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PROPERTIES OF TYRAMINE OXIDASE FROM *SARCINA LUTEA*: OXIDATION OF SH GROUPS AND QUALITATIVE ALTERATION IN SUBSTRATE AND INHIBITOR SPECIFICITY

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SUMMARY

1. Four SH groups per mole of enzyme were found in highly purified preparations of tyramine oxidase (monoamine:oxygen oxidoreductase (deaminating), EC 1.4.3.4) from *Sarcina lutea*.

2. After incubation with *o*-iodosobenzoate or with catalytic amounts of Cu^{2+} in aerobic conditions three of the SH groups lost their ability to react with 5,5′-dithiobis (2-nitrobenzoic acid) or with *p*-chloromercuribenzoate. At the same time the tyramine deaminating activity characteristic of this enzyme markedly decreased and a high lysine deaminating activity appeared.

3. Deamination of lysine by the enzyme that had been treated with Cu^{2+} was inhibited by carbonyl reagents. The deamination reaction was accompanied by consumption of O_2 and formation of H_2O_2 (about a mole of each per mole of NH_3 liberated).

4. Treatment of the modified (oxidized) enzyme with sodium borohydride partially restored the initial content of SH groups, and led to disappearance of the induced lysine deaminating activity and to partial restoration of the initial level of tyramine deaminating activity.

5. Tyramine oxidase treated with Cu^{2+} also acquired an ability to deaminate putrescine and spermine (but neither histamine nor serotonin).

6. The data obtained accord with previous observations concerning the effects of some oxidizing agents on mitochondria and partially purified preparations of mitochondrial monoamine oxidase from rat liver. According to these observations a decrease in monoamine deaminating activity after treatment with oxidizing agents is accompanied by the appearance of a diamine deaminating activity. In this connection the possibility is discussed of qualitative alteration of catalytic properties of mitochondrial monoamine oxidase accompanied by oxidation of SH groups of the enzyme.

INTRODUCTION

It has been established[1-3] that treatment in certain conditions of mitochondrial membranes from rat or beef liver with some oxidizing agents caused not only a decrease in monoamine oxidase activity but also led to the appearance of a new ability (never observed in the untreated mitochondrial membranes) to deaminate diamines, histamine and other nitrogenous compounds. The reaction of diamine deamination was inhibited[2,3] not by specific monoamine oxidase inhibitors but only by carbonyl reagents (isoniazid, hydroxylamine, cyanide) which do not influence the activity of monoamine oxidase. The addition of reducing agents to the mitochondrial membranes treated with oxidizers partially restored the initial ability of mitochondria to catalyze the reaction of monoamine deamination (which may be blocked by specific monoamine oxidase inhibitors) at the same time inhibiting the deamination of diamines and other nitrogenous compounds[3]. Treatment of mitochondrial membranes with oxidizing agents did not induce any diamine deaminating activity if the activity of mitochondrial monoamine oxidases was previously completely blocked by one of the irreversibly acting specific inhibitors of monoamine oxidase[1,2]. These observations suggested the possibility of an effect of oxidizing agents on mitochondrial monoamine oxidases with alteration (transformation) in their substrate and inhibitor specificity[2,3].

In order to investigate this possibility further it was planned to study the effects of some oxidizing agents on catalytic properties of purified preparations of monoamine oxidases. In experiments with partially purified (about 25-fold as compared with the homogenate) preparations of monoamine oxidase from rat liver[4], a histamine deaminating activity was induced in the course of treatment with Cu^{2+} in aerobic conditions[3]. At the same time a marked decrease in the content of SH groups in the enzyme preparations was observed[3]. These results, as well as the results of other experiments[3], led to a suggestion that the phenomenon of the observed transformation of monoamine oxidase may be accompanied by reversible oxidation of SH groups of the enzyme. Results supporting this hypothesis were also obtained in studies of the effects of some organic peroxides on catalytic properties of highly purified (about 200-fold as compared with homogenate) monoamine oxidases from bovine or rat liver[5]. However, the preparations of monoamine oxidases from mammalian tissues contained detergents used for solubilization of structure-bound monoamine oxidases. Additional experiments were therefore desirable with those monoamine oxidases prepared in highly purified form without the use of detergents. These requirements are met by a bacterial monoamine oxidase (monoamine: oxygen oxidoreductase, EC 1.4.3.4)—tyramine oxidase—isolated and purified[6-8] from *Sarcina lutea*. This soluble enzyme catalyzes the oxidative deamination of tyramine and dopamine only (out of 27 nitrogenous compounds studied)[7]. The activity of the enzyme was resistant to carbonyl reagents but sensitive to the monoamine oxidase inhibitor iproniazid[7]

