STUDY OF THE MONOAMINOXIDASE ACTIVITY OF MYCOBACTERIA B_5

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Translated from Byulleten Éksperimental'noi Biologii i Meditsiny, Vol. 56, No. 8, pp. 81-84, August, 1963
Original article submitted July 7, 1961

Numerous works have been devoted to investigating the properties of monoaminoxidase. Recently, there has been intensive study done on substances which can inhibit the activity of this enzyme through their ability to elevate the level of serotonin, adrenalin, and noradrenalin in the organism. Study of the monoaminoxidase inhibitors led to the discovery of new medicinal agents for the treatment of hypertension, cardiac and neuropsychic diseases.

The properties of monoaminoxidase and the monoaminoxidase inhibitors have been investigated chiefly in enzymatic material of animal origin. The data in the literature on bacterial monoaminoxidase are scarce [4], and there is no description in the literature of the monoaminoxidase of mycobacteria.

We attempted to elucidate whether or not a monoaminoxidase is present in the mycobacteria, and what its properties might be.

As a representitive of the mycobacteria, we used the acid-fast saprophyte, B_5 (Mycobacterium n. spec.). For the enzymatic preparation, we employed an acetone treated powder of the bacteria, obtained according to the method described earlier [1, 2]. Tyramine served as the substrate for deamination. The monoaminoxidase activity was determined from the amount of ammonia produced as a result of the enzymatic process.

We studied the relationship of monoaminoxidase activity to the pH of the medium, the concentration of the substrate, and the concentration of the enzymatic material. We also investigated the kinetics of the enzymatic process, and the effect of temperature and certain inhibitors on the monoaminoxidase activity.

EXPERIMENTAL METHOD

Determination of Monoaminoxidase Activity. In the central chamber of glass Conway dishes, we placed 2 ml of 0.01-0.015 N H_2SO_4. In the outer chamber of the control dishes, we introduced 1.5 ml of an acetone preparation of the B_5 bacteria, prepared in a 1/15 M phosphate buffer with a pH of 8.04 (the suspension of the preparation was calculated to provide 160 mg of the acetone preparation in each Conway dish), 1.5 ml of buffer solution, 1 ml of distilled water, and 2 drops of a 50% alcohol solution of phenol as an antiseptic. In the outer chambers of the dishes containing the experimental subjects, we placed 1.5 ml of the B_5 bacteria acetone preparation suspension, 1.5 ml of buffer solution, 1 ml of 0.02 N tyramine solution, and 2 drops of phenol solution. The dishes were closed and placed in an incubator at 37° for 20-24 h. After cooling to room temperature, 4 ml of a saturated potash solution were placed in the outer chambers, and the dishes were again placed in an incubator at 37°; after 4 h, the contents of the central chambers were titered with 0.01-0.015 N NaOH respectively, in the presence of a mixed indicator. The amount of acid used to neutralize the ammonia liberated as a result of the enzymatic process served as the measure of monoaminoxidase activity. In all cases, we also ascertained the activity of a boiled suspension of the bacterial acetone preparation. The latter did not possess any enzymatic activity.

Determination of the Effect of Various Preparations on the Monoaminoxidase Activity. In the outer chambers of Conway dishes, we placed 1.5 ml of the B_5 bacteria acetone preparation suspension and 1.5 ml of the substance being studied, prepared in a buffer solution; the mixture was carefully mixed, the dishes covered, and maintained for 6 h in an incubator at 37°. In calculating the concentration of the studied subject, attention was paid to the dilution that occurred following the addition of the B_5 bacterial acetone preparation suspension. Dishes containing buffer so-

