INFLUENCE OF THE ACTIVATION TEMPERATURE ON THE DEGRADATION OF RECOMBINANT PROTEIN IN *E. coli*

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The expression system based on the control of the λP_L promoter by the thermosensitive cIts regulator is one of the most widely used for *E. coli* large-scale protein production. This thermoinducible promoter is activated at temperatures around 42ºC and fully repressed at 30ºC. The major shortcomings of this temperature shift methodology are the problems commonly associated with protein expression above 37^{o}C (e.g., proteolysis, degradation, aggregation) that can negatively influence the production of particularly labile proteins.

In recent publication we described the synthesis of periplasmic human prolactin (hPRL) and human growth hormone (hGH) by utilizing the vector containing the λP_L promoter, but without the presence of the repressor protein and under optimized temperature conditions (Soares et al., J. Biotecnol. 2008; 133:27-35). It has been demonstrated that hPRL periplasmic secretion, under control of the constitutive λP_L promoter has a maximal efficiency at 37°C (1µg/mL/A_{600}) decreasing at higher temperatures. Since the bacterial periplasmic environment has been found to be quite detrimental to protein stability, especially above room temperature, we carried out western blot analysis of *E. coli* derived purified hPRL, total bacterial lysates and osmotic shock fluids obtained after activation at 37°C or at 42°C.

With basis on our results, we could conclude that hPRL is highly degraded in the cytoplasm, at 42°C, a degradation that, to a limited extent, is already occurring at 37°C. This seems be due to an increased activity of cytoplasmic proteases at 42ºC, because purified hPRL is stable at these temperatures. The levels of accumulated heterologous protein can thus be the result of an equilibrium between rate of protein synthesis and rate of degradation. For hPRL such equilibrium could be particularly in favor of the rate of synthesis, at 37°C.

We are in the process of analyzing other proteins, all produced in *E. coli* periplasm, such as hGH or the prolactin antagonist, S129D-hPRL.

Palavras-chave: hGH, prolactin, E.coli