A purpose of this work was to study: (1) the content of SH groups in highly purified preparations of tyramine oxidase from *S. lutea* and (2) the possibility of qualitative alterations of substrate and inhibitor specificity of this enzyme in conditions of oxidation of its SH groups.

MATERIALS AND METHODS

Microorganisms

A culture of *S. lutea* (strain N 486) was kindly presented by the Museum of the Institute of Microbiology, Academy of Sciences of the USSR (Moscow). The microorganisms were grown at pH 7 in a medium described previously[6] at 30° for 24 h with constant aeration (1 l of air per 1 l of medium per min) in 200 l containers. Bacterial cells were separated by centrifugation.

Reagents

Catalase and lysozyme were highly purified commercial preparations (Olaine Biochemicals, USSR). Hydroxylapatite was prepared as described by TISELIUS *et al.*[9]. A sample of lysine-rich histone (fraction f_1), isolated as described by JONES[10], was kindly presented by G. P. Georgiev. Samples of *o*-iodosobenzoate and N^{α}-bis(2-chloroethyl)-DL-lysine, synthesized in the Laboratory of Organic Chemistry, Institute of Biological and Medical Chemistry, were kindly presented by I. A. Redkin, A. I. Tochilkin and G. A. Davydova. Crystalline ergosterol peroxide was kindly presented by V. B. Spirichev. Dithiothreitol was synthesized according to a method modified from that of EVANS *et al.*[11]. Protamine sulphate (Spofa, Czechoslovakia) was purified by gel filtration through a Sephadex G-25 column. Sources and degrees of purification of other reagents used were stated previously[3].

Liberation of ammonia

Rates of enzymic reactions were usually evaluated by measuring NH_3 liberated in the course of incubation of enzyme preparations with amines. Samples contained, in a total volume of 3 ml, besides the enzyme preparations, catalase (50 μg), saturating concentrations of tyramine (5 mM) or of other amines (usually 10 mM). The mixtures were incubated in air at 30° for 30 min. Within this period of time the reaction of oxidative deamination followed apparent zero order kinetics. After fixation of samples by trichloroacetic acid (final concentration 5%) the content of NH_3 in protein-free supernatants was determined by means of isothermic diffusion with subsequent nesslerization[1-3].

Consumption of oxygen

We used a highly sensitive glass manometric vessel[12] with a thin sickle-shaped membrane, displacement of which was recorded by an inductive sensor connected with a two-channel pressure indicator and a recorder. The assembly allowed accurate measurements and continuous recording of comparatively small changes in pressure of the gaseous phase in presence of liquid phase.

Content of hydrogen peroxide

Quantitative determinations of the content of H_2O_2 in samples was carried out by a highly sensitive colorimetric method based on oxidation of phenolphthalin into phenolphthaleine in alkaline medium[13].

Content of protein

Content of protein was measured as described by LOWRY *et al.*[14] using crystal-

line bovine serum albumin (Koch-Light) as a standard. In chromatographic experiments the content of protein in samples was estimated approximately from the absorbance at 280 nm.

Content of SH groups

The content of SH groups was measured colorimetrically with 5,5'-dithiobis-(2-nitrobenzoic acid) as described by ELLMAN[15]. The spectrophotometric procedure of BOYER[16] with *p*-chloromercuribenzoate was used as an independent technique for the estimation of sulphhydryl groups. The results obtained by both methods were identical in all the experiments.

