Acta fytotechn zootechn, 27, 2024(4): 396-405
http://www.acta.fapz.uniag.sk

Original Paper

Kinetics of Acrylamide-Induced Changes in Ionome Composition in *Schizosaccharomyces pombe*

Marek Kovár[1*], Alica Navrátilová[2], Ivona Jančo[3], Lucia Klongová[3], Miroslava Požgajová[3]

[1] *Slovak University of Agriculture in Nitra, Faculty of Agrobiology and Food Resources, Institute of Plant and Environmental Sciences, Slovakia*

[2] *Slovak University of Agriculture in Nitra, Faculty of Agrobiology and Food Resources, Institute of Nutrition and Genomics, Slovakia*

[3] *Slovak University of Agriculture in Nitra, AgroBioTech Research Centre, Slovakia*

Article Details

Received: 2024-10-24 | Accepted: 2024-12-03 | Available online: 2024-12-31

https://doi.org/10.15414/afz.2024.27.04.396-405

In stressful situations, maintaining ionome homeostasis in the intracellular space is a key prerequisite for cell survival. This study investigates the role of ionome alteration due to acrylamide-induced oxidative stress in *Schizosaccharomyces pombe*. With the use of inductively coupled plasma-optical emission spectroscopy (ICP-OES) we have evaluated changes in the intracellular concentration of eight mineral elements (calcium, potassium, sodium, magnesium, manganese, zinc, iron, copper). In this study, the deleterious effect of acrylamide (1 and 10 mM) on the uptake and utilization of mineral elements and the disruption of ionome homeostasis has been revealed. We confirmed that the toxicity of acrylamide (mainly 10 mM concentration) for cells and their biological activity is associated with oxidative stress due to enhanced accumulation of reactive oxygen species (ROS) and increased formation of malondialdehyde (MDA). The addition of acrylamide into cultivation media in a final concentration of 10 mM led to 72.3% increase in total ROS production. At the same time, this resulted in statistically significant changes in the ionome, accompanied by an increase in the concentrations of monovalent ions K^+ and Na^+, divalent ions Ca^{2+}, Mg^{2+}, Cu^{2+}, and trivalent Fe^{3+}. These findings shed light on the complexity of the acrylamide-induced metabolic disorders that lead to the imbalance of ion homeostasis.

Keywords: acrylamide, ionomics, ROS, oxidative stress, fission yeast

1 Introduction

In addition to water, the most abundant components of every living cell are mineral ions. The organism is composed of many chemical elements of the periodic table, 17 of which are essential for life processes. In addition to the basic structural elements of organic matter (carbon, oxygen, nitrogen), there are mineral elements that participate in energetic and metabolic reactions (phosphorus, magnesium, sulphur, selenium). Minerals that are essential components of prosthetic groups in enzymes or participate in redox reactions, contributing to the functional integrity of enzymes include

[*] **Corresponding Author:** Marek Kovár, Slovak University of Agriculture in Nitra, Faculty of Agrobiology and Food Resources, Institute of Plant and Environmental Sciences, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia. E-mail: marek.kovar@uniag.sk ORCID: https://orcid.org/0000-0002-1478-8383

manganese, copper, iron, zinc, molybdenum, boron. Additionally, calcium acts as a second messenger in cellular signaling, while potassium, sodium, and chlorine help maintain ion balance and osmoregulation in the intracellular environment (Remick & Helmann, 2023). Homeostasis of ions in the cell is a basic requirement for the maintenance of life processes and survival for all organisms. The unicellular organism fission yeast *Schizosaccharomyces pombe* is a popular model for studying fundamental aspects of cell biology and toxicology (Álvarez-Herrera et al., 2024). Many studies in recent years have focused on the molecular mechanisms of nutrient uptake, transport, and utilization in unicellular organisms, including yeast (Aulakh et al., 2022; Jeyasingh et al., 2023). However, there remain gaps in our understanding of how changes in the cellular ionome affect metabolism, particularly regarding the influence of natural and food contaminants. This is especially important considering their role in activating oxidative damage.

Acrylamide (AA) is one of the compounds that induces oxidative stress in the cell. AA is an organic compound containing an unsaturated carbonyl group and a highly reactive vinyl monomer and is classified as a xenobiotic (Lipworth et al., 2012). In the last two decades, a huge research effort has been devoted to the study of the toxicity of AA, focusing mainly on its genotoxicity, neurotoxicity, immunotoxicity, and reproductive toxicity (Huchthausen et al., 2023).

