Abstract

Cellular utilization of nutrients often necessitates the handling of metabolic side-reactions and the detoxification of hazardous by-products and intermediates formed e.g. reactive oxygen species (ROS), byproducts of aerobic metabolism, or methylglyoxal produced e.g. enzymatically or non-enzymatically in glucose catabolism.

In this thesis the functionally uncharacterised genes *YML070W/DAK1* and *YFL053W/DAK2* in the yeast *Saccharomyces cerevisiae* were characterised as dihydroxyacetone kinases (DAKs), an enzyme present in most organisms. The substrate dihydroxyacetone (DHA), used by humans as an artificial self-tanning agent for medical and cosmetic reasons, was found to be toxic to yeast cells and detoxification to be dependent on the presence of a functional DAK. DHA affinities of the two DAK enzymes were in the μM range, in keeping with a role in DHA detoxification. Overexpression of either DAK enabled cells to grow on DHA as sole carbon and energy source. Protein expression studies of DHA growth revealed regulatory changes suggesting formate production and furthermore formaldehyde dehydrogenase (*SFA1*) deletion resulted in impaired adaptation to DHA growth as well as survival of DHA-dependent death, indicating DHA detoxification partly via formaldehyde and formate.

Functional differences between the two DAK isogenes were suggested by the absence of *DAK2* protein and mRNA when controlled by the same strong promoter on glucose, where *DAK1* was dominantly expressed, but not on ethanol or DHA medium. Initiation of transcription from the promoter seemed similar, indicating glucose-induced destabilisation of *DAK2* mRNA.

On a high DHA level, strains overexpressing the less efficient of the two isoenzymes, Dak1p, grew more slowly and showed induction of a ROS defense system, alkyl hydroperoxide reductase (Ahp1p), as well as accumulation of advanced glycation end (AGE) products, irreversible protein modifications associated with diabetic complications and aging. AGE formation correlated negatively to cell survival on lethal DHA levels, indicating a close linkage to mechanisms of death. DHA detoxification was found to be independent of central oxidative stress regulators, the main defense systems for the handling of the ROS superoxide anion and the main methylglyoxal detoxifying system, indicating no link of DHA toxicity to methylglyoxal or ROS formation. A *GSH1* mutant was, however, found extremely sensitive to DHA dependent death, indicating glutathione biosynthesis crucial for DHA detoxification.

It is proposed that detoxification of DHA might be a vital part of the physiological response during diverse stress conditions in many species.