Electrophoretic studies

Zonal electrophoresis was carried out by the method of DAVIS[17], with some modifications[18], in 7.5% polyacrylamide gel at pH 8.9 within 2 h (3 mA per 75 mm × 5 mm tube; 200–400 μg of protein per sample). After fixation in 5% trichloroacetic acid for an hour, the electrophoretograms were washed with water and stained for proteins with 0.1% solution of a dye Coomassie Brilliant Blue (ICI) in a mixture of methanol–water–acetic acid (10:30:1). Excess dye was washed out by this solvent within 16–18 h. Electrophoresis in density gradient (from 5 to 20%) of polyacrylamide gel was carried out as described previously[19].

Ultracentrifugation

Ultracentrifugation was carried out on an analytical ultracentrifuge Spinco, Model E, in AND rotor at $53 \cdot 10^3$ rev./min at 20°. Logarithms of the $s_{20,w}$ values obtained were used for estimation of log molecular weight by a modified Atassi–Gandhi equation[20].

RESULTS

Purification of the enzyme

The method for purification of tyramine oxidase developed by YAMADA *et al.*[6] was modified by us in order to exclude sonication of bacteria in aerobic conditions (*cf.* ELPINER[21]).

All the purification steps were carried out at 3°. The results obtained are presented in Table I.

Extract of bacterial cells. Cells of *S. lutea* washed with distilled water were suspended in 50 mM Tris–HCl buffer (pH 7.5). The content of cells per ml was about $5 \cdot 10^{12}$ (by a bacteriological density standard). Lysozyme, 1 mg in 0.1 ml of aqueous solution, and EDTA, 0.1 ml of 4% solution, were added per ml of the suspension. After incubation at 20° for 2 h the cells were disrupted by a bacterial press designed at the Institute of Chemical Physics, Academy of Sciences of the USSR. 1000 ml of the suspension were treated per hour at 2500 atm. Under these conditions destruction of all the bacterial cells in each of ten randomly selected fields of view of a light microscope was achieved. Cellular debris was removed by centrifugation (10 000 · *g* for 20 min.)

Ammonium sulphate fractionation. Solid $(NH_4)_2SO_4$ was added to the super-

TABLE I

PURIFICATION OF TYRAMINE OXIDASE FROM *S. lutea*

One unit of enzymic activity is defined as the amount of enzyme that catalyses the liberation of 1 nmole of NH_3 per min on incubation of tyramine (5 mM) under standard conditions (see MATERIALS AND METHODS).

I	*Step of purification*	*Total protein (mg)*	*Total activity (units)*	*Specific activity (units/mg protein)*	*Degree of purification*
I	Extract of cells	506 400	255 900	0.5	1
II	Fractionation with $(NH_4)_2SO_4$	55 860	111 720	2.0	4
III	Treatment with protamine sulphate	21 546	63 840	3.5	7
IV	Column chromatography on DEAE-cellulose	1 001	40 400	40.0	80
V	Treatment with hydroxylapatite	125	40 000	320.0	640
VI	Gel filtration through Sephadex G-200	62	40 000	645.0	1290

natant fluid obtained to give 0.3 saturation at pH 7.0 (the pH was kept constant by addition of 5% NH_3). The material was kept overnight at 4°. After separation of a sediment of inactive proteins by centrifugation (20 000 × *g* for 20 min) $(NH_4)_2SO_4$ was added to the supernatant fluid to give 0.6 saturation at pH 7.0 and the mixture was kept overnight at 4°. The sediment formed was removed by centrifugation (20 000 × *g* for 20 min), dissolved in a minimal volume of 10 mM potassium phosphate buffer (pH 7.0) and dialysed 4 times against 10-l amounts of buffer during 48 h.

Treatment with protamine sulphate. This procedure was carried out at pH 6. Other essential details are already described[6]. After the precipitate of inactive proteins was removed the pH of the supernatant was brought up to 7.0.

Column chromatography on DEAE-cellulose. This was carried out as described by YAMADA *et al.*[6].

