PROCEEDINGS OF THE YEREVAN STATE UNIVERSITY

Chemistry and Biology 2013, № 2, p. 40–43

Biology

OPTIMAL CONDITIONS OF INDUCTION OF L-AMINO ACID OXIDASE OF *ASPERGILLUS NIGER* R3 FUNGUS BY HYDROGEN PEROXIDE

H. M. DAVTYAN, S. P. HOVHANNISYAN*, H. M. KARAPETYAN

Chair of Biochemistry YSU (Scientific-Research Laboratory of Biochemistry of Nitrogen Compounds), Armenia

Our previous research has shown that L-amino acid oxidase activity in *Asp. niger* R3 yeast extracts was not detected and only some peroxisomal fractions have L-amino acid oxidase activity. Induction of L-amino acid oxidase is seen when hydrogen peroxide is added to the growth media. Our task was to investigate the induction of the enzyme by H_2O_2 addition to incubation media. The results revealed that adding H_2O_2 to *Asp. niger* R3 yeast extracts induce L-amino acid oxidase, especially it is more effective to use H_2O_2 in the concentration of 0.001 μM in the case of L-alanine as a substrate, and in the case of L-methionine – 0.003 μM respectively.

***Keywords*:** enzyme induction, L-alanine, L-methionine, deamination, inhibitory effect.

Introduction. According to the results obtained from our previous study about elaboration of induction of L-amino acid oxidase, addition of hydrogen peroxide in concentration 0.02 *M* to nutrient medium induces L-amino acid oxidase [1]. By increasing or decreasing the H_2O_2 concentration, enzyme induction is expressed more poorly. It has been previously shown, that high concentrations of H_2O_2 has inhibitory effect on the growth of *S. cerevisiae* yeasts, on the contrary low concentrations stimulate the growth [2]. It is interesting that static magnetic field and high power vibrations during first 20 *min* inhibit the growth of yeasts, but after 2 *h* stimulate the growth. According to the authors [3], static magnetic field and high power vibrations increase the level of hydrogen peroxide. It was shown, that extremely high magnetic field's rates (4 *Hz*) and hydrogen peroxide inhibit contractility of the heart by inducing Na-K ATP-ase, in the case when ultrasound stimulate ATP-ase activity. Authors concluded that H_2O_2 is a messenger by which accomplished the electromagnetic field's action on the heart, and suppression of the heart contractility by ultrasound is due to the decrease in CO_2 solubility [4]. Our previous investigations show, that there are low activity of L-amino acid oxidase in some peroxisomal fractions of *Aspergillus Niger* R3 [5]. According to

* E-mail: bio_chm@ysu.am

the researchers [6] high concentrations of H_2O_2 has cytotoxic effect, in the case when low concentrations induce many cellular functions, including enzyme induction. In order to make more effective induction of enzyme by H_2O_2, we investigated *Aspergillus Niger* R3 fungus's L-amino acid oxidase induction by adding H_2O_2 not only to nutrient medium but also by adding to incubation medium for enzyme activity determination.

Materials and Methods. Aspergillus Niger R3 is served as research object, which is used in citric acid production. Rolen's synthetic nutrient media is used with composition of glucose – 2.0 *g*, KH_2PO_4 – 0.05 *g*, $MgSO_4 \cdot 7H_2O$ – 0.05 *g*, $ZnSO_4 \cdot 7H_2O$ – 0.005 *g*, solved in 100 *Mm* tap water. For media growth we added 0.5 *ml* 33% H_2O_2. 0.4 *g* L-alanine was used as nitrogen source. Culture was made on 100 *ml* media (pH 7.0 42^0C). 4 day grown culture *Aspergillus Niger* R3 at 32^0C was homogenized in distilled water for 6 *min* in Potter-Elvedgiem's homogenizator [7]. To determine L-amino acid oxidase activity, enzyme preparation was incubated for 60 or 90 *min* in pyrophosphate buffer media in presence of corresponding substrates, L-alanine, L-methionine, L-valine and L-glutamate respectively, 10 *mM*. The reaction was stopped by 0.2% dinitrophenylhydrazine, then 0.1 *N* NaOH. Color intensity was measured at 440 *nm*. Enzyme activity was expressed in μM of produced ketoacid per 1 *g* of mycelium [8].

Results and Discussion. As it has been shown in our previous research (Tab. 1), in the absence of H_2O_2 in the media the activity of L-amino acid oxidase was not detected, in the case of adding 0.02 *M* concentration of H_2O_2 to the media the activity of L-amino acid oxidase was induced. Nevertheless, further increase of H_2O_2 concentration decreased the activity of enzyme by 40%.

Table 1

Effects of different H_2O_2 concentrations in the growth media on L-amino acid oxidase activity in the extracts of Asp. niger R3 fungus (μM ketoacid per 1 g mycelium n = 5, p < 0.05)

Composition of nutrient media	L-amino acid oxidase activity (substrate L-alanine)
without H_2O_2	0
0.01 *M* H_2O_2	6.04±0.3
0.02 *M* H_2O_2	9.5±0.4
0.03 *M* H_2O_2	7.6±0.2
0.045 *M* H_2O_2	6.1±0.2

It can be concluded, that H_2O_2 over or less than 0.02 *M* concentration has inhibitory effect on enzyme activity. According to the results obtained from our previous investigations adding H_2O_2 to the *Asp. niger* R3 yeast's growth media leads to the induction of L-amino acid oxidase.

