Relationship between Natural Concentration of Heavy Water Isotopologs and Rate of H_2O_2 Generation by Mitochondria

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Relationship between natural concentration of heavy water isotope homologs in natural water and the kinetics of H_2O_2 generation by isolated rat liver mitochondria in the presence of succinic acid as the substrate was studied. Natural concentrations of heavy water isotopologs significantly inhibited the studied reaction. The decrease in heavy isotopolog content in natural water leads to disinhibition and significant acceleration of the studied reaction.

Key Words: *heavy water isotopologs; mitochondrion; hydrogen peroxide; succinic acid; Michaelis-Mentain*

The isotope composition of natural water is heterogeneous [8,9]. It consists of about 99.732% molecules of $^1H_2{}^{16}O$ isotope homolog (isotopolog) and about 0.268% heavy water molecules, including at least one stable heavy isotope ^2H, ^{17}O or ^{18}O, the concentrations of the three main heavy isotopologs in natural water being 100 mM for $^1H_2{}^{18}O$, 19 mM for $^1H_2{}^{17}O$, and 16 mM for $^1H^{16}O^2H$.

Heavy water isotopologs differ from the main $^1H_2{}^{16}O$ isotopologs by physicochemical and biological characteristics [1]. The relationship between natural concentrations of heavy isotopologs in natural water and the course of enzymatic reactions was never studied previously. Evaluation of this relationship is practically interesting, because the content of heavy isotopologs in natural water varies depending on the region, climatic conditions, and relief (mountains or plain) from 0.243% in Antarctic water (SLAP standard) to 0.268% in oceanic water (VSMOW standard).

We studied the relationship between natural concentrations of heavy isotopologs in natural water and the kinetics of H_2O_2 generation by isolated rat liver mitochondria in the presence of succinic acid as the substrate.

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MATERIALS AND METHODS

The concentration of H_2O_2 was evaluated by oxidation of phenol red in the presence of horseradish peroxidase [7].

Experiments were carried out on mitochondria isolated from the liver of male Wistar rats. The mitochondria were isolated by differentiated centrifugation in a solution containing 0.25 M sucrose and 0.1 M EDTA. The nuclei were removed at 600*g*, the mitochondria were isolated at 10,000*g*. Isolated mitochondria were incubated for 15 min at 37°C in a medium containing 35 mM Tris-HCl (pH 7.6) and succinic acid in ascending concentrations, after which H_2O_2 concentration in the incubation medium was measured.

Distilled water was obtained by rectification of natural water from the Moscow region, with 0.265% natural summary content of heavy water isotopologs (142 ppm ^2H, 1982 ppm ^{18}O, 385 ppm ^{17}O; control) or water with summary content of heavy water isotopologs lower than the natural (0.221%; 52 ppm ^2H, 1735 ppm ^{18}O, 369 ppm ^{17}O; experi-

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ment), obtained by vacuum rectification of natural water, served as the base for incubation medium. The content of stable isotopes in water specimens was evaluated by mass spectrometry. The content of ^{2}H and ^{18}O was evaluated in water specimens, ^{17}O in gaseous mixture obtained by quantitative electrolytic degradation of water samples.

The significance of differences was evaluated using Student's *t* test. The Vm and Km parameters of Michaelis—Mentain equation were calculated by analysis of regressions.

RESULTS

Isolated experimental mitochondria were characterized by a significantly higher degree of H_2O_2 generation in comparison with that of control mitochondria (Fig. 1). This effect was observed also in the presence of physiological concentrations of succinic acid (1-9 μM) [5].

The observed relationship between the initial rate of H_2O_2 generation by isolated mitochondria and the concentration of succinic acid in the control and experimental samples was described by the Michaelis—Mentain's equation:

$$V=VmS/(Km+S),$$

where V is the initial rate of H_2O_2 generation, S the concentration of succinic acid, Vm maximum rate of H_2O_2 generation, and Km is the Michaelis' constant.

The Vm values in the control and experiment in the equation describing the kinetics of H_2O_2 generation were equal to 0.45 and 0.75 nmol/min/mg protein, respectively, Km were 21 and 9 μM, respectively.