Treatment with hydroxylapatite. A suspension containing 90 g of hydroxylapatite (wet weight) was added to a 240 ml sample of the material obtained at the previous purification step, mixed during 15 min and centrifuged (3000 × *g* for 10 min). The sediment was discarded. A new portion of hydroxylapatite was added to the supernatant fluid, and the whole procedure was repeated. To concentrate the enzyme in the supernatant fluid the latter was saturated with $(NH_4)_2SO_4$ up to 0.6; the precipitate formed was separated by centrifugation (20 000 × *g* for 20 min) and dissolved in a minimal volume of 10 mM potassium phosphate buffer (pH 7.0).

Gel filtration through Sephadex G-200. A 5 ml sample (125 mg of protein) was applied to a 2 cm × 100 cm column of Sephadex G-200 (Pharmacia, Uppsala; particle diameter 40–120 μ) equilibrated with 10 mM potassium phosphate buffer (pH 7.0). This buffer was passed through the column at a rate of about 20 ml/h. Fractions (10 ml each) containing tyramine oxidase with a specific activity higher than 500 units/mg protein were combined. To concentrate the enzyme the material

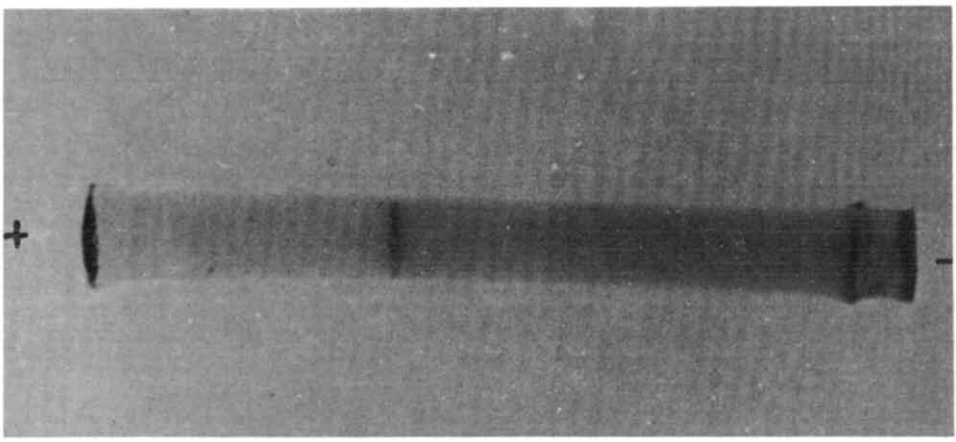

Fig. 1. Electrophoresis of tyramine oxidase in polyacrylamide gel. The sample contained 200 μg of purified enzyme (specific activity 645 units/mg protein).

obtained was saturated with $(NH_4)_2SO_4$ up to 0.7; the precipitate formed was separated by centrifugation (20 000 $\times$ *g* for 20 min), dissolved in a minimal volume of 10 mM potassium phosphate buffer (pH 7.0) and dialysed against three 3-l portions of this buffer containing dithiothreitol (final concentration 1 mM).

Electrophoresis

As shown in Fig. 1, purified preparations of tyramine oxidase were homogeneous in polyacrylamide gel electrophoresis. Similar results were obtained in electrophoretic studies carried out in density gradient of polyacrylamide gel[19].

Ultracentrifugation

Sedimentation patterns of tyramine oxidase (3.5 mg protein/ml) were similar to those described by KUMAGAI *et al.*[8]. A single symmetrical peak of sedimenting

TABLE II

CONTENT OF SH GROUPS IN TYRAMINE OXIDASE; EFFECT OF OXIDIZING AND REDUCING AGENTS

Samples containing 0.4 mg of protein (specific activity 645 units/mg protein) were brought to a total volume of 2 ml by 0.1 M potassium phosphate buffer (pH 8.0). The content of SH groups[15] was determined in triplicate. The increase in absorbance density at 412 nm was monitored at 30° in thermostated cuvettes of an SF-4A spectrophotometer. Reduction by 3 mg of $NaBH_4$ per mg of enzyme for 10 min at 20°. Oxidation by sodium *o*-iodosobenzoate: incubation for 48 h at 3° in 10 mM potassium phosphate buffer (pH 7.0), containing 0.1 mM *o*-iodosobenzoate. Catalytic oxidation by Cu^{2+}: incubation in air for 1.5 h at 20° and 48 h at 3° in 10 mM potassium phosphate buffer (pH 7.0), containing 6 μM $CuSO_4$.

