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EFFECTS OF BICARBONATE AND ALPHA-KETOGLUTARATE ON SENSITIVITY OF YEAST *SACCHAROMYCES CEREVISIAE* TO HYDROGEN PEROXIDE AND IRON IONS

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The effects of sodium bicarbonate on the sensitivity of yeast *Saccharomyces cerevisiae* to hydrogen peroxide and ferrous sulfate were studied. Viability of the yeast cells treated with 10–25 mM H_2O_2 and 0.1–0.2 mM $FeSO_4$ was significantly decreased when 25 or 50 mM $NaHCO_3$ was added to the medium. In the absence of bicarbonate, the levels of oxidative stress markers, namely protein carbonyls, total and oxidized glutathione in cells exposed to 0.2 mM $FeSO_4$ did not differ from ones in control cells (without $FeSO_4$). Yeast cells incubated with 0.2 mM $FeSO_4$ and 50 mM $NaHCO_3$ had similar levels of oxidized glutathione and carbonyl groups in proteins but lower level of total glutathione compared to cells treated with $FeSO_4$ in the absence of $NaHCO_3$. Yeast cells exposed to a mixture of "2 mM H_2O_2 + 2 mM $FeSO_4$" in 50 mM sodium bicarbonate buffer survived better than cells treated with these oxidants in 50 mM potassium phosphate buffer. The addition of 10 mM alpha-ketoglutarate led to the increased yeast survival in both buffers under the treatment with "Fe^{2+}/H_2O_2". The protective effect of alpha-ketoglutarate can be due to its H_2O_2-scavenging activity. The results suggest that bicarbonate ions can enhance or alleviate the toxic effects of redox-active compounds on *S. cerevisiae*. Pro/antioxidant effects of bicarbonate ions are likely to depend on the kinetics of an interaction between HCO_3^- and ROS produced.

Abbreviations: AKG, alpha-ketoglutarate; CP, carbonyl proteins; Cu,Zn-SOD, Cu,Zn-superoxide dismutase; KPi, potassium phosphate buffer; OD, optical density; ROS, reactive oxygen species.

Keywords: *Saccharomyces cerevisiae*; alpha-ketoglutarate; bicarbonate ions; carbonate radical; oxidative stress.

INTRODUCTION

Production of reactive oxygen species (ROS) and carbon dioxide (CO_2) is a part of normal aerobic cellular metabolism [19, 26]. ROS such as superoxide anion radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\bullet}OH$) are potentially dangerous

due to their high reactivity and capability to interact with virtually all cellular components. Toxicity of ROS is largely dependent on the presence of ions of transition metals, such as iron and copper. Transition metals can participate in the formation of highly reactive hydroxyl radical in the Fenton reaction [24]: $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^{-}$. Excessive ROS production and/or decrease in antioxidant defense leads to the development of oxidative stress, which is implicated in aging and many human diseases [19].

Carbon dioxide and its hydrated forms (HCO_3^- i CO_3^{2-}) are components of carbonate buffer system which plays an important role in pH regulation in biological liquids [23]. Bicarbonate buffer, which is composed of 1.3 mM CO_2 in equilibrium with 25 mM HCO_3^- in serum and 14 mM HCO_3^- intracellularly, has well-demonstrated redox effects [20, 23]. A number of studies demonstrated that HCO_3^- or CO_2/HCO_3^- can stimulate the oxidation, peroxidation, and nitration of various molecules [1, 2, 5, 6, 10, 14, 28]. Carbon dioxide and (bi)carbonate ions enhance metal-catalyzed decomposition of H_2O_2 [5, 14] and peroxidase activity of Cu,Zn-superoxide dismutase (Cu,Zn-SOD) [9, 15, 28]. At the same time, (bi)carbonate-mediated peroxidase activity of Cu,Zn-SOD leads to the formation of carbonate radical ($CO_3^{\bullet-}$), which has strong oxidizing properties [1, 3, 20, 26, 28]. $CO_3^{\bullet-}$ formation was shown to be responsible for the increased oxidation of proteins and lipids in carbonate buffer under exposure to transition metals [2]. It should be noted that articles cited above and many similar articles used *in vitro* systems. There is a little information about similar processes *in vivo*. We have previously shown that bicarbonate buffer sensitized yeast *Saccharomyces cerevisiae* to menadione, a redox-active compound which is able to generate superoxide anion radical [17]. The inactivation of aconitase and the decrease in glutathione level in yeast cells treated with menadione in bicarbonate buffer were observed.

