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EXPERIMENTAL ARTICLES

Arsenite as an Inducer of Lipid Peroxidation in *Saccharomyces cerevisiae* Cells

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Abstract—The ability of sodium arsenite at concentrations of 10^{-2}, 10^{-4}, and 10^{-6} M to induce lipid peroxidation in *Saccharomyces cerevisiae* cells was studied. Arsenite at the concentrations 10^{-2} and 10^{-4} M enhanced lipid peroxidation and inhibited the growth of yeast cells. Enhanced lipid peroxidation likely induced oxidative damage to various cellular structures, which led to suppression of the metabolic activity of cells. Arsenite at the concentration 10^{-6} M did not activate lipid peroxidation in cells. All of the tested arsenite concentrations inhibited the activity of α-ketoglutarate dehydrogenase and pyruvate dehydrogenase in cells. The inference is made that the toxicity of arsenite may be related to its stimulating effect on intracellular lipid peroxidation.

Key words: *Saccharomyces cerevisiae*, arsenite, lipid peroxidation, metabolism.

The contamination of the environment with arsenite is a problem of great ecological concern [1, 2]. Arsenic compounds are toxic to many metabolic processes [3]. Abdrashitova *et al.* [4] showed that the oxidation of arsenite to arsenate by *Pseudomonas putida* cells is accompanied by an enhancement of lipid peroxidation in these cells and the formation of reactive oxygen species, which impair cells through the oxidative modification of susceptible cellular structures [5]. The developing oxidative stress may cause cell death [6].

The tricarboxylic acid cycle plays an important part in the implementation of nonspecific cell resistance to various stress factors [7]. The rate-limiting enzymes of the tricarboxylic acid cycle (pyruvate dehydrogenase and α-ketoglutarate dehydrogenase) are very susceptible to reactive oxygen species [8, 9] and can be inhibited by arsenite [10]. The related changes in the functioning of the tricarboxylic acid cycle may affect almost all other metabolic pathways in cells.

This work was undertaken to verify the supposition that arsenite induces lipid peroxidation in eukaryotic cells.

MATERIALS AND METHODS

The effect of arsenite on lipid peroxidation in eukaryotes was studied using the yeast *Saccharomyces cerevisiae* Y-1173, which was kindly provided by V.M. Vagabov from the Institute of Biochemistry and Physiology of Microorganisms in Pushchino, Russia. The yeast was cultivated on a shaker (200 rpm) at 28°C in 700-ml flasks with 200 ml of synthetic Reader medium with 2% glucose. The medium was inoculated with an overnight yeast culture. Yeast growth was estimated by measuring culture turbidity at 540 nm using an SF-46 spectrophotometer. Spectrophotometric data were recast to the weight of dry biomass using a calibration curve.

The effect of arsenite was studied by growing *S. cerevisiae* cells in the presence of different concentrations of sodium arsenite (10^{-2}, 10^{-4}, and 10^{-6} M). After cultivation for 24 h, cells were harvested by centrifugation and washed with cold distilled water. The washed biomass (1 g) was disintegrated with quartz sand for 10 min in a mortar. The cell homogenate was centrifuged at 3000 *g* for 10 min. The supernatant was assayed for the activity of superoxide dismutase [11], catalase [12], pyruvate dehydrogenase [14], and α-ketoglutarate dehydrogenase [14], and for the intensity of lipid peroxidation, which was determined from the accumulation in cells of products reacting with 2-thiobarbituric acid (the so-called TBA-reactive products) [13].

Protein was quantified by the Lowry *et al.* method [15]. Glucose was assayed with the phenol–sulfuric acid reagent [16]. The reagents used in this work were purchased from Sigma (United States).

All experiments were carried out no fewer than three times, with no fewer than three replicate measurements being conducted in each experiment. The data presented in this paper are mean arithmetic values.

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RESULTS AND DISCUSSION

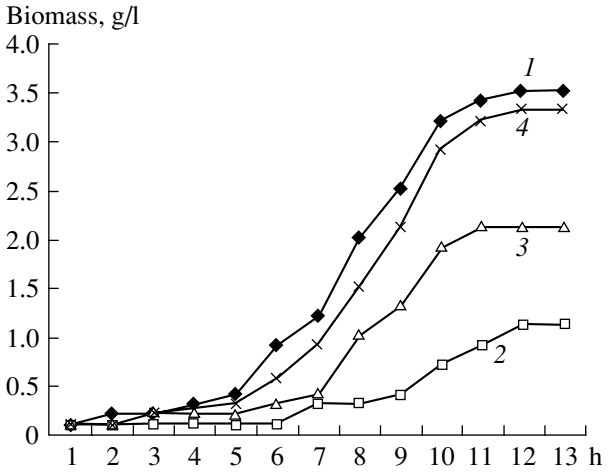
The addition of 10^{-2} M arsenite to the medium considerably inhibited the growth of *S. cerevisiae* (figure, curve *2*). At a concentration of 10^{-4} M, the inhibitory effect of arsenite was lower (figure, curve *3*). At a concentration of 10^{-6} M, arsenite had a minor effect on yeast growth (figure, curve *4*) as compared with the control (figure, curve *1*). After incubation in the presence of 10^{-2} and 10^{-4} M arsenite for 12–14 h, the yeast resumed its growth (data not presented), presumably due to the development of intracellular adaptive processes mitigating the toxic effect of arsenite.

Depending on its concentration, arsenite inhibited the consumption of glucose from the medium (data not presented) and stimulated the formation of TBA-reactive products in cells and the activity of superoxide dismutase and catalase (Table 1), whereas it inhibited the activity of the tricarboxylic acid cycle enzymes pyruvate dehydrogenase and α-ketoglutarate dehydrogenase (Table 2).