In the next stage of our experiments we attempted to investigate the activity of L-amino acid oxidase by adding the H_2O_2 to the incubation media. For that reason every 20 *min* 0.001 *M* concentration of H_2O_2 was added to the incubation media. The results are presented in Tab. 2.

As one can notice from the results the activity of enzyme is not detected in the sample without H_2O_2 in the incubation media. After 20 *min* incubation in the presence of 0.001 μM H_2O_2 and L-alanine used as a substrate, the activity of

L-amino acid oxidase can be detected (4.45 μM ketoacid/1 g mycelium). Further incubation for another 20 *min* with 0.002 μM H_2O_2 leads to a slight decrease in enzyme activity (1.9 μM ketoacid/1 g mycelium). Another 20 *min* incubation with 0.003 μM H_2O_2 causes a dramatic decrease in enzyme activity (from 1.9 μM to 0.6 μM ketoacid / 1 g mycelium). The results obtained from the same experiment which has been done by using L-methionine instead of L-alanine as deamination substrate have some differences. It is notable, that the highest deamination activity in the case of using L-alanine as a substrate is noticed after first 20 *min* incubation by H_2O_2 and the activity of enzyme when using L-methionine as a substrate equals 0 not only after first 20 *min* but also after 40 *min* incubation period. But, after 60 *min* incubation with 0.003 μM H_2O_2 L-amino acid oxidase activity increases to 2.8 μM ketoacid / 1 g mycelium.

Table 2

Effect of different concentrations of H_2O_2 in the incubation media on L-amino acid oxidase activity in the extracts of Asp. niger R3 yeasts ($n = 5$, $p < 0.05$)

Substrate	H_2O_2 concentrations, μM	Incubation duration, *min*	Enzyme activity (μM ketoacid / 1 g mycelium)
L-alanine	without H_2O_2	–	0
	0.001	20	4.45 ±0.046
	0.002	40	1.9 ± 0.02
	0.003	60	0.6 ± 0.01
L-methionine	without H_2O_2	–	0
	0.001	20	0
	0.002	40	0
	0.003	60	2.8 ± 0.02

It can be concluded, that H_2O_2 stimulates L-amino acid oxidase activity by adding it not only to the growth media but also to the incubation media. It is interesting, that there are some differences in the pattern of L-amino acid oxidase activity induction by H_2O_2 for different amino acids (L-alanine, L-methionine), and additional research is required for clarification.

Received 06.06.2013

REFERENCES

1. **Hovhannisyan S.P., Gabrielyan G.A., Davtyan M.A., Grigoryan A.G.** The Influence of Hydrogen Peroxide on the Induction of L-Aminoacid Oxidase in *Asp. niger* R-3 Yeast. // Vestnik IEALPS, 2010, v. 15, №5 (1), p. 56–58 (in Russian).
2. **Bagdasaryan N.S., Ayrapetyan S.N.** The effect of SMF, EHPP and Hydrogen Peroxide on the Development of Yeasts. In: Bioelectromagnetics: Current Concepts (eds. Ayrapetyan S. and Markov M.). NATO Science Series, Netherlands: Springer Press, 2006, p. 391–397.
3. **Ayrapetyan G., Grigoryan A., Dadasyan E., Ayrapetyan S.** The Comparative Study of the Effects of 4 *Hz* Electromagnetic Fields-, Infrasound- Treated and Hydrogen Peroxide Containing Physiological Solutions on Na Pump-Induced Inhibition of Heart Muscle Contractility. // The Environmentalist, 2007, v. 27, p. 483–488.

4. **Ayrapetyan G., Grigoryan A., Hambaryan G., Dadasyan E., Hayrapetyan H., Ayrapetyan S.** Exogenous Hydrogen Peroxide as a Messenger for Stimulation Effect of Magnetized Physiological Solution on Heart Contractility. // Bioelectromagnetics, 2007, v. 29, p. 549–558.
5. **Hovhannisyan S.P., Gabrielyan G.A., Grigoryan A.G.** Deamination of Aminoacides by L-amionoacids' Oxidase of Fungus *Asp. Niger* R-3. // Sovremennaya nauka, 2011, №1, p. 65–68 (in Russian).
6. **Ikebuchi Y., Masumoto N., Tasaka K., Koike K., Kasahara K., Miyake A., Tanizawa O.** Superoxide Anion Increases Intracellular pH. Intracellular Free Calcium, and Arachidonate Release in Human Amnion Cells. // J. Biol. Chem., 1991, v. 266, p. 13233–13237.
7. **Hovhannisyan S.P., Gabrielyan G.A., Davtyan M.A., Grigoryan A.G.** The Influence of Hydrogen Peroxide on the Induction of L-Aminoacid Oxidase in *Asp. niger* R-3 Yeast. // Vestnik IEALPS, 2011, v. 15, №5 (1), p. 64–67 (in Russian).
8. **Baudhuin P., Beaufay A., Rahman-Li Y., Sellinger O.Z., Wattiaux R., Jacques P., De Duve.,** Intracellular Distribution of Monoamine Oxidase, Aspartate Aminotransferase, Alanine Aminotransferase, D-Amino Acid Oxidase and Katalase in Rate-Liver Tissuse. // J. Biochem., 1964, v. 92, p. 179–184.