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Kinetics of Oxidative Deamination by Monoamine Oxidase Preparations

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Abstract

Assessing the activity of monoamine oxidase enzyme was performed for the reactions of serotonin, tryptamine and benzylamine deamination in different groups of animals according the ratio between the maximum deamination rate (w_{max}) and the Michaelis constant (KM). For the representatives of mammalian species studied the ratio of w_{max}/KM ranges within 1.0–3.6, for birds this range is wider amounting to 0.4–5.0, for fish this value ranges within 0.3?1.8. It was demonstrated that increasing the concentrations of substrates (up to 10^{-2} mol/L) or temperature (up to 42 °C) promotes the enzyme activation.

Key words: serotonin, tryptamine, kinetics, deamination, monoamine oxidase

INTRODUCTION

Enzyme monoamine oxidase (EC 1.4.3.4.) attracts researchers' attention as the main enzyme catalyzing the reaction of oxidative deamination of important biogenic monoamines such as indole derivatives and catecholamines [1–3]. These monoamines represent regulators, neurohormones, whose broad spectrum of action is not fully understood yet. They are considered as stress realizing factors being involved in the adaptation of organisms.

Monoamine oxidase enzyme activity depends to a considerable extent on environmental factors [1–3, 4] and on the action of inhibitors, whose individual representatives are used as medicinal preparations [5, 6].

The aim of this work consisted in determining the kinetic parameters and kinetic features for the reactions of oxidative deamination for the following substrates: serotonin as the specific substrate of monoamine oxidase A, benzylamine as the specific substrate of monoamine oxidase B, and tryptamine as the substrate of monoamine oxidase A and B in the mitochondrial fraction of some representatives of mammals, birds and fish.

EXPERIMENTAL

For the investigations, we chose the samples of liver and brain tissues from the representatives of mammals, fish and birds of both sexes, sexually mature age, living in West Siberia (Table 1). Birds were bagged by shooting; fish was caught in the lakes of the Tyumen Region (except for oceanic mackerel that was commercially deep-frozen). Bull's liver was taken from a slaughtering and meat processing plant, white rats and mice represented outbred laboratory animals. The organs of mammals, birds and fish under investigation were placed onto ice immediately after removal and stored in a freezer for less than 3 days.

The level of monoamine oxidase activity was determined from the amount of released ammonia [2, 7]. As the substrates we used serotonin creatinine sulphate, tryptamine hydrochloride (Sigma, the USA) and Russian chemically

TABLE 1

Kinetic parameters for the deamination of monoamines in the mitochondrial fraction of tissues taken from mammals, birds at 38 °C and fish at 20 and 38 °C (the average of 5 tests)

Animals (organ)	Serotonin		Tryptamine		Benzylamine	
	K_M, 10^{-3} mol/L	w_{max}/K_M, 10^6	K_M, 10^{-3} mol/L	w_{max}/K_M, 10^6	K_M, 10^{-3} mol/L	w_{max}/K_M, 10^6
Bull (liver)	1.8±0.2	1.8	1.4±0.1	2.3	0.4±0.0	6.25
Rat (brain)	1.6±0.1	2.1	1.3+0.0	1.8	ND	ND
Rat (liver)	2.2±0.4	1.8	1.1±0.0	3.6	ND	ND
Dog (liver)*	2.6±0.1	1.0	2.0±0.6	0.55	2.0±0.6	0.7
Dove (liver)	0.8±0.0	2.1	0.4±0.1	4.0	2.4±0.0	0.83
Dove (brain)	0.8±0.2	1.9	0.8±0.4	2.25	0.8±0.2	0.22
Rough-legged buzzard (liver)	1.3±0.0	1.5	0.9±0.3	2.1	0.8±0.1	4.1
Wood-grouse (liver)	0.4±0.0	5.0	0.6±0.1	4.2	0.8±0.1	4.1
Great tit (liver)	1.4±0.0	2.9	1.0±0.1	5.0	6.7±0.9	0.75
Silver Gull (liver)	1.4±0.5	1.4	2.0±0.2	0.65	2.5±0.0	1.2
Bullfinch (liver)	1.5±0.1	1.3	0.9±0.0	1.3	1.1±0.2	1.8
Ruff (liver)	4.5±0.3	0.4	4.0±0.1	0.25	1.5±0.3	1.7
Kestrel (liver)	2.5±0.0	1.0	2.0±0.1	1.25	5.0±0.6	0.7
Oceanic horse-mackerel (liver)	10.0±0.4**	0.3	2.5±0.3	2.0	ND	ND
	2.8±0.10	1.0	4.7±0.0	0.6	ND	ND
Golden carp (liver)	1.6±0.15**	1.8	1.25±0.3	1.8	ND	ND
	1.4±0.4	0.9	3.8±0.1	0.4	ND	ND

Note. ND – no data.

