Abstract

The aim of this thesis was to contribute to understanding of the functions of the alternative sigma factors of RNA polymerase, σ^H a σ^M, during stress response of *C. glutamicum*. The role of σ^H and σ^M in the transcription of the gene *sigM* encoding σ^M and the genes of the operon *dnaK-grpE-dnaJ-hspR* which encode proteins involved in heat-shock response of *C. glutamicum* was studied. The promoters of the tested genes were cloned into the *„promoter-probe“* vector pET2 and their activity was determined by the measuring of specific activity of the reporter enzyme chloramfenicol acetyltransferase. It was found, that the heat stress has a moderate positive effect on the activity of the promoter of the gene *sigM* (P-*sigM*), whereas no effect of the oxidative stress induced by diamide was found. It was proved, that deletion of the gene *sigH* or *sigM* itself does not lead to decrease of activity of the promoter P-*sigM* neither in standard conditions nor after heat-shock. On the other hand, complete abolition of the activity of the promoter P-*sigM* was observed in the strain *C. glutamicum* Δ*sigH*Δ*sigM*. The promoter of the gene *sigM* is thus recognized in standard conditions and after heat stress is recognized by both sigma factors σ^H and σ^M, but not by the sigma factor σ^A. It was shown, that mutation in the -10 region of the promoter P-*sigM* leads to its inactivation and that the trinucleotide GTT in the position 8 bp upstream of the transcriptional start point of the gene *sigM* is necessary for the function of P-*sigM*. These results lead us to conclusion that the gene *sigM* is transcribed from a single promoter. A significant decrease of activity of the promoters of the *dnaK* operon was found after the heat-shock in the strains *C. glutamicum* Δ*sigH* and *C. glutamicum* Δ*sigM*. This findings lead to conclusion that after heat-shock is functional mainly promoter P2-*dnaK* which is recognized (as well as P-*sigM*) not only by sigma factor σ^H, but also by sigma factor σ^M. This work shows for the first time that two alternative sigma factors, σ^H and σ^M , can be mutually substituted during transcription from a *C. glutamicum* promoter recognized by an alternative sigma factor.

Keywords: *Corynebacterium glutamicum*, sigma factor, promoter, transcription, *cat*, heat stress