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Protein response of insect cells to bioreactor environmental stresses

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Abstract

Protein expression of *Spodoptera frugiperda* (Sf9) insect cells was characterized upon exposure to environmental stresses typically present in bioreactors including heat shock, oxygen deprivation, shear stress, change of pH, and salinity or ethanol shock. This study fills the void in knowledge as to how bioreactor hydrodynamics, anoxia, small changes in pH as well as salinity alterations due to pH control or exposure to ethanol used in asepsis treatments affect protein expression in Sf9 cells. Heat shock at 43 °C induced proteins at 83 kDa, 68–78 kDa and six small heat shock proteins (hsps) at 23–15.5 kDa. Anaerobic conditions in CO_2 atmosphere reduced significantly the normal protein synthesis and induced a small subset of heat shock proteins at 70 kDa. Oxygen deprivation in nitrogen atmosphere transiently induces the 70 kDa proteins and had minor effects on the normal protein synthesis. Exposure to increased salinity or ethanol concentration failed to trigger the stress response, but may extensively inhibit the induction of normal proteins even though there was a negligible change in cell viability. Shear stress that had a major reducing effect on cell viability did not change the protein synthesis profile of Sf9 cells. Both long and short term exposures to small pH changes had negligible effects on protein synthesis.

Keywords: Heat shock protein; Stress protein; Bioreactor; Anoxia; Salinity; Ethanol; Shear stress; pH change

1. Introduction

Animal cells growing in a large-scale bioreactor face an environment completely different from the mild environment of a T-flask used in early steps of cell cultivation and product development. For example, cells are subjected to agitation and exposure to gas–liquid interface due to aeration. Cells are also exposed to temperature and salinity changes and pH alterations due to addition of acid or base for pH control. Cells are

Abbreviations: CHAPS, 3-((3-cholamidopropyl)-dimethylammonio)-1-propane-sulfonate; grp, glucose regulated protein; hap, hypoxia associated protein; hsp, heat shock protein; IEF, isoelectric focusing; LDH, lactate dehydrogenase; NADH, nicotinamide adenine dinucleotide-reduced form; NAD(P)H, nicotinamide adenine dinucleotide phosphate-reduced form; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate buffered saline; SDS, sodium dodecyl sulphate; Sf, *Spodoptera frugiperda*

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known to respond to various kind of stress situations by inducing a specific group of proteins, often called the heat shock proteins, which are now more commonly referred to as stress proteins. While effects of elevated temperatures on protein production have been investigated in a number of organisms, consequences of other stresses observed in a typical bioreactor environment have not been systematically investigated. Although loss of cell viability due to bioreactor hydrodynamics (agitation and aeration) has been extensively investigated (Tramper et al., 1986, 1988; Goldblum et al., 1990; Murhammer and Goochee, 1990; Ramirez and Mutharasan, 1990; Bavarian et al., 1991; Chalmers and Bavarian, 1991; Cherry and Hulle, 1992; Garcia-Briones and Chalmers, 1992; Trinh et al., 1994; Wu and Mutharasan, 1994; Wu and Goosen, 1995; Palomares et al., 2000; O'Connor et al., 2002), there is a void in knowledge as to how mechanical stresses as well as oxygen deficiency affect stress protein production. Furthermore, little is known about effects of small changes in extracellular pH on protein induction. Variability in medium composition affecting salinity and ethanol used in asepsis treatments are also potential inducers of stress proteins.

The heat shock response was first discovered by Italian geneticist Fernando Ritossa when he observed a new buffing pattern on the salivary glands chromosomes of *Drosophila* fruit fly as a response to a small increase in temperature and exposure to certain chemicals (Ritossa, 1962). In the early 1970s Tissières and colleagues demonstrated that the heat induced chromosomal buffing was accompanied by the high level expression of unique set of heat shock proteins (Tissières et al., 1974). These observations were among the first examples of altered gene expression caused by an environmental stressor and a start of a new field of research aimed at understanding why and how heat shock and other stressors modify the course of gene expression. Today it is well known that most cells respond to elevated temperatures by synthesizing a specific set of proteins known as heat shock proteins (hsps). Similar responses can be induced by several chemical stressors such as amino acid analogs, alcohols, heavy metals, sulfhydryl reagents, oxidizing agents, gene expression inhibitors, steroid hormones, and other agents such as suboptimal temperatures, anoxia, nutrient starvation, and viral infection (Nover, 1991). The effectiveness of these agents often varies. The rapidity, selectivity and extent of induction are usually much lower than upon heat shock. The increased expression of stress proteins has also been observed in cells and tissues representing a wide distribution of human diseases including cardiac hypertrophy, fever, inflammation, oxidant injury, metabolic diseases, and cell and tissue damage (Morimoto et al., 1990). Consequently due to fact that so many agents and treatments can induce similar responses in gene expression, "heat shock protein" or "heat shock response" are now more generally referred to as "stress protein" or "stress response".

Stress proteins are often divided into two major groups: heat shock proteins (hsps) and glucose regulated proteins (grps). Because their exact function was unclear when they were first discovered, stress proteins are usually referred to according to both their mode of induction and by their molecular sizes separated in sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The most widely recognized stress proteins, also called major heat shock proteins, are 60–70, 90–110 and 20–30 kDa (Subjeck and Shyy, 1986; Schlesinger, 1994). Of these, the 70 kDa family is usually strongly induced in most organisms. The 90 kDa protein is also widely observed in many organisms, but the 110 kDa-component has mainly been observed in mammalian cells. There may also be many secondary heat shock proteins, but most research has focused on those that are induced in higher levels, normally 0.5–1.5% of total cellular proteins (Lindquist, 1992). Although referred to as stress proteins, most of these proteins are in fact expressed constitutively in normal unstressed conditions, often in a tissue-specific manner and at particular state of development (Lindquist and Craig, 1988; Welch, 1990, 1993; Burel et al., 1992; Pauli et al., 1992).

The investigation of the function of stress proteins began with the observation that stress protein expression was correlated with inducible stress tolerance. When cells are exposed directly to an extreme temperature or to some other stress agent, cells will be damaged or may even die. But if the cells are first subjected to a sublethal stress, they will become more tolerant to extreme lethal stresses. Even cross-tolerance is possible. Stress tolerance induced by one stressor can protect cells against other stressors. For example, stress proteins induced in response to high temperature can protect cells against alcohols or heavy metals. Although the exact function of stress proteins was unclear for a