Taking into account that bicarbonate ions can intensify free radical processes, it seems to be possible, that the exogenous antioxidant compounds can alleviate these processes. Recently, the antioxidant properties for alpha-ketoglutarate (AKG) as an important intermediate in the Krebs cycle were demonstrated. In particular, the ability to scavenge hydrogen peroxide was shown for AKG [4].

This study aimed at studying the effects of sodium bicarbonate on sensitivity of yeast *S. cerevisiae* to hydrogen peroxide, iron ions and their mixture. The ability of AKG to prevent yeast death in bicarbonate buffer under combined treatment with H_2O_2 and Fe^{2+} was also studied.

MATERIALS AND METHODS

The *S. cerevisiae* strain YPH250 (*MAT***a** *trp1-Δ1 his3-Δ200 lys2-801 leu2-Δ1 ade 2-101 ura3-52*) was used in this study. The strain was kindly provided by Dr. Y. Inoue (Kyoto University, Japan). Cells were grown at 28 °C with shaking at 175 rpm in liquid medium containing 1% yeast extract, 2% peptone, 2% glucose (YPD). Exponential-phase cells were harvested after cultivation for 24 h (OD_{600}=1.4–1.5). In one series of experiments, cells were suspended in 100 mM HEPES buffer (pH 7.5)-contained 0.1% glucose and different concentrations of $NaHCO_3$. The resulted cell suspensions were exposed to (i) 10–25 mM H_2O_2 or (ii) 0.1–0.2 mM $FeSO_4$ for 2 h. In other series of experiments, cells were suspended in 50 mM potassium phosphate buffer (KPi) (pH 7.5) or in 50 mM sodium bicarbonate buffer (pH 7.5) and then were exposed to: (i) 2 mM H_2O_2 + 2 mM $FeSO_4$ or (ii) 2 mM H_2O_2 + 2 mM $FeSO_4$ + 10 mM AKG for 1 h. AKG was used in the form of disodium salt of alpha-ketoglutarate. The control cell suspensions

were incubated under the same conditions without stressors. Cell survival after stress exposure was monitored by counting of colony-forming units on YPD agar plates.

Cell extracts were prepared by vortexing yeast cells with glass beads (0.5 mm) as previously described [17]. The content of carbonyl groups in the proteins (CP) was measured by determining the amount of 2,4-dinitrophenylhydrazone formed upon the reaction with 2,4-dinitrophenylhydrazine. Carbonyl content was calculated from the absorbance maximum of 2,4-dinitrophenylhydrazone at 370 nm with molar extinction coefficient of 22 $mM^{-1} \cdot cm^{-1}$ [13]. The level of total glutathione was measured as described in the paper [18] Yeast cells were suspended in 1.3% dinitrosalicilic acid and disrupted by vortexing with glass beads (0.5 mm) for three cycles (1 min of disruption and 3 min of cooling on ice). For determination of oxidized glutathione, the aliquots of supernatants were incubated with 5% 2-vinylpyridine for 1 h at room temperature. Protein concentration was determined by Bradford [7] basing on binding of Coomassie brilliant blue G-250 dye with protein.

Experimental data are expressed as mean of 4-6 independent experiments ± the standard error of the mean (SEM), and statistical analysis used Dunnett's test and Student's *t*-test [8].

RESULTS AND DISCUSSION

The survival of yeast cells upon treatment with hydrogen peroxide or ferrous sulfate in the presence of sodium bicarbonate at different concentrations was studied (Fig. 1). Hydrogen peroxide decreased yeast survival in both control and bicarbonate-supplemented suspensions (Fig. 1*A*). The survival was decreased with increasing of H_2O_2 concentration. In particular, cell viability was 79 and 35% in the control suspensions treated with 10 and 25 mM H_2O_2, respectively. The addition of 10 mM $NaHCO_3$ did not influence yeast resistance to H_2O_2, whereas 25 mM $NaHCO_3$ enhanced sensitivity of yeast cells to 10 mM and 15 mM H_2O_2. Yeast cells were the most sensitive to H_2O_2 in the presence of 50 mM $NaHCO_3$ with 73% and 17% of survival after treatment with 10 and 25 mM H_2O_2, respectively.

The incubation of yeast cells with 0.1–0.2 мМ $FeSO_4$ did not affect cell survival in the control (without bicarbonate) and in the medium, containing 10 mM $NaHCO_3$ (Fig. 1*B*). However, the treatment with ferrous sulfate in the presence of 25 or 50 mM $NaHCO_3$ decreased yeast viability with more sensitizing effect of 50 mM $NaHCO_3$. Thus, the survival decreased by 19% and 56% after treatment with 0.2 mM $FeSO_4$ in the presence of 25 and 50 mM $NaHCO_3$, respectively. The obtained results suggest that bicarbonate ions can enhance sensitivity of *S. cerevisiae* cells to hydrogen peroxide and iron ions.