The production of reactive oxygen species (ROS) and their derivates is an integral part of the metabolism of a living organism (Halliwell, 2006). ROS comprise free radicals (hydroxyl radical $OH^{\bullet}$, alkoxy radical $RO^{\bullet}$, hydroperoxyl radical $HO_2^{\bullet}$, and superoxide anion $O_2^{\bullet-}$) and non-radical molecules such as hydrogen peroxide H_2O_2 and singlet oxygen $^1O_2$ (Shehzad & Mustafa, 2023). ROS are produced in response to several abiotic stimuli, including food contaminants (such as acrylamide) when excessive energy from the electron transport chain of mitochondria (or even chloroplasts in plants) and the activation of NADPH oxidases and peroxidases flow to the oxygen molecule. Cells have evolved several ways to detoxify ROS, which are based on their enzymatic or non-enzymatic degradation. If the production of ROS is higher than the ability of the metabolism to detoxify them, oxidative stress occurs. Intensive research has demonstrated that the induction of oxidative damage is a central phenomenon in many stress situations encountered by organisms, including yeast. These stressors include sub- and supraoptimal temperatures, high osmolality, salinity, and nutritional deprivation (Sies et al., 2022).

The low availability of nutrients, along with their high or toxic concentration, induces processes leading to oxidative stress and cell damage, as documented in numerous studies (López-Barneo et al., 2001). However, on the other hand, oxidative stress also causes disturbances in the intake and release of mineral substances and the maintenance of ionome homeostasis (Aulakh et al., 2022). The ionome is defined as the composition of mineral nutrients and trace elements of the organism. The mineral composition (often referred to as ash) represents the inorganic component of cellular systems and the entire organism.

Oxidative stress can not only damage metabolic processes in the cell, but also indirectly alter signal transduction through the activation of ion channels located in the plasma and tonoplast. Electrophysiological studies on animal cells (López-Barneo et al., 2001), yeast (Carraro & Bernardi, 2016), and plants (Demidchik, 2018) have demonstrated that ion channels play a significant role in ROS perception. Quantitatively, the activity of Ca^{2+} channels and the intracellular concentration of Ca^{2+} is associated with the degree of oxidative damage in yeast (Carraro & Bernardi, 2016; Evans et al., 2016).

In light of these considerations, this study attempted to quantify the dynamics of changes in the content of basic mineral elements in *S. pombe* cells affected by acrylamide and to reveal the role of oxidative stress in disrupting the maintenance of ion homeostasis using an ionomics approach. The research aim of this study was to understand the mechanisms that underline the harmful effect of acrylamide on the uptake and utilization of mineral nutrients that lead to changes in cell growth. Ultimately, this study may pave the way for the development of innovative mitigation strategies to alleviate the toxic effect of acrylamide on cellular metabolism.

2 Material and methods

2.1 Biological material and cultivation

The *Schizosaccharomyces pombe* strain used in this study was *SP72 h+ ade6-M210 ura4-D18 leu 1-32* derived from the original ancestor strain *972 h-*. Yeast cells were stored in a deep freezer box at −80 °C and cultivated (twice) on solid YES medium in the incubator at 30 °C before the start of

experiments. The solid medium consisted of 0,5% yeast extract, 3% glucose, and amino acids supplementation, including adenine, L-histidine, L-leucine, and L-lysine, at a concentration of 525 $mg \cdot L^{-1}$ and 2% agar. Subsequently, cells grew in a complete liquid YES medium under aerobic conditions at 30 °C and shaking at 150 rpm (incubator GFL 3031, GFL Gesellschaft für Labortechnik mbH, Burgwedel, Germany) overnight. The ionic composition of the liquid YES medium is presented in Table 1.

2.2 Yeast growth rate assessment

Overnight cell culture in the exponential phase of growth was used for the determination of growth rate assessment according to Kovár et al. (2022). Briefly, yeast cells culture with adjusted optical density OD_{600} to 0.30 were transferred to 24-well plates containing complete liquid YES medium supplemented with acrylamide (AA) (CAS 79-06-1, Sigma Aldrich) at final concentrations 0, 1, 10 mM. Plates were incubated at 30 °C and 150 rpm and OD_{600} was determined at time points 0, 3, 6, and 9 hours using the Glomax Multi Detection system (Promega Corporation, Madison, WI, USA). The generation time (gt) of yeast cells was calculated according to the formula:

$$\mathrm{gt} = \frac{\log(2)}{\mathrm{m}}$$

where m is the slope of the regression line between OD_{600} and time. Relative growth rate (RGR) was calculated as:

$$\mathrm{RGR} = \frac{\ln(\mathrm{OD}_{600})_{\mathrm{t1}} - \ln(\mathrm{OD}_{600})_{\mathrm{t0}}}{\Delta \mathrm{t}}$$

where index t1 indicates OD_{600} at time 9 h, t0 is OD_{600} at 0 h and Δt is time difference (h).

