Antioxidant activity of the peroxidase system in cell nuclei during germination of wheat seedlings

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Abstract. The dynamics of the antioxidant activity of the peroxidase system in the structures of cell nuclei during the induction of germination of wheat seedlings was tracked, which was synchronized with nuclear trypsin-like activity and mitotic activity in cells. The dynamics of the antioxidant activity of the peroxidase system in the fractions of cell nuclei seems to be related to the processes of cell division and stretching during the development of plant seedlings. Previously, these results can be considered as one of the complex multilevel regulatory networks of proteinases formed in cell nuclei, which can probably participate in the turnover of oxidized proteins.

1. Introduction

Reactive oxygen species (ROS) are natural products of cellular oxidative exchange in the cell and make a significant contribution to the processes of survival, death, differentiation and signal transmission in cells. Integrated antioxidant systems were developed by aerobic organisms in the course of evolution [1]. The scheme for the formation of various radicals consists in the transition of separate electrons to oxygen, which leads to the formation of different radicals in their reactivity: a relatively inert superoxide radical, a more reactive hydrogen peroxide and a highly reactive hydroxyl radical [2]. The type of ROS and its local concentration together determine whether the transmission of redox signals occurs or whether it is damage caused by oxidative stress [3-5]. One of the main targets of ROS exposure is proteins [6]. Protein oxidation can cause changes in the structure of amino acids (Lys, Arg, Pro, Thr, Cys, His), an increase in protein hydrophobicity, and loss of enzymatic activity. Modified proteins are recognized and disintegrated by intracellular proteinases, such as the proteosome complex [7, 8]. At the same time, free radicals can also react with these proteolytic complexes, causing a decrease in their functionality. If oxidized proteins are not removed effectively, their accumulation occurs and, as a result, cell function changes [9]. In the nucleus, free radicals are mainly accumulated both by transfer from other compartments of the cell, and by direct formation in the nucleus itself [10]. They have been shown to act as damaging molecules, but also present central hubs in cellular signalling networks [11]. There is growing evidence of their influence on chromatin structure, DNA methylation, and enzymatic and non-enzymatic posttranslational modifications of histones and DNA-binding proteins that combinatorial regulate chromatin function [2]. The peroxidase system is of undoubted interest for studying the biochemistry of the cell nucleus, since it is able to produce and consume ROS depending on the regulation of the activity direction [12].
The aim of this study was to determine the antioxidant activity of the peroxidase system in fractions of cell nuclei during germination of wheat seedlings. In addition, antioxidant activity of the peroxidase system was synchronized with trypsin-like proteolytic activity and mitotic activity in cells.

2. Materials and methods

The object of the study was wheat seeds of the Moscow-35 variety (Triticum aestivum L.). Seeds were sprouted at 22°C. Dry embryos were analyzed (0 h), 24 hours later and 48 hours after soaking the seeds. We have proposed a simple and effective method for distinguishing various compartments. Although the biochemical analysis is accompanied by a violation of the cell's anatomy, we have developed a soft fractionation method to separate the cell components while preserving their individual functions [13-15]. The purity of cell nuclei was monitored microscopically and spectrophotometrically. A salt concentration gradient was used to isolate the nuclear fractions: 0.14 M NaCl-nucleoplasm; 0.35 M NaCl-chromatin I; 2 M NaCl-chromatin II; 6 M GuHCl with 0.004% C_{6}H_{5}SO- nuclear residuum (NR). Antioxidant activity of peroxidase system (APS) and trypsin-like activity (TLA) were determined in the obtained fractions [13-15]. Mitotic activity was determined using the method [16]. Research was conducted on the equipment of the center "Agidel".

3. Results and discussion

ROS formation in plants occurs constantly in mitochondria, chloroplasts, peroxisomes, endoplasmic reticulum, microsomes, cytosol, cell walls, and apoplast [17]. Antiradical protection of biopolymers is supported by superoxide dismutase, ceruloplasmin, peroxidase, catalase, and glutathione-dependent groups found in all cell compartments. They spatially and temporarily change the redox status and affect the redox balance, controlling almost every aspect of cellular processes, such as cellular metabolism, growth and development, as well as adaptation to various environmental stresses in plants [18]. A number of studies have confirmed that the plant cell nucleus has its own antioxidant redox system [15, 19, 20]. As is known, the nuclear DNA of plants is well protected by histones and non-histone chromatin proteins [5, 7, 21]. At the same time, redox intermediates regulate the activity and expression of many enzymes involved in DNA methylation, histone methylation and acetylation, and chromatin remodeling, participating in the regulation of chromatin architecture, thereby being key regulators of epigenetic modifications in plants [5, 18]. The contribution of free radicals to the regulation of this complex structure is only partially investigated [5]. It is confirmed that the damaging effect of ROS increases with increasing ploidy in plant organisms, such as wheat [21].