Treatment of enzyme	*Average content of SH groups (moles/mole enzyme)*
Untreated	4
$NaBH_4$	4
o-Iodosobenzoate	1
$CuSO_4$	1
$CuSO_4$, then $NaBH_4$	3

protein was observed in conditions reported before[8]. The $s_{20,w}$ value was $5.65 \cdot 10^{-13}$ (cm/sec) in agreement with the published[8] value ($5.60 \cdot 10^{-13}$). The value for the molecular weight of tyramine oxidase calculated[20] by means of the Atassi–Gandhi equation was within the limits of 80 000–140 000. The value determined from the diffusion constant was reported[8] to be 129 000. This value was adopted in subsequent calculations.

Content of SH groups

Data presented in Table II show that purified preparations of tyramine oxidase contain 4 moles SH groups per mole. After treatment of the enzyme with oxidizing agents (*o*-iodosobenzoate, Cu^{2+} in aerobic conditions) three of the SH groups lost their ability to react with 5,5′-dithiobis(2-nitrobenzoic acid) (or *p*-chloromercuribenzoate). This phenomenon was to a certain degree reversible: treatment of enzyme preparations, previously incubated in presence of Cu^{2+} in aerobic conditions, with the reducing agent sodium borohydride partially restored the initial content of SH groups. Identical results were obtained in parallel experiments in which the estimation of SH groups was done by an independent method[16] with *p*-chloromercuribenzoate. The content of SH groups remained unaltered in presence of 6 M urea.

Catalytic properties of modified ("oxidized") tyramine oxidase

Induction of lysine deaminating activity. As shown in Table III, treatment of tyramine oxidase with oxidizing agents, *o*-iodosobenzoate or Cu^{2+} (in aerobic conditions), which partially oxidized SH groups of the enzyme (Table II), was accompanied by a sharp decrease in tyramine deaminating activity with simultaneous appearance of a new property for the tyramine oxidase—an ability to catalyze deamination of L-lysine. Another oxidizing agent—ergosterol peroxide, a compound chemically related to possible products of peroxidation of vitamin D_2 (refs. 22 and 23)—caused a considerably less marked decrease in tyramine deaminating activity but also induced the appearance of lysine deaminating activity in preparations of tyramine oxidase. In all cases, the rates of L-lysine deamination by the modified ("oxi-

TABLE III

ALTERATIONS IN CATALYTIC ACTIVITY OF TYRAMINE OXIDASE TREATED WITH OXIDIZING AGENTS

Samples contained 0.4 mg of protein (specific activity 645 units/mg protein) and 10 mM potassium phosphate buffer (pH 7.0) to a total volume of 3 ml. Conditions of treatment with *o*-iodosobenzoate and $CuSO_4$—see Table II. Oxidation of the enzyme by ergosterol peroxide: incubation for 40 h at 3° in 10 mM potassium phosphate buffer (pH 7.0) containing 0.1 mM ergosterol peroxide. Conditions for estimation of deamination rates, see MATERIALS AND METHODS. Final saturating concentrations of tyramine and lysine in samples: 5 and 12 mM, respectively. Mean values from 3–4 parallel experiments are presented.

Treatment of enzyme	*Deamination rate (nmoles NH_3/mg protein/min)*	
	tyramine	L-*lysine*
Untreated	17.6	0.0
Cu^{2+}	3.0	20.0
o-Iodosobenzoate	0.0	21.4
Ergosterol peroxide	15.0	11.0

TABLE IV

REVERSIBLE ALTERATION IN CATALYTIC ACTIVITY OF TYRAMINE OXIDASE TREATED WITH Cu^{2+} IONS

Composition of samples and experimental conditions, as for Tables 2 and 3. In experiments with histone 4 mg of lyophilized preparation of lysine-rich histone[10] were added per sample. Specific activity of the enzyme 645–670 units/mg protein. Mean values from two parallel experiments.