Table 1 Ion composition of liquid YES medium, determined by ICP-OES and analytical parameters for elements determination

Elements	Wavelength (nm)	Concentration ($g \cdot L^{-1}$)	LoD ($\mu g \cdot L^{-1}$)
Potassium (K)	766.491	1267.221 ± 35.880	0.39
Sodium (Na)	589.592	51.068 ± 6.161	0.15
Calcium (Ca)	315.887	17.171 ± 3.641	0.01
Zinc (Zn)	206.200	5.096 ± 0.128	0.21
Iron (Fe)	234.350	1.643 ± 0.120	0.10
Magnesium (Mg)	383.829	0.943 ± 0.104	0.01
Manganese (Mn)	257.610	0.052 ± 0.003	0.03
Copper (Cu)	324.754	0.036 ± 0.001	0.30

Values represent mean ± SD (n = 3). LoD – limit of detection

2.3 ROS and malondialdehyde assay

Cells incubated with different AA concentrations (0, 1, and 10 mM) were collected by centrifugation in a pre-cooled (4 °C) centrifuge (Hettich Rotina 420/420 R) at 8500 rpm and 90 seconds, 3 times washed with sterile cold water (4 °C) and resuspended in cold PBS buffer (pH 7.0). Cell lysis was performed with sonication using the Digital Sonifier 450 (Branson Ultrasonics Corp, Danbury, CT, USA) with setup according to Kovár et al. (2022). Finally, the lysate was centrifugated for 15 minutes at 14,000 × g and 4 °C. Malondialdehyde (MDA) content was determined using the TBA method. TBA solution containing 15% trichloracetic acid (CAS 79-03-9, Sigma Aldrich), 0.375% (w/v) thiobarbituric acid (TBA; CAS 504-17-6, Sigma Aldrich) and 0.25 M HCl. Absorbance was monitored at 532 and 600 nm using the Agilent Cary 60 UV/VIS spectrophotometer (Agilent Technologies, Switzerland) and for re-calculation of MDA concentration, the molar absorption coefficient 153 $mM^{-1} \cdot cm^{-1}$ was used. Protein content was determined spectrophotometrically at 600 nm by the Bradford method (Bradford, 1976) with the use of bovine serum albumin as the standard (Sigma Aldrich). Reactive oxygen species (ROS) production was determined according to Kovár et al. (2022). Cell culture was 1 h dark-incubated with 10 μM H_2DCFDA (Sigma–Aldrich) at 30 °C. After incubation, the cells were washed and resuspended in PBS (pH 7.4) and transferred into a 96-well plate. Fluorescence intensity was

monitored by Glomax Multi Detection System (Promega Corporation, Madison, WI, USA) using excitation (Ex) and emission (Em) filter Ex^{490} / $Em^{510-570}$ nm.

2.4 Cell ionome composition assay

The concentrations of eight ions (Ca, K, Na, Mg, Mn, Zn, Fe, Cu) were analyzed by the Agilent 720 ICP-OES spectrometer (Agilent Technologies Inc., Santa Clara, CA, USA) using auto-sampler SPS-3 (Agilent Technologies, Switzerland) according to Kovár et al. (2022) with minor modifications. Briefly, overnight cell culture was exposed to 0, 1, and 10 mM concentrations of AA for 3 and 9 h under aerobic conditions at 30 ºC. Next, the collected yeast culture (volume 25 mL) was washed three times with deionized water and dried at 55 °C for 12 h (UN55, Memmert GmbH, Schwabach, Germany). Weighted yeast pellets (accurate to 4 decimal places) were transferred into PTFE digestion tubes and 5 mL of trace pure 69% HNO_3 (Sigma Aldrich, Germany) and 1 mL of trace pure 30% H_2O_2 (Sigma Aldrich, Germany) were added. Mineralization of samples was realized by the ETHOS-One (Milestone, Srl., Sorisole BG, Italy) microwave digestion system. After mineralization, samples were filtered through Filtrak 390 (Munktell & Filtrak, GmbH, Bärenstein, Germany) into a volumetric flask and filled with deionized water (Synergy UV, Merck Millipore, France) to 25 mL final volume. Samples were stored at laboratory temperatures for final analysis. Setup and technical conditions of ICP-OES analysis are reported in Capcarova et al. (2024).

2.5 Statistical analysis

Statistical analysis was performed using R-Studio software (RStudio Team, PBC, Boston, MA, USA; http://www.rstudio.com/). The data were presented as mean ± standard deviation (SD). Statistically significant differences were determined using analysis of variance (ANOVA) and Duncan's post-hoc test and indicated as $p < 0.05$ (*), 0.01 (**), 0.001 (***). Correlation relationships between individual mineral elements were assessed by Pearson's correlation coefficient (r_P) with indicated level of significance (p).

3 Results and discussion

Acrylamide (AA) is an important food contaminant, arising mainly in the process of heat treatment of food by the Maillard reaction of reducing sugars and the amino acid asparagine. The cellular toxicity of AA and its metabolite glycidamide results from their ability to bind to DNA and proteins (Shipp et al., 2006), as well as to attack glutathione and accelerate the accumulation of ROS (Kwolek-Mirek et al., 2011; Kovár et al., 2022). In our previous study using fission yeast cells (Kovár et al., 2022), we found that the half-maximal inhibitory concentration (IC_{50}) of acrylamide is 30 mM. In this study, we investigated the mechanisms underlying the deleterious effect of relatively low concentrations of acrylamide (1 and 10 mM) on the uptake and utilization of mineral nutrients and the disruption of ion homeostasis.