* Data processed from [8].

** Data obtained at 20 °C.

pure (kh. ch.) grade benzylamine hydrochloride. As the enzyme source we used cell mitochondrial fraction. Mitochondria were precipitated by means of 10 % tissue homogenate differential centrifugation. The level of monoamine oxidase activity was expressed in micromoles of ammonia liberated per the amount of protein in the sample. Statistical data processing was performed: we determined the arithmetic mean of parallel experiments, standard error $\bar{x} \pm S_{\bar{x}}$. The confidence limits of the arithmetic mean were calculated as $S_{\bar{x}} \cdot t$, where t is the Student's test, $t = n - 1$ at $P = 0.05$ at the degree of freedom (f).

In this paper we used the Michaelis–Menten equation:

$$w^2 w_{max} = w^2_{max} w[S]/(K_M[S]) \quad (1)$$

where [S] is the substrate concentration. Equation (1) was transformed into the following form:

$$w = -K_M w/[S] + w_{max} \quad (2)$$

We obtained the Edie–Hofstee plot within coordinates w and $w/[S]$ that represented a straight line which intersects the ordinate at a point equal to w_{max} and the horizontal axis at the point w_{max}/K_M. As against the Lineweaver–Burk plot, this plot allows revealing the deviations of the deamination reaction course from linearity.

RESULTS AND DISCUSSION

We have investigated deamination kinetics for serotonin and tryptamine in the mitochondrial fraction of fish liver as a source of the enzyme monoamine oxidase. Figure 1 demonstrates the results of w_{max} and K_M calculation according to the Edie–Hofstee plot. It can be seen that the for the samples of the silver carp (Carassius auratus) liver at 20 and 38 °C the enzyme affinity level with respect to serotonin is higher than that with respect to tryptamine.

Similar conclusions were drawn earlier in the course of studying the liver of the golden carp [2].

From the analysis of the kinetic studies it follows that with increasing the temperature the affinity of the enzyme with respect to the substrate demonstrates an increase.

In addition, it was found that with increasing the temperature from 20 to 38 °C the liver samples of golden carp [2], for example, exhibit the ratio of w_{max}/K_M (characterizing the enzyme-to-substrate affinity level) demonstrates a two-fold increase for the deamination of serotonin, whereas in the case of tryptamine this value shows a 4.5-fold increase. The value of w_{max}/K_M ratio for mackerel liver samples at 38 °C is 3 and 5 times higher than that at 20 °C in the case of using the serotonin and tryptamine as substrates, respectively (see Table 1). Therefore, at 38 °C carp liver enzyme exhibits a higher affinity level with respect to tryptamine as the substrate of A and B type monoamine oxidase, whereas in the case of horse-mackerel liver the most high affinity level is exhibited with respect to serotonin as compared with that at 20 °C.

From the data presented in Fig. 1 one could see that the mitochondrial fraction of silver carp liver, as well as that of golden carp [2], the deamination of serotonin and tryptamine is activated by increasing the concentrations within about 10^{-2} mol/L.

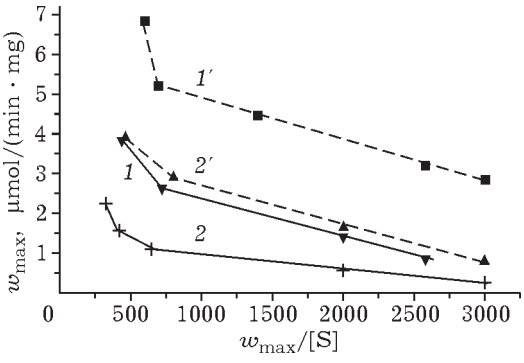

Fig. 1. Value of wmax depending on wmax/[S] under oxidative deamination of serotonin (*1*, *1′*) and tryptamine (*2*, *2′*) at temperature values of 20 (*1*, *2*) and 38 °C (*1′*, *2′*) in the mitochondrial fraction of silver carp liver. Here and in Figs. 3, 4 the number of experiments $n = 5$, error of the mean value error is less than 5 %.