Treatment of enzyme	*Deamination rate (nmoles NH_3/mg protein per min)*		
	tyramine	L-*lysine*	*histone*
Untreated	3.8	0	0
$CuSO_4$	0	7.5	8.0
$CuSO_4$, then $NaBH_4$	2.3	0.1	0.07

dized") preparations of tyramine oxidase were well comparable with the values o deamination rates of tyramine by the untreated preparations of tyramine oxidase.

When samples containing tyramine oxidase treated with Cu^{2+} were heated in aerobic conditions for 1 min at 100° the deamination of L-lysine was completely prevented.

Deamination of lysine by tyramine oxidase, subjected to catalytic oxidation in presence of Cu^{2+}, apparently followed the usual equation of oxidative deamination: for each mole of NH_3 liberated one mole of O_2 was consumed and about one mole of H_2O_2 was formed. In one of three parallel experiments 22 nmoles of NH_3 were liberated per min per mg of highly purified tyramine oxidase (specific activity 645 unit/mg protein) pretreated with $CuSO_4$ and incubated with L-lysine. At the same time 22 nmoles of O_2 were consumed and 18 nmoles of H_2O_2 were formed (the latter value was not corrected for non-enzymic decomposition of H_2O_2 in aqueous solution within 30 min at 30°).

Incubation of the copper-treated tyramine oxidase with a preparation of lysine-rich histone was accompanied by liberation of NH_3 (Table IV). In samples containing 4 mg (0.1 μmole) of the histone in standard conditions we observed

TABLE V

EFFECT OF SOME INHIBITORS ON THE RATES OF DEAMINATION OF TYRAMINE BY UNTREATED PREPARATION AND OF L-LYSINE BY PREPARATIONS OF TYRAMINE OXIDASE TREATED WITH COPPER IONS

Final concentration of inhibitors in samples 1 mM. Preincubation of inhibitors with the enzyme preparations for 60 min at 20°. Samples contained 50 μg of catalase and other components (see MATERIALS AND METHODS). In controls (without inhibitors), incubation with tyramine or L-lysine under standard conditions liberated 17.5 $\pm$ 1.0 nmoles of NH_3 per mg of protein per min, respectively. Mean values from 3–4 parallel experiments.

Inhibitor	*Inhibition of deamination (%)*	
	tyramine	L-*lysine*
Pargyline (*N*-methyl-*N*-benzylpropynylamine·HCl)	0	0
Tranylcypromine (trans-2-phenylcyclopropylamine·$\frac{1}{2}$ H_2SO_4)	0	17
Isoniazid (hydrazid of isonicotinic acid)	0	88
Hydroxylamine·HCl	0	100
KCN	0	90
N^{α}-bis(2-chloroethyl)-DL-lysine	0	79

liberation (during 30 min incubation) of 0.5 μmole of NH_3 which may be considered as a result of deamination of ε-amino groups[24] of five lysine residues per mole of histone.

Reversibility of the effect. As shown in Table IV, alterations in catalytic properties of tyramine oxidase, caused by oxidation of its SH groups in presence of Cu^{2+}, are to a certain degree reversible: enzyme preparations were incubated in presence of $CuSO_4$ then treated with sodium borohydride, which partially restored the initial level of tyramine deaminating activity and caused almost complete inhibition of the ability to deaminate lysine (or lysine-rich histone).

Effect of inhibitors. In the course of experiments presented in Table V we unexpectedly found that the specific inhibitors of monoamine oxidases from animal tissues, pargyline and tranylcypromine in conditions optimal for their effect[25,26], did not inhibit the activity of bacterial tyramine oxidase. The lysine deaminating activity, induced by catalytic oxidation of the enzyme in presence of Cu^{2+}, was only slightly inhibited by tranylcypromine whereas pargyline was absolutely inactive. Powerful inhibitors of this reaction were the so-called "carbonyl reagents" (isoniazid, hydroxyl-

TABLE VI

DEAMINATION OF VARIOUS NITROGENOUS COMPOUNDS BY TYRAMINE OXIDASE TREATED WITH Cu^{2+}

Samples contained 0.4 mg of protein of purified tyramine oxidase (specific activity before treatment with $CuSO_4$:645 units/mg protein). Mean values from 3–4 parallel experiments.

