70) [9, 10]. We know that hsp are involved in the repairing of stress-induced injuries through disaggregation of abnormal protein–protein interactions [9, 10, 13]. Expression of individual genes coding for isoforms of inducible hsp has been shown to depend on the strength and character of the stressor [1, 6, 12, 13]. It has also been shown that the content of inducible hsp 70 in cells depends on the time elapsing after exposure to stress and, in particular, in the case of rat cardiomyocytes, two phases in the accumulation of hsp 70 have been found after pressure loading [3]. Meanwhile, the important question of how the isoform spectrum of hsp 70 depends on the time after stress has not yet been answered, i.e., essentially it remains unclear in what order expression of the genes of particular hsp 70 isoforms takes place after stress.

The aim of this investigation was to study the isoform spectrum of hsp 70 accumulating in the rat myocardium at different times after heat shock.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 200-250 g. To produce heat shock, initially the rats were anesthetized with pentobarbital (50 mg/kg, intraperitoneally), after which the animals were placed in a special thermostat at 80°C for 20 min. Under these conditions, the rectal temperature of most rats reached 42°C after 5 min and stayed at that level for the remaining 15 min [2]. The content of hsp 70 was determined in the cytosol fraction. For this purpose the heart was quickly isolated, then washed thoroughly to remove blood in a Langendorff perfusion system. The heart tissue was then kept for 10 min at 4°C in hypotonic buffer: 10 mM Tris, pH 7.4, 10 mM KCl, 1 mM PMSF, and then homogenized in the same solution with a ratio buffer:tissue 5:1. The resulting homogenate was filtered through eight layers of gause and centrifuged for 10 min at 12,000*g*. The supernatant was used for analysis. Two-dimensional electrophoresis was carried out as in [8]. Isoelectric focusing was carried out at 500 V for 18 h. Electrophoresis in the second direction was undertaken in 10% PAG as in [5]. After electrophoresis the gels were stained with silver as in [7]. As markers for isoelectric focusing, a preparation of carbamoylated carbonic anhydrase ("LKB Pharmacia") was used. As markers of molecular weight pure proteins ("LKB, Pharmacia") were used. Isoforms of inducible polypeptides of hsp were identified and characterized according to molecular weight pI [4, 9]. The content of hsp 70 was determined in the myocardium of the control group of rats, in a group of rats 24 h after heat shock, and in a group 48 h after heat shock.

Research Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, Moscow, Research Institute of Medical Genetics, Russian Academy of Medical Sciences, Tomsk. (Presented by Academician of the Russian Academy of Medical Sciences S. S. Debov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 113, No. 6, pp. 586-587, June, 1992. Original article submitted November 15, 1991.

0007-4888/92/0006-0781$12.50

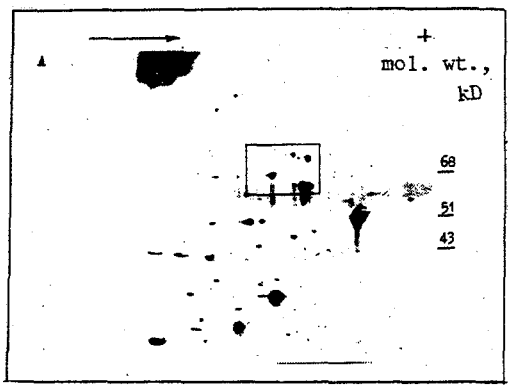

Fig. 1

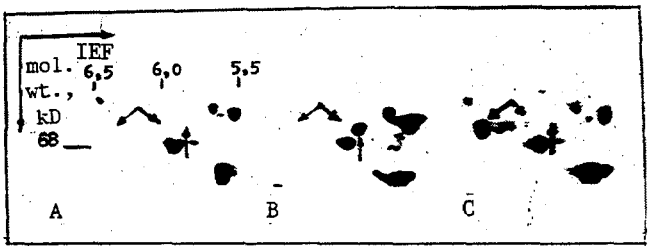

Fig. 2

Fig. 1. Typical appearance of gel after electrophoresis of cytosol proteins of rat heart cells. Horizontal arrow – direction of isoelectric focusing. Rectangle describes region of location of hsp 70.

Fig. 2. Effect of time after heat shock on isoform composition of hsp 70 accumulating in cytosol of heart cells. Fragments of gel correspond to region identified in Fig. 1 by rectangle; A) control, B) 24 h after heat shock, C) 48 h after heat shock. Arrows indicate location of isoforms of hsp 70: arrows from top to bottom indicate inducible hsp 70; downward pointing arrows indicate inducible hsp 70, upward pointing arrows – inducible hsp 70 with pI about 5.8; bent arrows indicate HSC.

EXPERIMENTAL RESULTS

The typical appearance of the gels after two-dimensional electrophoresis of cytosol proteins of the heart is shown in Fig. 1. The pattern of distribution of the polypeptide fractions was highly reproducible during analysis both of the same specimen and of specimens obtained from different animals. The region described by a rectangle, with boundaries: mol. wt. 62 and 78 kD; pI 6.5 and 5.5, in Fig. 1 corresponds to the localization of hsp 70 [4, 9]. The pattern of distribution of the polypeptide fractions in this region was constant, and none of the control specimens tested contained any additional protein fractions. One typical fragment of the gel after electrophoresis is shown in Fig. 2A.

A major inducible hsp 70 with mol. wt. of 71 kD and with pI of about 5.8, which is completely absent from the cytosol of the heart of control animals (Fig. 2A, B) was found 24 h after heat shock among the cytosol proteins of the heart. In addition, an increase in content of the polypeptide fraction with mol. wt, 73 kD and with pI of about 5.6 was found. This fraction probably corresponds to HSC 73 [9].

A considerable change in the spectrum of hsp 70 took place 48 h after heat shock (Fig. 2c). First, the inducible hsp 70 with mol. wt. 71 and pI about 5.8, which was found after 24 h, had disappeared, and second, the content of HSC 73 fell to the control level; third and last, there was a marked degree of accumulation of several isoforms of inducible hsp 70 with mol. wt. of about 71 kD and pI within the range 5.9-6.3, among which the most alkaline was also the most representative (Fig. 2C).

Thus the isoform composition of stress proteins induced by heat shock depends on the time after exposure to stress. Initially a polypeptide with pI 5.8 accumulates, followed by several more alkaline polypeptides. This trend of the isoform composition of hsp 70 probably reflects the time sequence of activation of expression of the genes of different isoforms of hsp 70 after heat shock.

Evidence in support of this hypothesis is given by data obtained on Drosophila, HeLa, yeast, and man [6, 11, 12], showing that the latent period between the end of exposure to stress and the beginning of transcription of hsp 70 genes depends on the sensitivity of the promoter region of the gene to activating influences. This, in turn, is determined by the structural organization of the promoter, namely by the number of regulatory sequences, such as HSE, Spl, etc., and their arrangement relative to the TATA box [12]. Correspondingly, it might be supposed that the sequential induction of hsp 70 isoforms in the rat myocardium after heat shock is connected with the particular features of organization of the promoter zone of the genes of different hsp 70 isoforms. The possibility likewise cannot be ruled out that sequential activation of genes of different hsp isoforms reflects definite cooperative interactions within the family of hsp 70 genes itself, more especially because similar interactions in other families of genes are widely represented in nature [14].

It must be emphasized that the temporary coordinated appearance of different hsp 70 isoforms in cells may reflect the functional role of a particular hsp 70 isoform at a definite stage of recovery after heat shock, and it is therefore evident that further research in this direction could be very promising.

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