Our results are consistent with previous reports *in vitro* which showed the ability of bicarbonate ions participate in redox-processes [3, 20, 23, 26]. In particular, the increase in peroxidase activity of Cu, Zn-SOD was shown in the presence of bicarbonate. The enzyme decomposes H_2O_2 with the formation of superoxide anion radical which is a direct substrate of SOD: $SOD\text{-}Cu^{2+} + H_2O_2 \rightarrow SOD\text{-}Cu^{1+} + O_2^{\bullet-} + 2H^+$; $SOD\text{-}Cu^{1+} + H_2O_2 \rightarrow SOD\text{-}Cu^{2+}\text{-}{}^{\bullet}OH + OH^-$. At this process, the enzyme is converted to intermediate inactive form $SOD\text{-}Cu^{2+}\text{-}{}^{\bullet}OH$ which can undergo further oxidative inactivation or can be restored to initial form ($SOD\text{-}Cu^{2+}$) by interaction with (bi)carbonate ions. HCO_3^- and/or CO_3^{2-} undergo one-electron oxidation to carbonate radical $CO_3^{\bullet-}$: $SOD\text{-}Cu^{2+}\text{-}{}^{\bullet}OH + HCO_3^- \rightarrow SOD\text{-}Cu^{2+} + H_2O + CO_3^{\bullet-}$ [9, 15, 20, 26, 28]. It was also shown that *in vitro* $CO_3^{\bullet-}$ can be formed in the reaction of carbonate ions with peroxinitrite ($ONOO^-$) or directly

with hydroxyl radical (•OH) [3, 16, 20]. It was previously shown that the sensitivity of bacteria *Escherichia coli* and yeast *S. cerevisiae* to γ-radiation was significantly increased in bicarbonate buffer. It was due to the formation of carbonate radical in the reaction of HCO_3^- with products of water photolysis [12]. Our results suggest that the enhanced cytotoxic action of H_2O_2 and Fe^{2+} in the presence of bicarbonate ions can be associated with the intracellular generation of carboxyl radical, because there is no information regarding direct non-enzymatic reaction between HCO_3^- and H_2O_2 or iron ions [11]. It was assumed that bicarbonate ions can enter yeast cells through mammalian Slc4-like proteins which were also indentified in yeast as bicarbonate transporters [21]. In cells, HCO_3^- can enhance H_2O_2-scavenging activity of Cu,Zn-SOD, as it was shown *in vitro* [15]. Thus, $CO_3^{\bullet-}$ can be produced in this reaction. $CO_3^{\bullet-}$ is more reactive compound than H_2O_2, and this fact can explain a higher sensitivity of yeast cells to hydrogen peroxide in the presence of bicarbonate ions. The enhanced sensitivity of *S. cerevisiae* to ferrous sulfate treatment in the presence of bicarbonate (Fig. 1*B*) can also be explained by $CO_3^{\bullet-}$ formation. It is known, the toxicity of Fe^{2+} is connected with its ability to generate hydroxyl radical in the Fenton reaction [24]. In turn, hydroxyl radical can react with HCO_3^-/CO_3^{2-} to form $CO_3^{\bullet-}$ [3, 16, 20]. Despite $CO_3^{\bullet-}$ is less reactive compound than •OH, $CO_3^{\bullet-}$ has a much longer half-life and can therefore diffuse further and oxidatively modify distant cellular targets [16].

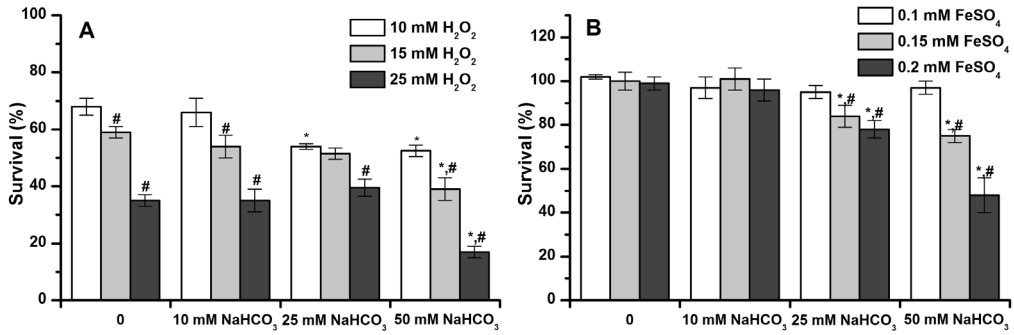

Fig. 1. Survival of *S. cerevisiae* YPH250 cells treated with з H_2O_2 (*A*) or $FeSO_4$ (*B*) for 2 h in the presence of $NaHCO_3$. * – significant different from respective values of the group without $NaHCO_3$, # – from respective values of the group treated with 10 mM H_2O_2 (A) or with 0.1 mM $FeSO_4$ (B) with $P<0.05$ using Dunnett's test, n = 4–6