Table 2 Acrylamide-induced changes in generation time (gt), relative growth rate (RGR), concentration of total reactive oxygen species (ROS), and concentration of malondialdehyde (MDA) in *S. pombe* cells

Acrylamide (mM)	gt (h)	RGR ($\Delta OD_{600} \cdot h^{-1}$)	ROS (a.u. per μg^{-1})	MDA ($mM \cdot mg^{-1}$)
0	2.643 ± 0.024	0.130 ± 0.001	202 ± 4.538	0.256 ± 0.051
1	2.939 ± 0.045***	0.117 ± 0.002***	315 ± 15.046***	0.551 ± 0,077***
10	3.336 ± 0.104***	0.103 ± 0.003***	348 ± 3.959***	1.698 ± 0,109***

Values represent mean ± SD (n = 4). Significant differences were evaluated in Duncan's post-hoc test. *** represents significance for $p < 0.001$

Balanced cell growth requires strict coordination of all biological processes, including nutrient assimilation, energy generation and utilization, primary and secondary metabolism, and the cell division cycle. AA leads to retardation of the growth of *S. pombe* cell culture. An increase in the concentration of acrylamide significantly prolongs the generation time (gt) and reduces the relative

growth rate (RGR) (Table 2). Under normal physiological conditions, the gt (represents the time required for the cell doubling) of *S. pombe* cells is around 2.0 to 4.0 hours, strongly depending on cultivation temperature and other conditions (Vyas et al., 2021). Cultivation (for 9 h) of fission yeast cells in the presence of 10 mM AA caused prolongation of gt by more than 26% with a high statistical significance ($p < 0.001$). The observed gt prolongation resulted from a significant ($p < 0.001$) reduction of RGR by almost 21% (Table 2). The observed growth retardation of many model organisms, including yeast, in the presence of acrylamide is mainly attributed to its cytotoxic and genotoxic effects (Huchthausen et al., 2023). Mechanistically, in addition, to direct nucleophilic conjugation of AA with nucleic acids or proteins, AA leads to the formation and accumulation of ROS (Zhang et al., 2023) in cell metabolism. Fluorescence spectroscopy was used in this experimental setup to measure the production of total ROS. The addition of AA into cultivation media in final concentrations 1 and 10 mM led to an increase ($p < 0.001$) in total ROS production compared to the untreated control (55.9% and 72.3%, respectively) (Table 2). It is well known from the literature that increased production of ROS leads to lipid peroxidation, especially in biological membranes. The end product of lipid peroxidation is malondialdehyde (MDA). As assumed, MDA concentration during AA exposure dramatically and significantly ($p < 0.001$) increased (Table 2), mainly in cells exposed to 10 mM AA (6.6 times higher than control). These results of the increase in ROS production and MDA formation prompted us to conclude that the exposure of yeast cells to acrylamide at concentrations of 1 and 10 mM led to the induction of oxidative stress.

To answer the main goal of this work, i.e. how oxidative stress induced by acrylamide affects ion homeostasis, we used the ICP-OES spectroscopic method (Komaromy-Hiller, 1999), which allows the simultaneous determination of the content of eight different elements in the fission yeast cells. Fundamental biological macromolecules in cells, including DNA, RNA, and proteins, possess many charges on their surfaces. The majority of these charges are negative, predominantly attributable to the many phosphate groups in DNA and RNA molecules. Consequently, the structural integrity and functionality of these macromolecules predominantly necessitate positively charged metal ions to maintain charge equilibrium (da Silva & Williams, 2016).