This relationship between the inverse reaction velocity (1/V) and inverse concentration of succinic acid (1/S) indicates that inhibition of H_2O_2 generation reaction by heavy water isotopologs was not competitive (Fig. 2). In accordance with this scheme heavy water isotopologs in natural concentrations as components of natural water act as inhibitors of H_2O_2 generation by mitochondria, while the decrease in concentrations of these isotopologs below the values observed in nature leads to disinhibition and acceleration of this reaction.

Our results do not contradict the data on $^{2}H_2{}^{16}O$ heavy isotopolog (heavy water) inhibition of succinic acid oxidation by rat liver mitochondria [6].

The fact that heavy water isotopologs in natural concentrations as components of natural water inhibit the reaction playing the role of a positive regulator of the insulin receptor signal transmission cascade [2,3] indicates the practical significance of studying the biological effects of heavy water isotopologs in natural water. The biological effects of natural mineral waters can be due to not only their mineral composition, but also the concentrations of heavy isotopologs, a factor heretofore neglected. It is known that the content of heavy isotopologs is usually low in mineral waters of mountainous origin [4].

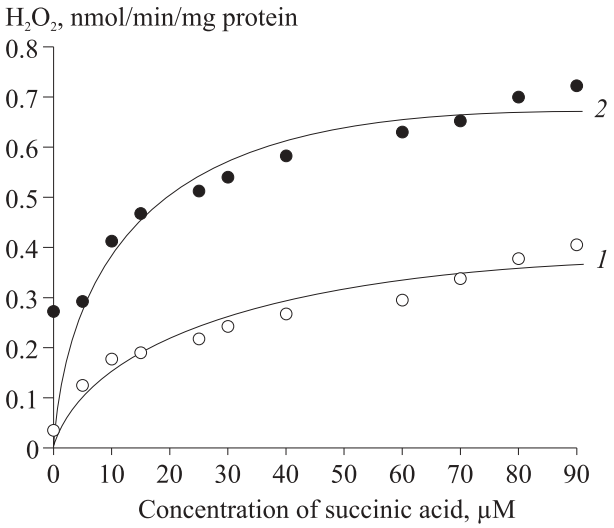

Fig. 1. Relationship between initial rate of H_2O_2 generation by isolated mitochondria and the concentration of succinic acid. **p*<0.05 *vs.* control (*n*=4). Here and in Fig. 1: *1*) control; *2*) experiment. Symbols: experimental data; lines: estimated data.

Hence, we found that heavy water isotopologs in natural concentrations as components of natural water exhibit a significant common inhibitory effect on H_2O_2 generation by the mitochondria. Reduction of heavy isotopolog content in the water to the level below the natural concentration disinhibits and significantly accelerates the studied reaction.

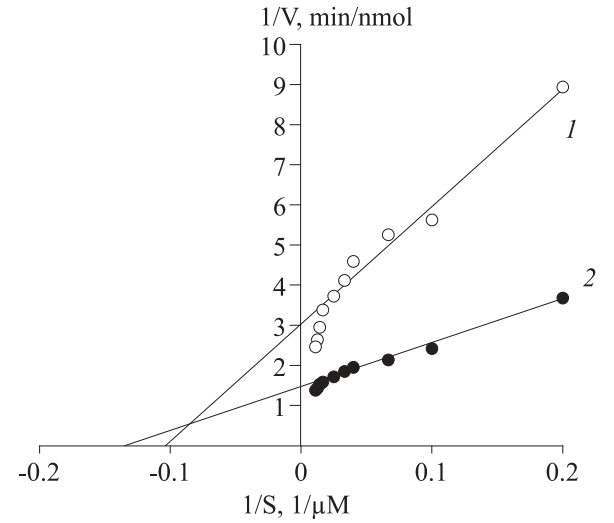

Fig. 2. Relationship between initial rate of H_2O_2 generation by isolated mitochondria and the concentration of succinic acid in double inverse coordinates.

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