ALTERED GRAVITY EFFECT ON THE HEAT SHOCK PROTEIN LEVEL IN PLANTS

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ABSTRACT

Significant environmental deviations often cause activation of synthesis of heat shock proteins (HSPs). We supposed a participation of HSPs in plant adaptation to altered gravity. In this work, we tested whether clinorotation causes an activation of HSP90 and HSP70 synthesis. 3-day old pea seedlings were subjected to short (2-10 h) clinorotation (2 rpm). The increased HSP70 and HSP90 levels in response to clinorotation were determined by Western-blot analysis. Such increasing was more significant for HSP70 than for HSP90. The obtained data testify upregulation of the stress proteins HSP90 and HSP70 in pea seedlings under clinorotation.

1. INTRODUCTION

HSP70 and HSP90 are highly conserved and abundant in cells molecular chaperones that are essential for viability in eukaryotes. Under normal growth conditions, they serve in several basic biological functions, including protein folding, transport and assembly of nascent proteins, maintenance of the proper conformation and regulation of proteins [1, 2]. HSP expression increases differentially in response to different kinds of stress including elevated temperature, desiccation, anoxia, salinity, heavy metals, chilling and many others [1]. So, the HSPs are related to the components of the multiple stress resistance mechanism [3].

Altered gravity (clinorotation) causes changes in the metabolism and ultrastructre of plants [4], so it may be considered as a stress factor. A number of studies with human cells have indicated influence of altered gravity on HSP70 expression [5, 6]. We supposed a participation of HSPs in plant adaptation to altered gravity.

We have recently determined that HSP70 and HSP90 were abundant in embryos of dry pea seeds and decreased significantly during seed germination, as it is characteristic for seeds [1]; however, their levels were higher in the seedlings permanently grown on the slow horizontal clinostat. It has been supposed this HSP70 and HSP90 level increasing may be caused by a delay of hydrolysis of the HSPs preformed in dry seeds or activation of their expression under clinorotation. The aim of this study is elucidation whether clinorotation causes an activation of HSP90 and HSP70 synthesis.

2. MATERIAL AND METHODS

Pea (Pisum sativum L., cv. “Damir”) seeds were germinated and seedlings grew in the dark at 22 ± 1°C for 3 days in the stationary growth conditions and then were subjected to 2-, 4-, 6-, 8- and 10-h horizontal clinorotation. Seedlings grown in the stationary conditions for 3 days + 2 h and 3 days + 10 h served as the control. HSP90 and HSP70 levels in the seedlings were analyzed by Western-blot analysis after 1-dimentional denaturing polyacrylamide gel electrophoresis of total soluble proteins. Monoclonal antibodies against HSP70, HSP90, actin and secondary anti-mouse antibodies coupled to biotin (Sigma) were used. Immunoreactive spots on the blots were visualized by ExtrAvidin-peroxidase system (Sigma). Quantification of the blots was done by measuring the spot integrated density value corrected for background using ImageMaster™ TotalLab, 2.00 (Amersham). The experiment was carried out 3 times.

3. RESULTS AND DISCUSSION

An increase in HSP90 and HSP70 levels as a result of short-term clinorotation was revealed in pea seedlings (Fig. 1). Their higher amounts were already shown after 2-h clinorotation, but the increasing in the HSP70 was more significant than in the HSP90. The HSP90 level remained high over the whole 10-h period of clinorotation with a tendency for normalization after 10 h. At the same time, the HSP70 level was maximal after 2-4-h clinorotation and declined to the control by 10 h. Decreasing in the HSP levels towards the end of the 10-h period of clinorotation may indicate that processes in which HSP90 and HSP70 take part become less intensive.

This is in keeping with the results of other authors that show the alterations in gene expression and protein turnover in plants exposed to clinorotation for several hours. Thus, 15- and 24-h clinorotation induced significant modifications in ubiquitination of some Vicia faba chloroplast proteins indicating selective changes in protein turnover [7]. Moreover, upregulation of HSP expression under altered gravity was shown in animal cells. Time-dependent HSP70 expression was determined in rat brain after exposure to hypergravity [8]. Endothelial cells, which maintained the capability to proliferate under simulated microgravity, upregulated HSP70 [5]. The
authors have proposed HSP70 may take part in cell growth modulating. Apparently, altered gravity may disturb some processes, and their maintenance may need refolding and reassembly of some proteins or straining of other HSP functions.

Fig. 1. Immunoblots of HSP90, HSP70 and actin (as an internal control of protein loading) after SDS-PAGE of total soluble protein of the pea seedlings grown in the stationary conditions (control) for 3 days + 2 h (1) and 3 days + 10 h (7) or subjected to 2-, 4-, 6-, 8- and 10-h clinorotation (2, 3, 4, 5, 6, correspondently). Relative protein quantity was calculated for each protein as a ratio of the integrated density value of every spot to the least one. Mean values ± SE (p=0.95) of the relative protein quantities of HSP90 and HSP70 from three experiments are shown at the graphs.

The obtained results testify our supposition that clinorotation may cause time-dependent HSP90 and HSP70 overexpression in plant cells, which may be involved in plant adaptation.

4. REFERENCES