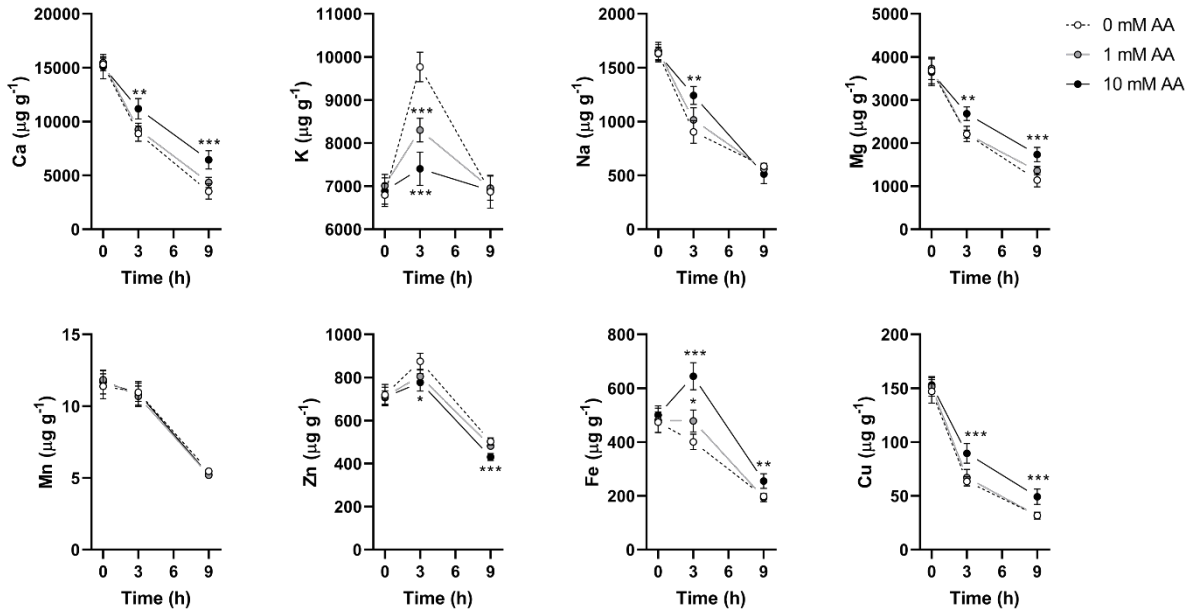

Fig. 1 Acrylamide induced time dependence changes in ionome composition in *S. pombe* cells
Point (empty circle – 0 mM AA, gray circle – 1 mM AA, and black circle 10 mM AA) is mean ± SD (n = 4). *, **, and *** represented (Duncan's post-hoc test) significance difference to 0 mM AA for $p < 0.05$, 0.01, and 0.001, respectively

The mineral composition of yeast cells can change significantly during growth in a liquid medium, depending on various factors such as nutrient availability, growth phase, and environmental conditions. These changes can affect cellular metabolism, enzyme activities, and overall cell physiology, as reported in numerous ionomics studies (Salt et al., 2004; Eide et al., 2005; Požgajová

et al., 2020). Among other tasks, yeasts have been used to study many aspects of ion homeostasis based on their advantage as an experimental system and the high level of conservation during the evolution of many membrane-bound ion-transporting proteins (Eide et al., 2005). In our previous works, we observed changes in the ionic composition of *S. pombe* cells induced by environmental contaminants (Požgajová et al., 2020), and by AA (Kovár et al., 2022). In this experiment, we aimed to quantitatively investigate and understand the dynamic and time-dependent changes in the ionic composition of the cell after exposure to AA. Time-dependent changes in the content of eight mineral nutrients are presented in Figure 1. During 9 h of cultivation in the rich YES medium (Table 1 shows the ion composition), a statistically highly significant ($p < 0.001$) decrease in the concentration of ions (except for potassium after 3 h of cultivation) in the dry matter of the cells has been recorded. The most marked changes have been determined with the basic divalent cations Ca^{2+} and Mg^{2+}. From a physiological point of view, the content of individual mineral substances in the dry matter of cells decreases during cultivation. This, so-called dilution effect is a function of time and is the result of an increase in the number of cells in the culture medium. Assessment of dynamic, time-dependent changes in ion content during cultivation is often neglected in many studies (e.g., Lin et al., 2023). At the same time, from a physiological point of view, the absolute intracellular concentration of ions is a static data, with hardly defined causal relationships between the ionome of the cell and the stress effect (Zhang et al., 2020). Therefore, it is desirable in studies to emphasize the monitoring of ionome changes over time, as in our study. In the case of intensively dividing yeast cells, the concentration of ions decreases, while the mutual ratios between ions remain preserved (Figure 1).

A significant ($p < 0.001$) increase in the intracellular Ca^{2+} concentration was observed already three hours after *S. pombe* cells were exposed to AA compared to unaffected cells. A similar increase ($p < 0.01$) in the intracellular concentration was also observed in the case of Mg^{2+}. Exposure of cells to environmental contamination (heavy metals, food contaminants), as well as many stress-inducing situations (osmotic stress, high temperature, salinity) leads to enhanced ROS production and causes an increase in the concentration of free Ca^{2+} in the cytosol (Ruta et al. 2014; Požgajová et al., 2020). It has been well-documented that the maintenance of cellular processes is closely linked to Ca^{2+} availability. The accumulation of Ca^{2+} observed in our study already 3 hours after AA addition confirms this fact. Oxidative stress causes an increased influx of Ca^{2+} into the cytoplasm from the extracellular environment and from the endoplasmic reticulum. The increasing concentration of Ca^{2+} in the cytoplasm causes an influx of Ca^{2+} into the intracellular compartments (mainly mitochondria and nucleus) by activating calcium transporters. In mitochondria, Ca^{2+} disrupts normal metabolism to an increased extent and triggers processes associated with apoptosis (Görlach et al., 2015; Evans et al., 2016). Ca^{2+} in the nuclei and the cytosol can regulate the phosphorylation activity of proteins and thus modulate signal transduction pathways, as shown by Lyu et al. (2022). Therefore, the ionic and electrochemical imbalance caused by the accumulation of basic divalent cations, especially in the mitochondria, may be another cause of damage to the metabolism of cells affected by AA.