Рис. 1. Виживання клітин *S. cerevisiae* YPH250 після двохгодинної інкубації з H_2O_2 (*A*) та $FeSO_4$ (*B*) у присутності $NaHCO_3$. * – вірогідно відрізняється від відповідних значень у пробах без $NaHCO_3$, # - від значень у пробах, підданих дії 10 мМ H_2O_2 (А) або 0,1 мМ $FeSO_4$ (Б) з $P<0,05$ за тестом Даннета, n = 4–6

Since the ability of bicarbonate ions to potentiate toxicity of hydrogen peroxide and iron ions could be connected with the intensification of free radical processes, the levels of oxidative stress markers such as protein carbonyl groups and glutathione were measured in yeast cells. Content of carbonyl group in proteins (CP) is a widely used parameter of oxidative damages of proteins [9, 17, 22]. Glutathione (GSH) is a low molecular mass antioxidant which plays an important role in the maintenance of redox homeostasis in *S. cerevisiae* [25]. CP levels and levels of oxidized glutathione (GSSG) were similar control cells and in cells treated 0.2 mM $FeSO_4$ in the absence or presence of

$NaHCO_3$ (See table). At the same time, total GSH was decreased in cells treated with 0.2 mM $FeSO_4$ in the presence of $NaHCO_3$ at higher concentrations. Accordingly, the total GSH was 22% lower in cells treated with 0.2 mM $FeSO_4$ and 50 mM $NaHCO_3$.

Similar results were obtained when the ability of bicarbonate to modulate sensitivity of yeast cells to menadione was studied [17]. Bicarbonate enhanced cytotoxicity of menadione that was accompanied by decreased GSH level in cells without changes in CP levels. The absence of changes in CP level could suggest that $CO_3^{\bullet-}$ generated in bicarbonate buffer might promote other types of protein damages which are different from carbonylation. For example, $CO_3^{\bullet-}$ was found can form tyrosyl radical and tyrosine cross-links and oxidize SH-groups of cysteine [1, 6, 26]. $CO_3^{\bullet-}$ can also damage DNA by reacting with guanine base producing 8-oxoguanine [27]. The decrease in level of GSH which is a cysteine-containing tripeptide seems not to be connected with its oxidation because the level of GSSG was unchanged in cells co-treated with ferrous sulfate and $NaHCO_3$ (See table). Obviously, the synthesis GSH *de novo* can be decreased under these conditions. The decreased GSH level can lead to disturbing redox balance in cells and reduce antioxidant defense. It could enhance yeast sensitivity to oxidative stress inductors in bicarbonate buffer.

Level of glutathione and carbonyl proteins in *S. cerevisiae* YPH250 cells treated with $FeSO_4$ in the presence of $NaHCO_3$ (M ± m, n = 4–5)

Вміст глутатіону та карбонільних груп білків у клітинах *S. cerevisiae* YPH250, проінкубованих з $FeSO_4$ за наявності $NaHCO_3$ (M ± m, n = 4–5)

Conditions	Parameter	$NaHCO_3$, mM			
		0	10	25	50
Control	Total GSH, μM/OD_{600}	2.61±0.04	2.51±0.12	2.36±0.17	2.25±0.14
	GSSG, μM/OD_{600}	0.626±0.049	0.642±0.112	0.526±0.064	0.611±0.052
	CP, nmol/mg protein	3.93±0.21	4.04±0.25	3.86±0.61	3.65±0.16
0.2 mM $FeSO_4$	Total GSH, μM/OD_{600}	2.74±0.04	2.36±0.19	2.28±0.05*	2.13±0.18*
	GSSG, μM/OD_{600}	0.655±0.034	0.660±0.075	0.601±0.041	0.580±0.045
	CP, nmol/mg protein	4.17±0.29	3.84±0.25	4.15±0.17	3.86±0.28

Comment: *Significantly different from respective values of the group without $NaHCO_3$ with $P<0.05$ using Dunnett's test

Примітка: *Вірогідно відрізняється від відповідного значення у пробах без $NaHCO_3$ з $P < 0,05$ за тестом Даннета