The dynamics of the antioxidant activity of the peroxidase system in nuclei is shown in figure 1. Interestingly, it appears only in the nucleoplasm and chromatin, but it was not detected at the level of the nuclear residuum (figure 1). To eliminate the suspicion that in fact guanidine hydrochloride has the ability to affect the APS, an experiment was conducted with Horseradish peroxidase (Reanal), which did not show the slightest effect of guanidine hydrochloride.

Oxygen penetrates into the cell through channels bounded by various membranes, and thus is inactivated and altered by the cell's molecular components, which prevent oxygen or its reactive forms from accessing the intracellular structures. Mitotic chromosomes are more susceptible to the action of secondary metabolites than interphase ones, primarily because they do not have a nuclear envelope. According to some authors, in the course of evolution, a system of cell membranes was appeared to protect genetic material from the effects of oxygen toxicity. It has been demonstrated that the nuclear membrane, in contrast to the membranes of other eukaryotic cell organelles, is more resistant to peroxidation due to its phospholipid composition [21].

Analysis of figure 1A shows that a low degree of APS in nuclei is maintained at the level of dry embryos in the nucleoplasm, chromatin I and chromatin II, apparently to regulate peroxidation of lipids. 24h seedlings still retain a relatively low degree of antioxidant activity of the peroxidase system in the nucleoplasm, chromatin I, and chromatin II. It may be related to the beginning of proliferative activity and early cell expansion by stretching. Seedlings (48h) have a higher level of APS associated with the root systems and mesocotyls (Fig. 1A: nucleoplasm, chromatin I and chromatin II; 48 h), where, as is
known, cell division occurs in the areas of the apical shoot meristem and apical root meristem, and some cell layers grow by stretching. The catalytic cycle of peroxidases is complex, and they can induce reactions of formation of hydroxyl radicals [22]. In turn, the hydroxyl radical acts on cell wall polymers, weakening the bonds between them and promoting stretching of cells during growth [23].

![Antioxidant activity of the peroxidase system (A) and trypsin-like proteolytic activity (B) in nuclear structures (nucleoplasm; chromatin I; chromatin II; nuclear residuum).](image)

**Figure 1.** Antioxidant activity of the peroxidase system (A) and trypsin-like proteolytic activity (B) in nuclear structures (nucleoplasm; chromatin I; chromatin II; nuclear residuum).

Figure 1B shows the dynamics of TLA in nuclear structures. As can be seen from the figure, a high degree of TLA is present in the nuclear residuum of 24h seedlings, which is apparently associated with the processes of cell growth by stretching. During the 48h growth of individual organs in the embryo, weak activity can be observed, which is initiated in the nucleoplasm of the coleoptile, mesocotyl and roots and increases simultaneously in the structures of chromatin I and chromatin II.

Test on figure 2 shows that numerous mitoses occur in the root, and significantly fewer mitoses occur in coleoptile and mesocotyl. There is no doubt that the processes of assembling and disassembling of chromatin with the participation of nuclear proteins are especially intense during this period in the roots.
and much less intense in the coleoptile and mesocotyl. As for TLA, a high level of activity is also observed in the roots in fractions of chromatin (I and II) and being somewhat lower in the mesocotyls and coleoptiles (48 h).

![Figure 2. Mitotic activity in the 48 h seedlings.](image)

It is known that nuclear proteins are actively modified by ROS, which affects their folding, and stability. This is especially important in the case of histones, for example, since all possible variations will significantly affect the structure and function of chromatin [2]. Currently, there is insufficient research on the specificity and functions of nuclear proteinases, so it is not possible to clearly describe the turnover of nuclear proteins. It is confirmed that one of their functions is to control the folding of proteins. As mentioned earlier, there are more and more studies on redox biology that consider low concentrations of ROS as a way to activate signaling pathways to trigger various biological processes. They have undeniable advantages: they are quickly formed, have a high reactivity, react with membrane lipids, carbohydrates, proteins and DNA. ROS, such as hydrogen peroxide, can simply pass through biomembranes using aquaporins. In turn, the cells have effective antioxidant systems for strong ROS control. In combination, these factors make the AFC committed participants in cell signaling [24].

4. Conclusions
This paper presents primary data on the dynamics of the antioxidant activity of the peroxidase system and trypsin-like proteolytic system in the structures (nucleoplasm, chromatin, nuclear matrix) of cell nuclei upon the induction of growth processes. Future studies of these systems at the level of histone and non-histone proteins will probably bring us closer to understanding the nature of their functional conjugation in the process structural and dynamic chromatin reorganization, both locally and on a large scale. Previously, these results can be considered as one of the complex multilevel regulatory networks of proteinases formed in the nuclei, which are likely to participate in the turnover of oxidized protein.

Acknowledgments
We are thankful to Dr. Ivanova E.A. at Ufa Institute of Biology for expert advice and encouragement.

The study was performed within the framework of the state assignment of the Ministry of Education and Science of the Russian Federation (075-00326-19-00) of the theme AAAA-A18-118022190104-7.

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