Very interesting is the significantly increased Fe^{3+} concentration already after 3 hours of AA exposure to fission yeast. The Fe^{3+} content was increased by 65 % following treatment of 10 mM AA ($p < 0.001$). When redox-active iron atoms (and also copper) are free, they generate highly reactive hydroxyl radicals via the Fenton reaction. This reaction can accelerate further pressure on the cell's antioxidant system and induce irreversible damage (Eide et al., 2005; Sies et al., 2022).

Visualization of the magnitude of changes in the ionome was performed for each mineral ion as a difference between the variant after AA application and the control variant (Figure 2). Cell exposure to 10 mM AA-induced more significant changes in the content of individual elements than 1 mM. A decrease in the intracellular content was detected for the ion K^+, and divalent Mn^{2+} and Zn^{2+}.

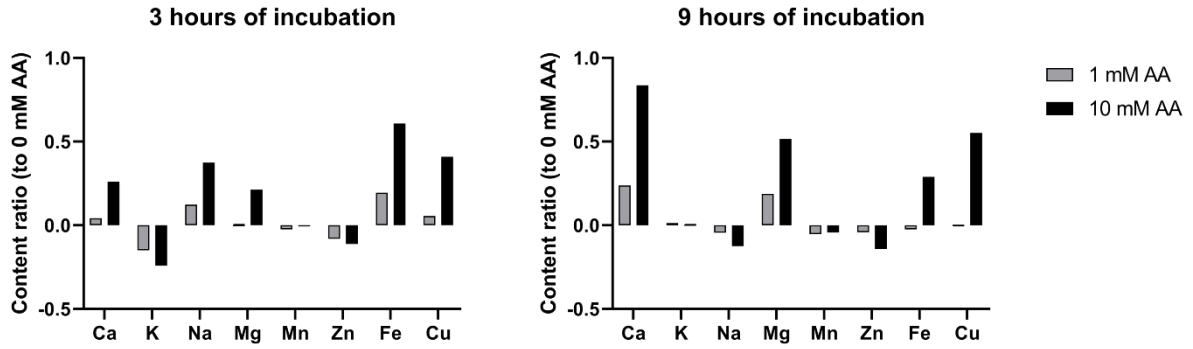

Fig. 2 Changes in the ionome (expressed as a content ratio relative to 0 mM AA) in *S. pombe* cells during 3 h and 9 h of incubation with acrylamide

Positive numbers (+) represent an increase and negative numbers (−) represent a decrease in ion concentration, respectively. The column represents the mean (n = 4)

Longer exposure of *S. pombe* cells (9 hours of cultivation) to AA at a concentration of 10 mM induced statistically significant ($p < 0.001$) changes in the ionome, accompanied by an increase in the concentrations of divalent ions Ca^{2+}, Mg^{2+}, Cu^{2+}, and ($p < 0.01$) trivalent Fe^{3+}. On the other hand, the content of monovalent element K^{+} together with Mn^{2+} has changed non-significantly and the concentration of Zn^{2+} and Na^{+} decreased ($p < 0.001$) (Figure 2 and 3). A lower concentration of acrylamide (1 mM) did not significantly affect the content of the studied ions.

Pearson's correlation analysis, which expresses the strength of positive or negative mutual interactions between the concentrations of all eight monitored mineral ions, revealed that there is a positive correlation between the ions under normal physiological conditions, except for monovalent K^{+} and divalent Zn^{2+} to some extent (r_p 0.2 to 0.5 for individually monitored ions). The strength of the negative correlation of potassium with other ions with the increase in acrylamide concentration weakens, which indicates changes in ion homeostasis (Figure 3).

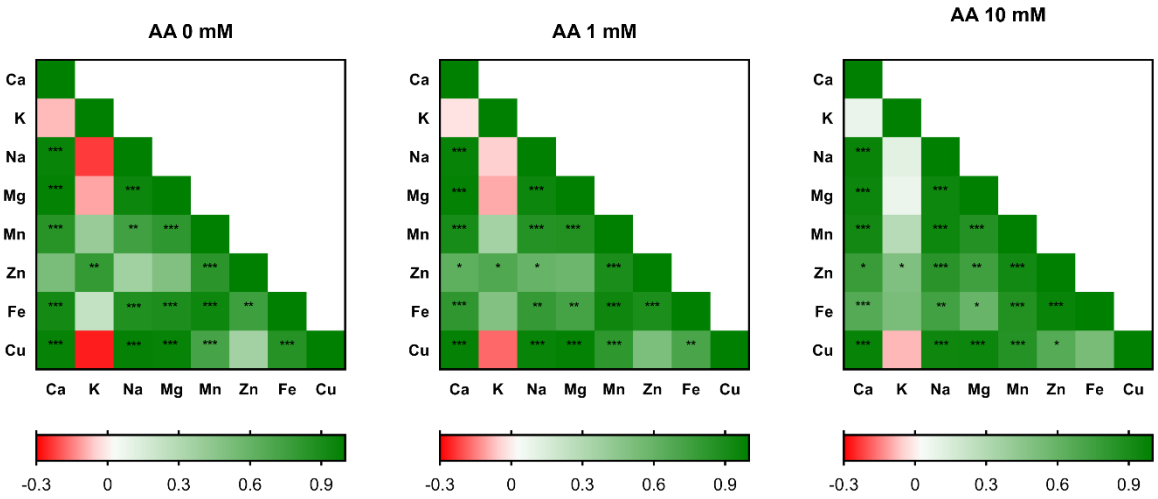

Fig. 3 Heatmap matrix of mutual ion interactions analyzed by Pearson's correlation coefficient (r_P) for 0, 1, and 10 mM concentrations of acrylamide