In next step of experiments, the survival of yeast cells treated with mixture of "2 mM H_2O_2 + 2 mM $FeSO_4$" was studied. Hydroxyl radicals are directly generated in this mixture. The survival of YPH250 cells treated with "Fe^{2+}/H_2O_2" in 50 mM KPi (pH 7.5) or in 50 mM sodium bicarbonate buffer (pH 7.5) was calculated (Fig. 2). The number of the viable cells was significantly decreased in both buffers but the cells treated in sodium bicarbonate buffer were more resistant to "Fe^{2+}/H_2O_2" with 1.6-fold higher survival compared to the one in KPi. The results suggest that bicarbonate can alleviate toxic action of Fe^{2+}/H_2O_2 system. Given that HCO_3^- can react with $^{\bullet}OH$ forming $CO_3^{\bullet-}$, it can be supposed, that $CO_3^{\bullet-}$ is less toxic, than $^{\bullet}OH$, and therefore cells survived better. At the same time, the experiments above showed that bicarbonate enhanced toxicity of H_2O_2 and

Fe^{2+} if yeast cells were treated with these compounds separately. Somewhat similar results previously were observed on *E. coli* та *S. cerevisiae* exposed to radiolysis products [12]. Yeast and bacteria were more sensitive to $CO_3^{\bullet-}$, than to $^{\bullet}OH$, but under the combined treatment with these radicals cells survived better than in the medium where $^{\bullet}OH$ was only generated. The protective effects of bicarbonate were increased when high amounts of $^{\bullet}OH$ were produced [12]. The authors explained these results by complicated kinetics of an interaction between HCO_3^-/CO_3^{2-}, $CO_3^{\bullet-}$ and $^{\bullet}OH$. It can be supposed, if $^{\bullet}OH$ and $CO_3^{\bullet-}$ are produced in relatively moderate amounts, the combination of these radicals enhances their toxic action. When $^{\bullet}OH$ is produced in high concentrations, it is more dangerous than $CO_3^{\bullet-}$ due to very short life time. Under treatment with system "Fe^{2+}/H_2O_2" in bicarbonate buffer, most $^{\bullet}OH$ produced can rapidly react with bicarbonate ions with formations of large amounts of radical $CO_3^{\bullet-}$. On the other hand, at high amounts, $CO_3^{\bullet-}$ can react with each other to form non-radical ions: $CO_3^{\bullet-} + CO_3^{\bullet-} \rightarrow CO_2 + CO_4^{2-}$ [3]. This can explain the decreased toxicity of "Fe^{2+}/H_2O_2" in bicarbonate buffer but not in KPi. When yeast cells were exposed to Fe^{2+} and H_2O_2 separately, it seems that production of $^{\bullet}OH$ was lower, therefore toxicity of $CO_3^{\bullet-}$ was more expressed and yeast viability was reduced.

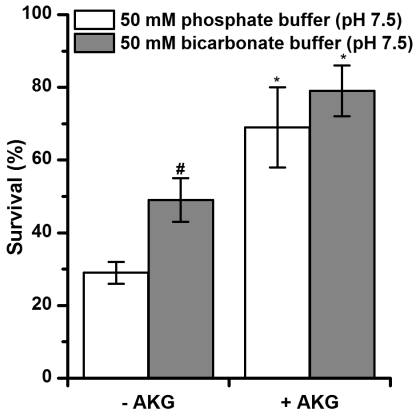

Fig. 2. Survival of *S. cerevisiae* YPH250 cells treated with "2 mM H_2O_2 + 2 mM $FeSO_4$" for 1 h in 50 mM potassium phosphate buffer (pH 7.5) or 50 mM sodium bicarbonate buffer (pH 7.5) without or with 10 mM AKG. * – significant different from respective values of the group without AKG, # – from respective values in KPi with $P<0.05$ using Student's t-test, n = 5–6

Рис. 2. Виживання клітин *S. cerevisiae* YPH250 після інкубації протягом 1 год у системі «2 мМ H_2O_2 + 2 мМ $FeSO_4$» у 50 мМ калій-фосфатному (pH 7,5) або 50 мМ натрій-бікарбонатному буфері (pH 7,5) без та з додаванням 10 мМ АКГ. * – вірогідно відрізняється від відповідних значень у пробах без АКГ, # – від відповідних значень проб у КФБ з $P<0,05$ за тестом Стьюдента, n = 5–6

Since pro-oxidant and protective effects of bicarbonate ions could depend on the intensity of $^{\bullet}OH$ production, the ability of antioxidant compounds to modulate these bicarbonate activities was studied. Alpha-keroglutarate, an important intermediate of the Krebs cycle, was chosen as an antioxidant. In our previous works, the powerful H_2O_2-scavenging *in vitro* activity of AKG was demonstrated [4]. As seen from Fig. 2, the addition of 10 mM АКГ enhanced yeast survival in system "2 mM H_2O_2 + 2 mM $FeSO_4$" in both KPi and bicarbonate buffers. The protective effects of AKG can be attributed its ability to non-enzymatically react with H_2O_2 and to prevent OH production [4]. The protective effect of AKG was more expressed in KPi buffer (cell survival increased from 29 to 68%), than in sodium bicarbonate buffer (cell survival increased from 49 to 79%). Thus, the presence of antioxidant compounds interferes partly with protective effects of bicarbonate ions.