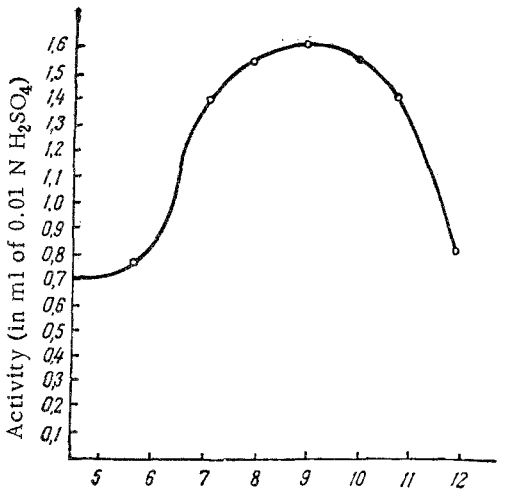

Fig. 1. Dependence of the monoaminoxidase activity on the pH of the medium (acetone preparation of B_5 bacteria and tyramine).

lution instead of the experimental substance were also placed in the incubator for 6 h: they served in determining the original monoaminoxidase activity of the B_5 bacteria acetone preparation.

After the 6-hour preliminary incubation, 1 ml of a tyramine solution was placed in the outer chambers of the Conway dishes, and in the control dishes – 1 ml of distilled water; in the central chambers – 2 ml of 0.01 or 0.015 N H_2SO_4. The monoaminoxidase activity was determined according to the described method.

The amount of the enzymatic preparation corresponding to the activity obtained in the control was taken as 100%. From the curve depicted in Fig. 2, we determined the amount of enzymatic preparation that corresponded to the activity obtained in the presence of the inhibitor and in the control. The initial figure was divided by two and the quotient was multiplied by 100. The obtained figure was then subtracted from 100. The difference was expressed in percents depression of monoaminoxidase in the presence of the inhibitor.

The calculation can be done according to the formula:

$$X = 100 - a \cdot 100/b,$$

where X – depression in percents; a – the amount of enzymatic preparation corresponding to the activity in the tests with inhibitor, and b – the amount of enzymatic preparation corresponding to the activity in the control.

EXPERIMENTAL RESULTS

Properties of the Monoaminoxidase. The acetone preparation obtained from the acid-fast saprophyte, B_5, possesses significant monoaminoxidase activity, which depended on the pH of the medium. Maximum activity of the enzyme was observed at pH of 8.0-10.0; lowering the pH to 6 or raising it to 12 decreased the monoaminoxidase activity by a factor of approximately 2 (Fig. 1).

Studying the dependency of the enzymatic activity on the concentration of the substrate showed that with an increase (up to a certain limit) in the amount of substrate used in the experiment, the monoaminoxidase activity rose, but the percent of amine entering into the reaction simultaneously dropped (Table 1).

The monoaminoxidase activity of the suspension of B_5 bacteria acetone preparation changed, depending on the concentration of the enzymatic material. Maximum activity was obtained with a concentration of the acetone treated powder of 40-50 mg/ml; in this case, the maximum amount of substrate entered into the reaction (Fig. 2).

Studying the kinetics of the reaction showed that, at 37°, under optimal conditions of, the pH, concentration of the substrate, and concentration of the bacterial acetone preparation, the enzymatic process reaches its equilibrium within 16-20 h.

The B_5 bacteria acetone preparation suspension completely lost its monoaminoxidase activity after heating in a water bath at 65° for 5 min. Heating the suspension at 55° for 5 min partially inactivated the enzyme (by 55%); 5 min heating at 50° did not alter the activity of the enzyme (Fig. 3).

The Effect of Certain Preparations on the Activity of Monoaminoxidase. In order to characterize the microbial enzyme, we studied the action of certain preparations on the B_5 bacteria acetone treated powder suspension, which, according to the data in the literature [3, 5, 6], depress monoaminoxidase activity.

It was established that the investigated compounds depress the monoaminoxidase activity of the B_5 bacteria to a varying degree β-phenylisopropylhydrazine suppresses the activity of the B_5 bacteria monoaminoxidase markedly more intensely than does ipraside (1-isonicotinoyl-2-isopropylhydrazine). An analogous picture was also observed in relation to the monoaminoxidase of animal tissues. This fact permits concluding that the monoaminoxidase of the B_5 bacteria is similar in its properties to the monoaminoxidases of animal origin.