Trends significance (p) is reported by asterisks (*, **, and *** that represents significance for $p < 0.05$, 0.01, and 0.001, respectively)

The analysis of interactions and the significance of the tested factors (cultivation time (t), effect of acrylamide (AA) and their mutual interactions (t × AA)) revealed a highly significant effect of the factors on changes in the intracellular concentration of the individual investigated mineral ions (Table 3).

Table 3 Total test of significance for ionome changes in *S. pombe* cells for the time of cultivation (t), acrylamide concentration (AA), and time and AA (t × AA) interaction

Element	t	AA	t × AA
Ca	***	***	**
K	***	***	***
Na	***	*	***
Mg	***	**	*
Mn	***	ns	ns
Zn	***	***	ns
Fe	***	***	***
Cu	***	***	*

ns – non-significant. *, **, and *** represents significance for $p < 0.05$, 0.01, and 0.001, respectively

The presented results of changes in intracellular ion concentrations (Figure 2) support the idea that acrylamide modulates ion homeostasis, either directly through the regulation of transport processes, or indirectly through modulation of the redox state in the cytoplasm due to induced oxidative stress. Mineral ions, in contrast to metabolic organic molecules, cannot be synthesized or destroyed by cells for maintenance of metabolic and physiological systems. Thus, cells may alone manage their ionic composition by importing or exporting ions from their extracellular environment. It is suspected that AA, by changes in the intracellular ion composition and ion homeostasis in the cell, not only changes the redox potential and the electrochemical potential during oxidative stress, but also alters the transmission of signals by secondary messengers and thus the cell's response to stress.

4 Conclusions

The toxicity of acrylamide for cells and their biological activity is associated with oxidative stress. In this study, using fission yeast *Schizosaccharomyces pombe*, we provide evidence that acrylamide-induced oxidative damage alters the intracellular concentration of major mineral ions and thus the entire ionic homeostasis. As revealed by quantitative analysis of the cell ionome using inductively coupled plasma optical emission spectroscopy (ICP-OES), an increase in the concentration of acrylamide in the cellular environment statistically significantly increases the intracellular concentration of mainly divalent ions Ca^{2+}, Mg^{2+}, as well as the concentration of iron and copper.

Acknowledgments

This research was supported by the Slovak Research and Development Agency under the contract APVV-22-0294, VEGA under the grant 1/0583/23.

References

Álvarez-Herrera, C. et al. (2024). Schizosaccharomyces pombe as a predictor toxicity tool. *MethodsX*, 13, 102823. https://doi.org/10.1016/j.mex.2024.102823

Aulakh, S.K. et al. (2022). Metal ion availability and homeostasis as drivers of metabolic evolution and enzyme function. *Current Opinion in Genetics & Development*, 77, 101987. https://doi.org/10.1016/j.gde.2022.101987

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72(1–2), 248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Capcarova, M. et al. (2024). Levels of essential and trace elements in mozzarella available on the Slovak market with the estimation of consumer exposure. *Biological Trace Element Research*, 202, 2357–2366. https://doi.org/10.1007/s12011-023-03813-x

Carraro, M. & Bernardi, P. (2016). Calcium and reactive oxygen species in regulation of the mitochondrial permeability transition and of programmed cell death in yeast. *Cell Calcium*, 60(2), 102–107. https://doi.org/10.1016/j.ceca.2016.03.005

da Silva, J.J.R.F. & Williams, R.J.P. (2016). The biological chemistry of the elements: the inorganic chemistry of life. Oxford University Press, 600 p. ISBN 978-0198508489

Demidchik, V. (2018). ROS-activated ion channels in plants: biophysical characteristics, physiological functions and molecular nature. *International Journal of Molecular Sciences*, 19(4), 1263. https://doi.org/10.3390/ijms19041263

Eide, D.J. et al. (2005). Characterization of the yeast ionome: a genome-wide analysis of nutrient mineral and trace element homeostasis in Saccharomyces cerevisiae. *Genome Biology*, 6, R77. https://doi.org/10.1186/gb-2005-6-9-r77

Evans, M.J. et al. (2016). A ROS-assisted calcium wave dependent on the AtRBOHD NADPH oxidase and TPC1 cation channel propagates the systemic response to salt stress. *Plant Physiology*, 171(3), 1771–1784, https://doi.org/10.1104/pp.16.00215