CONCLUSIONS

The obtained results suggest that bicarbonate ions at physiological concentrations (25–50 mM) can enhance and alleviate the toxic effects of hydrogen peroxide and iron ions on yeast *S. cerevisiae*. Both effects are likely to be caused by the formation of carbonate radicals. The level of ROS produced and their complicated interaction with bicarbonate ions seems to determine the direction of bicarbonate action. Bicarbonate ions sensitize yeast cells to the oxidants when $^{\bullet}OH$ is produced in relatively low levels. When $^{\bullet}OH$ is produced in high amounts, the protective effects of bicarbonate can be observed. Alpha-ketoglutarate protects yeast cells under exposure in system "Fe^{2+}/H_2O_2" in KPi and bicarbonate buffers, although the protective effect is lower in bicarbonate buffer. Thus, HCO_3^- and CO_3^{2-} ions which are widely distributed in biological systems, can show both prooxidant and antioxidant properties. The latter depend largely on the intensity of ROS production and the activity of other antioxidant compounds. The redox-activity of CO_2, HCO_3^- and CO_3^{2-} ions suggests that the main physiological buffer can modulate oxidative injuries resulting from ROS generated endogenously *in vivo* under physiological or pathological conditions. For example, carbon dioxide retention due to hypoventilation resulting from airway obstruction, emphysema, respiratory muscle paralysis and pulmonary fibrosis increases bicarbonate-carbon dioxide levels above the physiological ones and this may be relevant to the oxidative damage associated with these clinical conditions. Even at physiological levels, the bicarbonate-carbon dioxide pair stimulates oxidations mediated by Cu,Zn-SOD, hydrogen peroxide or iron ions. Thus, the study of the oxidants derived from the bicarbonate-carbon dioxide pair is likely to provide new mechanistic insights into the understanding and control of numerous pathological states.

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1. *Andrekopoulos C., Zhang H., Joseph J.* et al. Bicarbonate enhances α-synuclein oligomerization and nitration: intermediacy of carbonate radical anion and nitrogen dioxide radical. **Biochemical Journal**, 2004; 378: 435–447.
2. *Arai H., Berlett B.S., Chock, P.B.* et al. Effect of bicarbonate on iron-mediated oxidation of low-density lipoprotein. **PNAS**, 2005; 102(30): 10472–10477.
3. *Augusto O., Bonini M.G., Amanso A.M.* et al. Nitrogen dioxide and carbonate radical anion: two emerging radicals in biology. **Free Radical Biology and Medicine**, 2002; 32(9): 841–859.
4. *Bayliak M.M., Lylyk M.P., Vytvytska O.M.* et al. Assessment of antioxidant properties of alpha-keto acids *in vitro* and *in vivo*. **European Food Research and Technology**, 2016; 242(2): 179–188.
5. *Berlett B.S., Chock P.B., Yim M.B.* et al. Manganese(II) catalyzes the bicarbonate-dependent oxidation of amino acids by hydrogen peroxide and the amino acid-facilitated dismutation of hydrogen peroxide. **Proceedings of the National Academy of Sciences (USA)**, 1990; 87: 389–393.
6. *Bonini M.G., Fernandes D.C., Augusto O*. Albumin oxidation to diverse radicals by the peroxidase activity of Cu,Zn-superoxide dismutase in the presence of bicarbonate or nitrite: diffusible radicals produce cysteinyl and solvent-exposed and -unexposed tyrosyl radicals. **Biochemistry**, 2004; 43: 344–351.