Thus, at the concentration 10^{-2} M, arsenite exerted the maximum effect on yeast cells. At the concentration 10^{-4} M, the effect was lower. The effect exerted by 10^{-6} M arsenite on *S. cerevisiae* cells was minimal.

Upon penetrating into yeast cells, arsenite may stimulate lipid peroxidation in two ways. The first way is associated with the possible enzymatic oxidation of arsenite to arsenate, which happens in many microorganisms, including bacteria and fungi [2]. The second way is related to the ability of As^{3+} to release Fe^{2+} from its complexes with proteins [17]. The resultant free Fe^{2+} ions considerably activate lipid peroxidation in cells, giving rise to reactive oxygen species, such as the superoxide radical. The accumulation of superoxide radicals in yeast cells activates superoxide dismutase, the enzyme that catalyzes the dismutation of the superoxide radical to molecular oxygen and hydrogen peroxide, the latter being the substrate of the catalase.

In spite of the high activity of the antioxidant enzymes, superoxide dismutase and catalase, in yeast cells grown in the presence of 10^{-2} and 10^{-4} M arsenite, such cells contain an elevated amount of TBA-reactive products. Consequently, the elevated activity of the antioxidant enzymes cannot prevent the induction of lipid peroxidation in yeast cells by arsenite mediated by reactive oxygen species. The aforementioned resumption of yeast growth after 12–14 h of incubation in the presence of arsenite may be related to an increase in the activity of superoxide dismutase and catalase. It is widely recognized that the maximum induction of these antioxidant enzymes, as well as glutathione peroxidase and glutathione reductase, by a reactive oxygen species is possible only in the stationary growth phase [18], when the effect of glucose repression is absent. We believe that the enhanced activity of superoxide dismutase and catalase in the yeast cells grown in the presence of arsenite is one of the important factors responsible for the implementation of adaptive processes aimed at mitigating the toxic effect of arsenite on eukaryotic cells.



The effect of arsenite on the growth of the yeast *S. cerevisiae*. Curves *1, 2, 3,* and *4* show yeast growth in the presence of 0, 10^{-2}, 10^{-4}, and 10^{-6} M arsenite, respectively.

The detected inhibition of pyruvate dehydrogenase and α-ketoglutarate dehydrogenase by arsenite may be associated not only with the direct action of this compound on these enzymes [10], but also with the inhibitory action of the products of lipid peroxidation, which can nonspecifically inhibit many enzymes through their oxidative modification [8, 9].

Despite inducing some inhibition of pyruvate dehydrogenase and α-ketoglutarate dehydrogenase activi-

Table 1. The effect of different concentrations of arsenite on the activity of antioxidant enzymes and the accumulation of TBA-reactive products in *S. cerevisiae* cells

Parameter	Enzymes and TBA-reactive products in the presence of arsenite (M)			
	0	10^{-2}	10^{-4}	10^{-6}
Superoxide dismutase, U/mg protein	44.1 ± 2.3	93.8 ± 3.2	65.5 ± 3.1	47.3 ± 2.1
Catalase, U/mg protein	32.4 ± 1.2	67.5 ± 2.3	42.1 ± 1.3	34.6 ± 2.3
TBA-reactive products, nmol/mg protein	18.9 ± 0.9	37.1 ± 1.5	27.4 ± 1.9	22.1 ± 0.8

Table 2. The effect of different concentrations of arsenite on the activity of the enzymes of the tricarboxylic acid cycle in *S. cerevisiae* cells

Enzyme	Specific activity of enzyme in the presence of arsenite (M)			
	0	10^{-2}	10^{-4}	10^{-6}
α-Ketoglutarate dehydrogenase, nmol NADH/(min mg protein)	73.56 ± 3.89	20.32 ± 1.17	40.79 ± 2.82	62.53 ± 2.15
Pyruvate dehydrogenase, nmol NADH/(min mg protein)	33.72 ± 2.34	10.15 ± 0.97	15.56 ± 0.82	27.82 ± 1.14

ties by 10^{-6} M arsenite, this concentration of arsenite had only a minor effect on the growth parameters of *S. cerevisiae*. It can be suggested that the inhibition of the activity of pyruvate dehydrogenase and α-ketoglutarate dehydrogenase, which may lead to a deficiency of ATP in cells, was compensated for by an enhancement of other activities of the tricarboxylic acid cycle or by the appearance of the respective bypass metabolic routes. The understanding of the mechanisms of cell resistance to arsenite was considerably improved by the discovery of a family of *ACR* genes [19]. The *ACR3* gene encodes an ATP-dependent protein involved in the transport of arsenite through the plasma membrane [20]. The mutation of this gene in *S. cerevisiae* considerably augments the sensitivity of the yeast to arsenite, whereas the transformation of yeast cells with a plasmid bringing about enhanced expression of the *ACR3* gene imparts them with a high arsenite resistance. The *ACR2* gene codes for glutathione- and glutaredoxin-dependent arsenate reductase, the enzyme that reduces arsenate to arsenite. The mutants lacking *ACR2* remain resistant to arsenite but become sensitive to arsenate. The *ACR1* gene codes for the transcriptional factor that regulates the expression of the *ACR2* and *ACR3* genes. This factor is very similar to the transcriptional factor YAP-1, which is the major regulator of the expression of antioxidant genes in *S. cerevisiae* cells [20].

Thus, arsenite likely induces lipid peroxidation in yeast cells. The resultant oxidative damage to various cellular structures may be responsible for the inhibition of many metabolic processes in yeast cells. The arsenite-enhanced activity of antioxidant enzymes in cells plays an important part in the development of their resistance to this toxic compound. It is the induction of lipid peroxidation by arsenite that may be responsible for the toxic effect of this compound or eukaryotic cells.

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