Görlach, A. et al. (2015). Calcium and ROS: a mutual interplay. *Redox Biology*, 6, 260–271. https://doi.org/10.1016/j.redox.2015.08.010

Halliwell, B. (2006). Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life. *Plant Physiology*, 141(2), 312–322. https://doi.org/10.1104/pp.106.077073

Huchthausen, J. et al. (2023). Reactivity of acrylamides causes cytotoxicity and activates oxidative stress response. *Chemical Research in Toxicology*, 36(8), 1374–1385. https://doi.org/10.1021/acs.chemrestox.3c00115

Jeyasingh, P.D. et al. (2023). Adaptation to a limiting element involves mitigation of multiple elemental imbalances. *Journal of Royal Society Interface*, 20(198), 2020220472. https://doi.org/10.1098/rsif.2022.0472

Komaromy-Hiller, G. (1999). Flame, flameless, and plasma spectroscopy. *Analytical Chemistry*, 71(12), 338R–342R. https://doi.org/10.1021/a1999907z

Kovár, M. et al. (2022). Acrylamide-derived ionome, metabolic, and cell cycle alterations are alleviated by ascorbic acid in the fission yeast. *Molecules*, 27(13), 4307. https://doi.org/10.3390/molecules27134307

Kwolek-Mirek, M. et al. (2011). Yeast Saccharomyces cerevisiae devoid of Cu,Zn-superoxide dismutase as a cellular model to study acrylamide toxicity. *Toxicology in Vitro*, 25(2), 573–579. https://doi.org/10.1016/j.tiv.2010.12.007

Lin, G. et al. (2023). Gene regulation and ionome homeostasis in rice plants in response to arsenite stress: potential connection between transcriptomics and ionomics. *Biometals*, 36, 1157–1169. https://doi.org/10.1007/s10534-023-00510-z

Lipworth, L. et al. (2012). Review of epidemiologic studies of dietary acrylamide intake and the risk of cancer. *European Journal of Cancer Prevention*, 21(4), 375–386. https://doi.org/10.1097/CEJ.0b013e3283529b64

López-Barneo, J. et al. (2001). Cellular Mechanism of Oxygen Sensing. *Annual Review of Physiology*, 63, 259–287. https://doi.org/10.1146/annurev.physiol.63.1.259

Lyu, K. et al. (2022). The sensitive genes in response to various metal ion stresses in the yeast Saccharomyces cerevisiae. *Protein & Peptide Letters*, 29(3), 231–241. https://doi.org/10.2174/0929866529666220126102348

Požgajová, M. et al. (2020). Cadmium-induced cell homeostasis impairment is suppressed by the Tor1 deficiency in fission yeast. *International Journal of Molecular Sciences*, 21(21), 7847. https://doi.org/10.3390/ijms21217847

Remick, K.A. & Helmann, J.D. (2023). The elements of life: A biocentric tour of the periodic table. In Poole, R.K. & Kelly, D.J. (Eds), *Advances in Microbial Physiology*. Academic Press, 82, (pp. 1–127). https://doi.org/10.1016/bs.ampbs.2022.11.001

Ruta, L.L. et al. (2014). Calcium signaling mediates the response to cadmium toxicity in saccharomyces cerevisiae cells. *FEBS Letters*, 588(17), 3202–3212. https://doi.org/10.1016/j.febslet.2014.07.001

Salt, D.E. (2004). Update on plant ionomics. *Plant Physiology*, 136(1), 2451–2456. https://doi.org/10.1104/pp.104.047753

Shehzad, J. & Mustafa, G. (2023). Mechanism of reactive oxygen species regulation in plants. In Faizan, M. et al. (Eds), *Reactive oxygen species*. Springer, Singapore, (pp. 17–41). https://doi.org/10.1007/978-981-19-9794-5_2

Shipp, A. et al. (2006). Acrylamide: review of toxicity data and dose-response analyses for cancer and noncancer effects. *Critical Reviews in Toxicology*, *36*(6–7), 481–608. https://doi.org/10.1080/10408440600851377

Sies, H. et al. (2022). Defining roles of specific reactive oxygen species (ROS) in cell biology and physiology. *Nature Reviews Molecular Cell Biology*, 23, 7, 499–515. https://doi.org/10.1038/s41580-022-00456-z

Vyas, A. et al. (2021). Fission yeast Schizosaccharomyces pombe: a unicellular "micromammal" model organism. *Current Protocols*, 1(6), e151. https://doi.org/10.1002/cpz1.151

Zhang, L. et al. (2023). Acrylamide-induced hepatotoxicity through oxidative stress: mechanisms and interventions. *Antioxidants & Redox Signaling*, 38(16–18), 1122–1137. https://doi.org/10.1089/ars.2022.0055

Zhang, Y. et al. (2020). Disease ionomics: understanding the role of ions in complex disease. *International Journal of Molecular Sciences*, 21(22), 8646. https://doi.org/10.3390/ijms21228646