7. *Bradford M.M*. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. **Analytical Biochemistry**, 1976; 72: 289–292.
8. *Brooks S.P.* A simple computer program with statistical tests for the analysis of enzyme kinetics. **BioTechniques**, 1992; 72: P. 906–911.
9. *Davies M.J.* Protein oxidation and peroxidation. **Biochemical Journal**, 2016; 473(7): 805–825.
10. *Elam J.S., Malek K., Rodriguez J.A.* et al. An alternative mechanism of bicarbonate-mediated peroxidation by copper-zinc superoxide dismutase: rates enhanced via proposed enzyme-associated peroxycarbonate intermediate. **Journal of Biological Chemistry**, 2003; 278(23): 21032–21039.
11. *Jansson P.J., Del Castillo U., Lindqvist C.* et al. Effects of iron on vitamin C/copper-induced hydroxyl radical generation in bicarbonate-rich water. **Free Radical Research,** 2005; 39 (5): 565–570.
12. *King D.A., Sheafor M.W., Hurst J.K*. Comparative toxicities of putative phagocyte-generated oxidizing radicals toward a bacterium (*Escherichia coli*) and a yeast (*Saccharomyces cerevisiae*). **Free Radical Biology and Medicine**, 2006; 41(5): 765–774.
13. *Levine R.L., Wehr N., Williams J.A.* et al. Determination of carbonyl groups in oxidized proteins. **Methods in Molecular Biology**, 2000; 99: 15–24.
14. *Liochev S.I., Fridovich I.* Carbon dioxide mediates Mn(II)-catalyzed decomposition of hydrogen peroxide and peroxidation reactions. **Proceedings of the National Academy of Sciences (USA)**, 2004; 101: 12485–12490.
15. *Liochev S.I., Fridovich I.* Mechanism of the peroxidase activity of Cu, Zn superoxide dismutase. **Free Radical Biology and Medicine**, 2010; 48(12): 1565–1569.
16. *Luc R., Vergely C*. Forgotten radicals in biology. **International Journal of Biomedical Science: IJBS**, 2008; 4(4): 255–259.
17. *Lushchak O.V., Bayliak M.M., Korobova O.V.* et al. Buffer modulation of menadione-induced oxidative stress in *Saccharomyces cerevisiae*. **Redox Reports**, 2009; 14: 214–220.
18. *Lushchak V., Lushchak L., Mota A.* et al. Oxidative stress and antioxidant defences in goldfish *Carassius auratus* during anoxia and reoxygenation. **American Journal of Physiology**, 2001; 280: R100–R107.
19. *Lushchak V.I.* Free radicals, reactive oxygen species, oxidative stress and its classification. **Chemico-Biological Interactions**, 2014, 224C: 164–175.
20. *Medinas D.B., Cerchiaro G., Trindade D.F*. et al. The carbonate radical and related oxidants derived from bicarbonate buffer. **IUBMB Life**, 2007; 59: 255–262.
21. *Parker M.D., Boron W.F.* The divergence, actions, roles, and relatives of sodium-coupled bicarbonate transporters. **Physiological Research**, 2013; 93(2): 803–959.
22. *Prokopiv T.M., Fedorovych D.V., Boretsky Y.R.* et al. Oversynthesis of riboflavin in the yeast *Pichia guilliermondii* is accompanied by reduced catalase and superoxide dismutases activities. **Current Microbiology**, 2013; 66(1): 79–87.
23. *Queliconi B.B., Marazzi T.B., Vaz S.M.* et al. Bicarbonate modulates oxidative and functional damage in ischemia-reperfusion. **Free Radical Biology and Medicine**, 2013; 55:46–53.
24. *Stadtman E.R., Levine R.L.* Free radical-mediated oxidation of free amino acids and amino acid residues in proteins. **Amino Acids**, 2003; 25(3-4): 207–218.
25. *Toledano M.B., Delaunay-Moisan A., Outten C.E.* et al. Functions and cellular compartmentation of the thioredoxin and glutathione pathways in yeast. **Antioxidants and Redox Signaling Journal**, 2013; 18(13): 1699–711.
26. *Vesela A., Milhelm J.* The role of carbon dioxide in free radical reactions of the organism. **Physiological Research**, 2002; 51: 335–339.
27. *Yadav A., Mishra P.C.* Carbonate radical anion as an efficient reactive oxygen species: Its reaction with guanyl radical and formation of 8-oxoguanine **Chemical Physics,** 2012; 405: 76–88.
28. *Zhang H., Joseph J., Gurney M.* et al. Bicarbonate enhances peroxidase activity of Cu,Zn-superoxide dismutase. Role of carbonate anion radical and scavenging of carbonate anion radical by metalloporphyrin antioxidant enzyme mimetics. **Journal of Biological Chemistry**, 2002; 277: 1013–1020.