The effect of monoaminoxidase inhibitors on the monoaminoxidase activity of the acid-fast saprophyte, B_5, depends not only on the nature of the inhibitor, but also on the duration of contact of the B_5 bacteria acetone treated powder suspension with the solution of the test substance. With an increase in the contact time (from 2 to 4-6 h), the effect of the monoaminoxidase inhibitors becomes more intense (Table 2).

TABLE 1. Dependence of Monoaminoxidase Activity on the Concentration of the Substrate

Amount of substrate in the test (in moles)	Activity (in ml of 0.01 N solution of H_2SO_4)	Activity (in γ of NH_3/1 ml of acetone treated powder)	Deamination (in %)
$0,5 \cdot 10^{-5}$	0,39	0,42	78
$1 \cdot 10^{-5}$	0,79	0,84	79
$2 \cdot 10^{-5}$	1,42	1,51	71
$3 \cdot 10^{-5}$	1,45	1,54	48

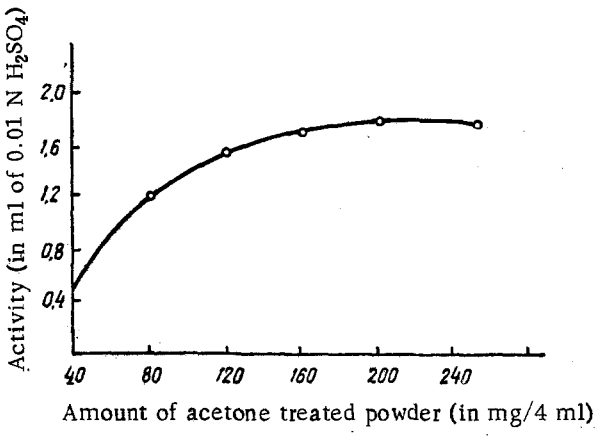

Fig. 2.

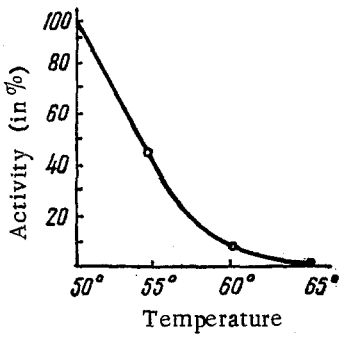

Fig. 3.

Fig. 2. Dependence of monoaminoxidase activity on the amount of enzymatic material.

Fig. 3. The effect of temperature on the monoaminoxidase activity of the B_5 bacteria acetone preparation (duration of heating – 5 min).

TABLE 2. Depression of Monoaminoxidase Activity of the Mycobacteria B_5 under the Influence of Various Preparations

Preparation	Concentration of the preparation	Depression of monoaminoxidase activity (in %) with a duration of contact between the enzyme and inhibitor of		
		2 h	4 h	6 h
β-phenylisopropylhydrazine	1:1 000	100	100	100
	1:10 000	100	100	100
	1:100 000	32	46	55
	1:1 000 000	0		20
Ipraside	1:1 000	49		52
	1:10 000	32		42
	1:100 000	0		0

SUMMARY

Acetone preparation of mycobacteria B_5 possesses a monoaminoxidase activity (substrate – thyamine). The greatest activity of the enzyme is manifested when the pH of the medium is 8-10. At 37°C and the optimal pH of the medium, of the substrate concentration, and of the acetone B_5 mycobacteria preparation the enzymatic process becomes balanced in 16-20 h. Monoaminoxidase of B_5 bacteria is a thermolabile enzyme: suspension of the acetone bacterial preparation completely loses the monoaminoxidase activity following heating for 5 min at 65°C. β-phenylisopropylhydrazine and ipraside (1-isonicotinoyl-E-isopropylhydrazine) depress the monoaminoxidase activity of mycobacteria B_5; β-phenylisopropylhydrazine is a stronger enzyme inhibitor than ipraside. Monoaminoxidase of mycobacteria B_5 is similar to the monoaminoxidase of the animal origin by its properties.

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