Compound	*Final saturating concentrations (mM)*	*Deamination rate (nmole NH_3/mg protein per min)*	
		before treatment	*after treatment with $CuSO_4$*
Tyramine·HCl	5	17.4	3.0
L-Lysine·HCl	12	0.0	20.0
Putrescine·2HCl	10	0.0	11.0
Spermine·4HCl	10	0.0	9.0
Histamine·2HCl	10*	0.0	0.0
Serotonin creatinine sulphate	5*	0.0	0.0
Adenosine-5′-monophosphate	10	0.0	14.0

* These concentrations were not selected on the basis of special preliminary experiments (as was done with the other compounds) but taken from a previous work[3].

amine, cyanide), which had no effect on deamination of tyramine by the untreated tyramine oxidase. Enzymic deamination of lysine was alo inhibited by a structural analogue of lysine, N^{α}-bis(2-chloroethyl)-DL-lysine synthesized by A. I. Tochilkin and G. A. Davydova, as a possible selective inhibitor of enzymic deamination of ε-amino groups of lysine[24] by liver mitochondria treated with oxidized oleic acid.

Deamination of various nitrogenous compounds. As shown in Table VI, preparations of tyramine oxidase (only after treatment by Cu^{2+}) catalysed the deamination of putrescine and spermine at a considerable rate (but of neither histamine nor serotonin). On incubation of adenosine-5′-monophosphate with preparations of tyramine oxidase pretreated by $CuSO_4$ we observed the unexpected liberation of large amounts

of NH_3 (Table VI). This peculiar phenomenon was never observed in similar experiments with untreated preparations of the enzyme (Table VI).

DISCUSSION

We have shown that tyramine oxidase (EC 1.4.3.4) from *S. lutea* contains four SH groups per mole. Oxidation of the SH groups was accompanied by qualitative alterations in catalytic properties of the enzyme.

In our modified method for purification of tyramine oxidase, destruction of bacterial cells was achieved by a combined effect of lysozyme and mechanical pressure to avoid the use of sonic waves[6–8] which might cause alterations in properties of protein molecules especially in aerobic conditions[21]. Our enzyme preparations were homogeneous as shown by electrophoresis in polyacrylamide gel (including the electrophoresis in density gradient[19] of polyacrylamide gel) and analytical ultracentrifugation. Compared with the crystalline tyramine oxidase of YAMADA *et al.*[6–8], our enzyme preparations possessed lower specific activity, probably due to decrease in activity of our enzyme in the concluding steps of purification.

There are no free SH groups in diamine oxidases[27]. The bacterial tyramine oxidase possesses free SH groups as do mitochondrial monoamine oxidases from rat liver[28,29], bovine liver[30] and bovine kidney[31]. The bacterial tyramine oxidase, however, is characterized by a remarkably sharp substrate specificity[7], whereas even purified[32–37] monoamine oxidases of mammalian origin catalyse the deamination of many monoamines. The possibility exists of the occurrence in certain animal species of separable structure-bound monoamine oxidases[38] each of which possesses a relatively narrow substrate specificity.

There is also a difference between tyramine oxidase from *S. lutea* and mammalian monoamine oxidases in sensitivity towards some inhibitors. The specific non-hydrazine monoamine oxidase inhibitors pargyline and tranylcypromine do not inhibit the activity of the bacterial tyramine oxidase. However, a hydrazine monoamine oxidase inhibitor iproniazid, as reported by YAMADA *et al.*[6–8], inhibited tyramine oxidase activity. We have also confirmed the data of YAMADA *et al.*[6–8] on the inability of the carbonyl reagents (isoniazid, hydroxylamine, cyanide) to inhibit the activity of tyramine oxidase.

