Denaturation of membrane proteins and hyperthermic cell killing

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Hyperthermia (treatment of cells a few degrees above their growth temperature) can lead to loss of the cells reproductive capacity (cell kill), and sensitizes cells for radiation and chemotherapeutics. The mechanisms underlying hyperthermic cell killing are unclear as yet. Data from the literature indicate an important role for heat induced protein denaturation, and the involvement of membrane damage in the process of cell killing. Manipulation of the lipid composition of cellular membranes may have a modulating effect on cell killing. Cellular membranes consist of two major components: lipids and proteins. It has been shown that the lipid fraction is not directly influencing the heat sensitivity of cells. Lipids may however affect the heat sensitivity of the membrane proteins in an indirect way.

The experiments described in this thesis are performed to gain more information on the role of membrane proteins in the process of hyperthermic cell killing. For this purpose, heat-induced protein denaturation is studied in an isolated subcellular fraction, containing the cellular membranes, except the nuclear membrane: this fraction is named the particulate fraction (PF).

During heating, changes were observed in the proteins of the membrane fractions (chapter 2). Using Electron Spin Resonance (ESR) conformational changes in the proteins were shown to occur between 38 and 44°C, between 47 and 53°C and above 58°C. The last two conformational changes appeared to be irreversible; after cooling and subsequent reheating of the membrane preparation, these changes were not observed (chapter 2). Aggregation, caused by formation of intermolecular disulphide bridges, of three heat-labile proteins was observed using Thermal Gel Analysis (TGA). Aggregation of these proteins took place during heating from 42-50°C. As stated in the introduction (1.3.1), denaturation and aggregation temperatures are no absolute entities, but depend on the heating rate. When heating is performed at a higher heating rate, denaturation temperatures will be higher.

When the membrane fractions are heated in the presence of different concentrations of chemical agents which increase the heat sensitivity of whole cells (chapter 4), the denaturation temperature of the membrane proteins is decreased in a concentration dependent manner.

When the membrane fraction, isolated from heat-induced thermotolerant cells (HTT) is heated, the denaturation temperature of the proteins was higher as compared to control cells (chapter 2). The degree of thermotolerance in the cells (cell survival) was reflected in the magnitude of the shift of the
denaturation temperatures. When membrane fractions isolated from cells in which thermotolerance was induced by ethanol (ETT), the correlation between the denaturation temperature of the proteins in this fraction and the thermal sensitivity of the cells did not hold (chapter 5). This in contrast to the situation of arsenite induced thermotolerance (ATT). In ATT cells, the proteins in the membrane fraction were found to be resistant against heat induced denaturation (as found for HTT cells), but in ETT cells, no enhanced heat resistance of the proteins in the membrane fraction was found. To explain these observations a model is proposed in which resistance is only induced in proteins that are damaged during the thermotolerance inducing treatments. In this model, ethanol is supposed to damage heat sensitive proteins in other subcellular fractions (e.g. the nucleus).

Cells exposed to stress (e.g. heat, sodium-arsenite or ethanol) respond by synthesizing a specific set of proteins, the so called "heat shock proteins" or HSPs. These proteins are thought to be involved in the development of thermotolerance, and thought to protect the cells against damage induced by that particular stress. These proteins are also present in the cell under physiological conditions (albeit in lower concentrations), which indicates a role for these proteins in unstressed conditions (e.g. in protein folding and translocation of proteins across cellular membranes). Inhibition of stress-induced synthesis of HSPs (by cycloheximide (CHX)) does not always prevent (partial) development of thermotolerance (chapter 5). When heat or ethanol are used as inducers, even in the presence of CHX, thermotolerance can develop to some extent (protein synthesis independent thermotolerance). However, development of arsenite induced thermotolerance can completely be inhibited by CHX (chapter 5). Assuming the involvement of HSPs in the development of protein synthesis independent thermotolerance, a model is proposed to explain why the constitutively present HSPs are available for the development of thermotolerance in HTT+CHX and ETT+CHX cells, but not in ATT+CHX cells. According to this model, the availability of the constitutive HSPs depends on the manner in which the thermotolerance inducing agents affect (inhibit) the cells protein synthesis (chapter 5).

In chapter 6, a possible relation between an enhanced HSP72 content of the membrane fraction and the increase of the denaturation temperature of the proteins in these fractions was investigated. In all fractions in which previously an increase in the denaturation temperature of proteins was observed (HTT, HTT+CHX, and ATT), an increase in the HSP72 content was also found. However, also in the membrane fraction of ETT cells an increase in the HSP72 content was seen, although no increase in the denaturation temperature of the proteins was found. Binding of HSP72 to
Thermotolerance was found in the denaturation and thermal sensitivity of the membrane fraction of arsenite-resistant cells. The induced denaturation (as a measure of the heat resistance of the membrane) is consistent with the observations in proteins that are ethanol-sensitive but heat-insensitive. In this model, heat shock proteins (HSPs) respond by increasing the availability of proteins in the membrane. This indicates that ethanol-induced heat resistance should be found in proteins of other subcellular fractions, e.g., nuclear proteins as already indicated above.

For further elucidation of a possible role of HSP72 in the increased heat resistance of the proteins in the membrane fractions, it will probably be necessary to determine to which proteins the HSPs are bound. Assuming roles for HSPs in the induced resistance against protein denaturation as well as in the disaggregation process, a model is described in the general discussion.

Summarizing: heat induced denaturation of membrane proteins is probably related to hyperthermic cell killing. Induced resistance of heat sensitive proteins seems to be involved in the development of thermotolerance. Although many questions remain to be answered, it appears that HSP72, when bound to membrane proteins, is capable of providing heat resistance to these proteins.