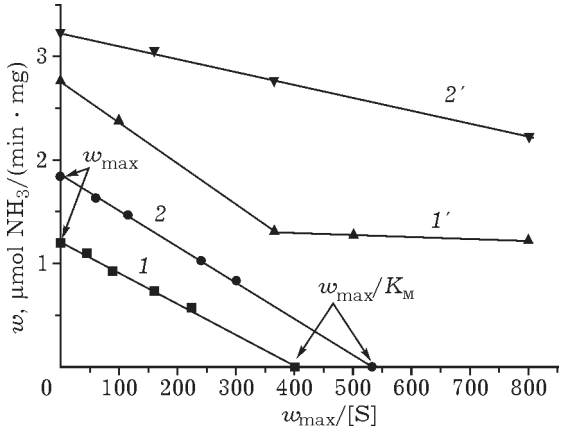

Fig. 2. Deamination rate of tryptamine (*1*, *1′*) and serotonin (*2*, *2′*) in the mitochondria of dog's liver without hyperthermia (*1*, *2*) and with hyperthermia (*1′*, *2′*). A point stands for the average of three experiments.

Similar results were obtained earlier [8] in studying the representatives of mammals (dogs). We calculated the kinetic parameters of oxidative deamination in the liver of dogs according to the Eddie – Hofstee plot (Fig. 2). We have found that when the animals were not subjected to hyperthermia one can observe a linear behaviour of the oxidative deamination of serotonin and tryptamine in the mitochondrial fraction of dog's liver [8], however under hyperthermia conditions (elevated body temperature, about 40.3–42 °C), and the linearity of the reaction course is disrupted.

Next, we studied the activity of the deamination enzyme in birds. It should be noted that the birds could serve as a naturally occurring "hyperthermia model" because their body temperature is normally much higher than that inherent in mammals, ranging within 38–45.5 °C (the average value being equal to approximately 42 °C). As a consequence, the birds generally have a greater rate of metabolism as compared to mammals.

We revealed that the oxidative deamination reaction indeed actively occurs in the liver and in the brain of rock-pigeon of a great tit, as well as in the liver of great tit and bullfinch (Fig. 3). The deamination rate increases with substrate concentrations, and in the liver the majority of birds this process occurs more intensely as compared to mammals (see Table 1).

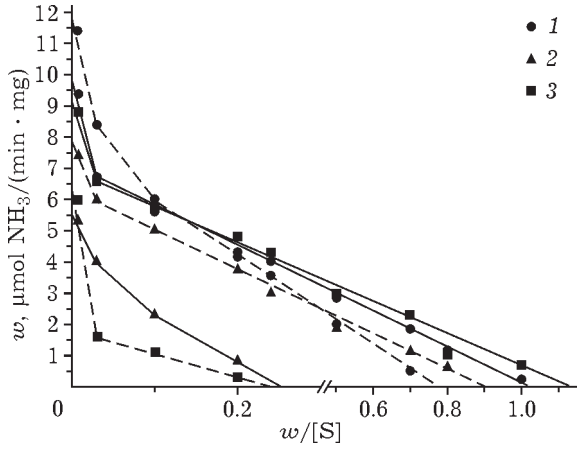

Fig. 3. Deamination rate (w) of serotonin, tryptamine and benzylamine deamination depending on the ratio the between w and the concentration of these substrates w/[S] in the mitochondrial fraction of liver for bullfinch (solid curves) and great tit (dashed curves): *1* – serotonin, *2* – tryptamine, *3* – benzylamine.

Monoamine oxidase activation in the case of increasing the substrate concentrations was noted by other authors, too. For example, in [9] this phenomenon is explained by the presence of allosteric centres in different types of monoamine oxidase.