Incubation of tyramine oxidase with either *o*-iodosobenzoate (which specifically oxidizes[39] SH groups in proteins) or catalytic amounts of Cu^{2+} in aerobic conditions led to a decrease in content of SH groups which was probably caused by their reversible oxidation. Special studies are required to establish the nature of oxidation products of the SH groups. Oxidation by *o*-iodosobenzoate of SH groups in glyceraldehyde-3-phosphate dehydrogenase[40,41] (EC 1.2.1.12) led to the formation of stabilized derivatives of sulphenic acid –SOH but not disulphide bonds as might be expected[39] and as was previously supposed[42]. ZH. I. AKOPYAN[5] showed that treatment with arsenite (which did not reduce disulphide bonds but reduced the residues of sulphenic acid[40]) of highly purified preparations of bovine liver mitochondrial monoamine oxidase, preincubated with oxidized oleic acid, partially restored the initial level of SH groups as well as the initial rate of monoamine deamination and, at the same time, inhibited the diamine deaminating activity induced after preincubation of the enzyme with oxidized oleic acid.

Reversible oxidation of SH groups of tyramine oxidase was accompanied by qualitative alteration in enzymic properties. Tyramine oxidase treated either with *o*-iodosobenzoate or catalytic amounts of Cu^{2+} in aerobic conditions acquired a distinct ability to catalyse deamination of some nitrogenous compounds that are not substrates of tyramine oxidase. These deamination reactions were also inhibited by the carbonyl reagents which did not influence the activity of tyramine oxidase.

Reversible alteration of enzymic properties after treatment of enzyme preparations with various physical or chemical agents was often observed when more then one substrate was used for estimation of enzymic activity[43–49]. After oxidation by *o*-iodosobenzoate of SH groups in glyceraldehyde-3-phosphate dehydrogenase, inhibition of the ability to catalyse oxidative phosphorylation of 3-phosphoglyceraldehyde was observed with simultaneous appearance of a phosphatase activity[43]. The v_{max} value for the phosphatase reaction, appearing as a result of transformation of glyceraldehydephosphate dehydrogenase[41], however, did not exceed 10% of the v_{max} value for conventional triosephosphate dehydrogenase activity. In our experiments the apparent v_{max} values for deamination of putrescine, spermine and lysine (*i.e.* for the reactions catalysed by transformed tyramine oxidase) were comparable to or even exceeded the v_{max} value for deamination of tyramine by the native enzyme.

We are certainly dealing not with transformation of a flavin enzyme (tyramine oxidase) into a pyridoxalphosphate-containing enzyme (diamine oxidase); there occurs a reversible modification of catalytic properties of tyramine oxidase accompanied by reversible oxidation of SH groups. The general pattern of qualitative alterations in enzymic properties of tyramine oxidase after treatment with oxidizing agents very much resembles similar effects observed previously after treatment with oxidizing agents of mitochondria from the liver of rat[1,2] or beef[3] and of purified preparations of mitochondrial monoamine oxidases[3,5]. However, treatment with oxidizing agents of bacterial tyramine oxidase, characterized by sharp substrate specificity, is never accompanied by the appearance of a histamine deaminating activity which is readilly detected in similar experiments with mitochondrial monoamine oxidases[1–3]. It may be supposed that treatment with oxidizing agents of various mitochondrial monoamine oxidases[38] could induce the appearance of abilities to catalyse deamination of various diamines and other nitrogenous compounds.

Although the induction of a hydrolytic AMP-deaminating activity, after treatment of an oxidative enzyme tyramine oxidase with some oxidizing agents, is striking, there are precedents of this kind in the literature[43]. Treatment of glyceraldehydephosphate dehydrogenase with *o*-iodosobenzoate induced the appearance of a hydrolytic phosphatase activity[41,43]. Treatment of bovine liver mitochondria with oxidizing agents induced the appearance of a high AMP-deaminating activity[3] as well as of an ability to deaminate many coenzyme nucleotides[3] in molecules of which a residue of AMP is represented as a structural element. The data presented in this paper, as well as the results of analogous experiments by ZH. I. AKOPYAN[5] with highly purified bovine liver mitochondrial monoamine oxidase, suggest the possibility of existence of some, presently unrecognized relationship between monoamine oxidases and the AMP-deaminating activity.

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