Cytosolic acidification as a signal mediating hyperosmotic stress responses in Dictyostelium discoideum


ABSTRACT
Together, these results indicate that hyperosmotic stress triggers pleiotropic effects, which are partially mediated by a pH signal and which all contribute to the downregulation of cellular activity. The comparison of our results with the effect of hyperosmolarity and intracellular acidification on receptor-mediated endocytosis in mammalian cells reveals striking similarities, suggesting the hypothesis of the same mechanism of inhibition by low internal pH.


INTRODUCTION
Background Cells steadily face fluctuations of the external osmolarity due to dehydration. Occasionally, dramatic changes in osmolarity can occur, resulting in a stress condition. Hyperosmolarity of the external medium leads to the extrusion of water and the concomitant shrinkage of cells. Within a few minutes, the cells activate mechanisms, termed "regulatory volume increase" (RVI), to regain their volume. Under prolonged hyperosmotic conditions, compatible osmolytes, e.g. polyols or amines are accumulated inside the cells. These osmolytes exhibit a stabilizing effect on proteins and thereby avoid the deleterious effect of protein aggregation. In addition, the expression of stress proteins, as chaperones and DNA repair proteins was observed in various organisms in response to hypertonicity. Recently it could be shown, that the amoeba Dictyostelium discoideum exhibits an unusual response to hypertonic stress which is distinct from the response observed in other organisms: the cells remain shrunken after water extrusion and they neither accumulate compatible osmolytes nor express stress proteins. Instead, Dictyostelium largely rearranges cellular proteins between compartments. Especially the enhanced recruitment of major cytoskeletal proteins to the cell cortex, i.e. the layer beneath the plasma membrane plays a pivotal role, as increased amounts of actin and myosin II co-localize at the cell cortex of hyperosmotically shocked cells and form a rigid network. The formation of this shell-like structure is believed to be the major osmoprotective mechanism in Dictyostelium. The osmoprotective role of the cytoskeletal reinforcement is also supported by the fact that several cytoskeletal proteins are essential for viability under hypertonic conditions. The knowledge about the signalling pathways involved in osmoregulation is scarce. In response to hyperosmotic stress, guanylyl cyclase is activated, resulting in an increase in cGMP concentration. This induces the phosphorylation of the myosin II heavy chain kinase, which in turn results in the phosphorylation of myosin II heavy chain. Thereby, the myosin II filaments are disassembled as a prerequesite for their rearrangement to the cell cortex. In addition, DokA, a homologue of bacterial histidine kinases, was shown to be essential under hypertonic conditions, suggesting a role for 2-component regulatory elements in osmoregulation. Furthermore, cAMP is believed to play an essential role during the spore state, which represents a naturally occurring hyperosmotic condition induced by the developmental program of the amoeba. The adenylyl cyclase G was shown to be an osmosensor, which suppresses germination under hyperosmotic conditions in spore cells. This correlates with the observation that high cAMP concentrations are maintained during spore dormancy. In addition, acidification of the external medium has been observed in response to hypertonicity, which was attributed to the secretion of protons. We therefore addressed the question, whether this increase in

external proton concentration correlates with a change in pH in an intracellular compartment. Cytosolic pH changes have been shown to act as signals in regulating proteins and cellular processes in various organisms. The processes regulated by cytosolic pH changes include e.g. glycolysis, protein synthesis, DNA synthesis, motility and activation from dormant states. Generally, the acidification of the cytosol correlated with a decrease, whereas the alkalinization correlated with an increase in activity or efficiency of cellular processes. In addition to cytosolic pH, homeostasis in the endo-lysosomal pH has been shown to be essential for cellular processes: perturbations of the pH affect endocytosis as well as the post-translational modification and sorting of proteins in Dictyostelium cells. In this paper we present evidence that high osmolarity results in a significant acidification and a concomitant decrease in endocytic activity, as well as a depletion of the NTP pool, indicating a general decrease in cellular activity.

## CONCLUSION

We could demonstrate that Dictyostelium cells which are exposed to hypertonic conditions exhibit a significant internal acidification, a depletion of the internal NTP pool as well as a downregulation of vesicular mobility and a total inhibition of fluid-phase endocytosis and exocytosis. In addition, our results show that the cytosolic acidification can be responsible for the block of fluid-phase endocytosis, thereby acting as signal mediator. Together these results suggest, that hyperosmotic stress elicits pleiotropic effects in Dictyostelium cells resulting in a general downregulation of cellular activity.