The data obtained indicate that the substrate and specific variability of monoamine oxidase species is possible in birds. At the same time, the deamination of serotonin in the liver of the most of bird species studied (such as wood-grouse, tit, bullfinch and gull), as well as in the preparations of pigeon brain occurs in a more intense manner than it does in the case of mammals. This fact is indicated by the kinetic parameters of the deamination process not only for serotonin and tryptamine, but also for benzylamine (see Table 1).

The investigation of the substrate deamination processes in the mitochondrial fraction of the brain and liver of mammals, birds and fish demonstrated that the enzyme that catalyzes serotonin deamination in the mitochondrial fraction of the brain and liver of some mammals exhibits the w_{max}/K_M ratio to vary to an insignificant extent, *i. e.* within the range of 1.0–2.1. For birds, the parameter varies to a greater extent, *i. e.* from 0.4 (for ruff) to 5.0 (for wood-grouse). For fish the ratios are about the same value as for mammals (see Table 1).

For the deamination of tryptamine the w_{max}/K_M parameter inherent in liver samples of fish and mammals is comparable with that for serotonin deamination. In birds this ratio for tryptamine varies over a wide range (0.25–5.0).

The experiments with the substrate benzylamine demonstrated that the greatest affinity with respect to the enzyme among birds is observed in the case of upland buzzard and wood-grouse the liver (4.1).

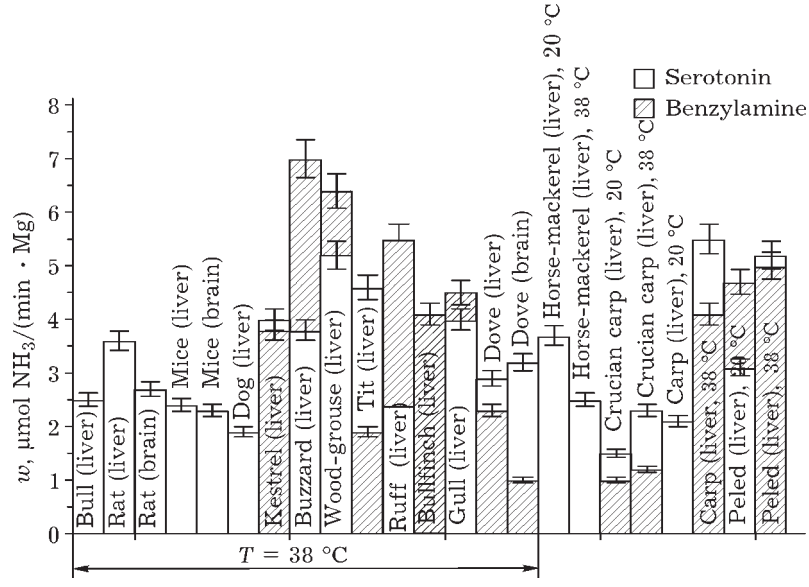

Fig. 4. Comparison of monoamine oxidase activity in the mitochondrial fraction of liver and brain (rat, mouse, dove) for mammals, birds and fish, depending on the substrate and the temperature (in fish).

The maximum value of the K_M parameter is inherent in the enzyme that catalyzes the deamination of benzylamine in the liver mitochondria fraction of great tit ($6.7 \cdot 10^{-3}$ mol/L). In general, the K_M value for the liver mitochondrial fraction taken from the majority of birds is lower as compared to that inherent in mammals (see Table 1). It should also be noted that in comparison with the mammals under investigation the monoamine oxidase enzyme in birds is much more active.

From data presented in Fig. 4 one could see that at the same saturating substrate concentration the monoamine oxidase activity in the liver and brain of mammalian, fish and bird species is different. However, for carp and peled it was found that under the temperature conditions unnatural for them (38 °C), the activation level of deamination enzymes in the liver is comparable with that inherent in birds.

CONCLUSION

The efficiency of catalyzing the transformations of monoamines as the monoamine oxidase substrates in the mitochondrial fraction of evolutionarily distant fauna representatives was studied. Birds and fish were found to exhibit substrate deamination selectivity in the mitochondrial fraction of the liver, whereas an organ specificity of this process in birds demonstrated. There were found some features of this catalysis such as deviation in the course of the deamination reaction from linearity in the liver of fish, birds and mammal representatives with increasing either substrate concentration or temperature.

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