ВПЛИВ БІКАРБОНАТІВ ТА АЛЬФА-КЕТОГЛУТАРАТУ НА ЧУТЛИВІСТЬ ДРІЖДЖІВ *SACCHAROMYCES CEREVISIAE* ДО ДІЇ ПЕРОКСИДУ ВОДНЮ ТА ІОНІВ ЗАЛІЗА

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Досліджено вплив бікарбонату натрію на чутливість дріжджів *Saccharomyces cerevisiae* до пероксиду водню та сульфату заліза. Життєздатність дріжджів, підданих дії 10–25 мМ H_2O_2 та 0,1–0,2 мМ $FeSO_4$, значно знижувалася за додавання у середовище інкубації 25 або 50 мМ $NaHCO_3$. За обробки 0,2 мМ $FeSO_4$ при відсутності бікарбонатів, вміст у клітинах маркерів оксидативного стресу, а саме білкових карбонільних груп, загального та окисленого глутатіону не відрізнявся від відповідних показників у контролі (без $FeSO_4$ та $NaHCO_3$). У клітинах дріжджів, інкубованих з 0,2 мМ $FeSO_4$ та 50 мМ $NaHCO_3$, вміст загального глутатіону був нижчим за рахунок зниження його синтезу, а вміст карбонільних груп у білках та вміст окисленого глутатіону не відрізнявся від відповідних показників у клітин, які піддавалися дії $FeSO_4$ за відсутності бікарбонатів. Клітини дріжджів, піддані дії суміші "2 мМ H_2O_2 + 2 мМ $FeSO_4$" у 50 мМ натрій-бікарбонатному буфері виживали краще, ніж клітини, оброблені даними оксидантами у 50 мМ калій-фосфатному буфері. Додавання 10 мМ альфа-кетоглутарату підвищувало виживання клітин, оброблених у системі "Fe^{2+}/H_2O_2", в обох буферах. Захисний ефект альфа-кетоглутарату, очевидно, пов'язаний з його здатністю знешкоджувати H_2O_2. Отримані результати свідчать про те, що бікарбонатні іони можуть як посилювати, так і послаблювати токсичну дію редокс-активних сполук на клітини *S. cerevisiae*. Ймовірно, що про-/антиоксидантна дія бікарбонатів залежить від кінетики взаємодії HCO_3^- з АФК, які утворюються при дії оксидантів.

Ключові слова: *Saccharomyces cerevisiae*; альфа-кетоглутарат; бікарбонат-іони; карбонатний радикал; оксидативний стрес.

ВЛИЯНИЕ БИКАРБОНАТОВ И АЛЬФА-КЕТОГЛУТАРАТА НА ЧУВСТВИТЕЛЬНОСТЬ ДРОЖЖЕЙ *SACCHAROMYCES CEREVISIAE* К ПЕРЕКЕСИ ВОДОРОДА И ИОНАМ ЖЕЛЕЗА

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Исследовано влияние бикарбоната натрия на чувствительность дрожжей *Saccharomyces cerevisiae* к перекиси водорода и сульфата железа. Жизнеспособность дрожжей, обработанных 10–25 мМ H_2O_2 и 0,1–0,2 мМ $FeSO_4$, значительно уменьшалась при добавлении в среду инкубации 25 или 50 мМ $NaHCO_3$. При обработке 0,2 мМ $FeSO_4$ в отсутствии бикарбонатов, содержание в клетках маркеров окислительного стресса, а именно белковых карбонильных групп, общего и оки-

сленного глутатиона не отличалось от соответствующих показателей в контрольных клетках (без $FeSO_4$ и $NaHCO_3$). В клетках дрожжей, инкубированных с 0,2 мМ $FeSO_4$ и 50 мМ $NaHCO_3$, содержание общего глутатиона было ниже за счет снижения его синтеза, а содержание окисленного глутатиона и карбонильных групп в белках не отличалось от соответствующих показателей у клеток, которые подвергались воздействию $FeSO_4$ при отсутствии бикарбонатов. Клетки дрожжей, обработаны смесью "2 мМ H_2O_2 + 2 мМ $FeSO_4$" в 50 мМ натрий-бикарбонатном буфере, выживали лучше, чем клетки, обработанные данными оксидантами в 50 мМ калий-фосфатном буфере. Добавление 10 мМ альфа-кетоглутарата повышало выживаемость клеток, обработанных в системе "Fe^{2+}/H_2O_2", в обоих буферах. Защитный эффект альфа-кетоглутарата, очевидно, связанный с его способностью обезвреживать H_2O_2. Полученные результаты свидетельствуют о том, что бикарбонатные ионы могут как усиливать, так и ослаблять токсическое действие редокс-активных соединений на клетки *S. cerevisiae*. Вероятно, что про-/антиоксидантное действие бикарбонатов зависит от кинетики взаимодействия HCO_3^- с АФК, которые образуются при воздействии оксидантов.

Ключевые слова: *Saccharomyces cerevisiae*; альфа-кетоглутарат; бикарбонат-ионы; карбонатный радикал; окислительный стресс.

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