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THYROIDAL BIOSYNTHESIS OF IODOTHYRONINES

II. GENERAL CHARACTERISTICS AND PURIFICATION OF MITOCHONDRIAL MONOAMINE OXIDASE*

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SUMMARY

1. Monoamine oxidase (monoamine:O_2 oxidoreductase, EC 1.4.3.4) has been partially purified from bovine thyroid mitochondria. The enzyme appeared to require Cu^{2+} and sulfhydryl groups for activity. Unlike the plasma oxidase, the thyroid enzyme was not inhibited by carbonyl reagents which suggests that the thyroid enzyme may not contain pyridoxal phosphate. Flavin analogs were inhibitory.

2. Thyroid monoamine oxidase exhibited a rather high degree of substrate specificity. Of the amines tested, only tyramine and phenylethylamine were oxidized at an appreciable rate. 3-Iodotyramine was not oxidized, but was a potent non-competitive inhibitor. Enzymic activity was insensitive to 0.5 M urea, but this concentration of urea obviated iodotyramine inhibition.

3. A tentative reaction mechanism has been proposed based on initial velocity and product inhibition studies.

INTRODUCTION

It has been shown that bovine thyroid glands contain monoamine oxidase[1,2] and that the addition of tyramine to the thyroid microsomal fraction can provide the H_2O_2 necessary for iodination and the coupling of iodotyrosine molecules to form iodothyronine(s)[2]. Both the mitochondria and the microsomes of bovine thyroid glands contain monoamine oxidase activity[3]. We have undertaken an investigation of this enzyme because of its possible importance in the biosynthesis of the thyroid hormone. In this communication, we wish to report the partial purification of monoamine oxidase

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from bovine mitochondria and also present a tentative reaction sequence based on kinetic studies.

MATERIAL AND METHODS

Materials

Bovine thyroid glands were obtained from a local slaughter house and the mitochondria were isolated as previously described[2]. Monoiodotyrosine, tyramine hydrochloride, phenylethylamine, spermine tetrahydrochloride, histamine dihydrochloride, acriflavin hydrochloride, *p*-hydroxymercuribenzoate, amytal, isoniazid and horseradish peroxidase (donor:hydrogen peroxide oxidoreductase, EC 1.11.1.7) were purchased from Mann Research Laboratories, Inc. Catalase (hydrogen peroxide: hydrogen peroxide oxidoreductase, EC 1.11.1.6) and dithiothreitol were obtained from Calbiochemicals. Sodium diethyldithiocarbamate, neocuproine, cupferron, 2,2'-bipyridine, phenylacetaldehyde, *o*-dianisidine, butylamine, *N*-methylbutylamine, and *sec.*-butylamine were obtained from Matheson Coleman and Bell. Cuprizone was purchased from G. Frederick Smith Chemical Company; adrenalin and nor-adrenalin were obtained from Parke, Davis and Co. The detergents, Triton X-100 and Tergitol-NPX were gifts from Rohm and Hass Company and the Union Carbide Corporation, respectively. Parnate was generously supplied by Smith, Kline and French Laboratories; Marsilid phosphate and Marplan were gifts from Hoffmann-LaRoche. 3-Iodotyramine was synthesized as previously described[2].

Methods

The free amines were converted to the hydrochlorides by the addition of 2 ml of each base to 5 ml of ethyl ether and treatment of the resultant solution with hydrogen chloride gas until no further hydrochloride appeared to be formed. Each salt was filtered and recrystallized from absolute ethanol. The salts were again filtered, washed with ethyl ether and dried under vacuum.

O-Dianisidine as the free base was dissolved in acetone, decolorized with charcoal and crystallized by the addition of distilled water to give a 25% acetone solution. 1 g of the recrystallized free base was dissolved in 20 ml of distilled water to which 1 ml of concentrated HCl had been added. The hydrochloride was crystallized by the addition of 100 ml of acetone. The salt was filtered, washed with acetone and ethyl ether and dried *in vacuo* over KOH.

Monoamine oxidase assay. Monoamine oxidase activity was determined either by photometric assay of H_2O_2 production or by manometry. The photometric assay has been described previously[2].

The initial velocities in most of the experiments to be reported were measured with a Cary Model 15 Spectrophotometer at 450 mμ. The media were equilibrated with 100% oxygen prior to the addition of the enzyme. In the experiments in which the effect of oxygen concentration was studied, the reactions were conducted in Thunberg cuvettes. Prior to the addition of the enzyme from the side-arm, the Thunberg cuvettes were evacuated with a water aspirator for 2 min followed by aeration for 5 min with a gas mixture containing the desired oxygen concentration. Pilot experiments were conducted to establish that neither ammonia nor phenylacetaldehyde was lost from

the media during the evacuation and aeration. To test the possible loss of ammonia, a boric acid-trap was inserted in the line. No ammonia was detected in the boric acid solution under the conditions employed. A phenylhydrazine-trap was placed in the line to trap phenylacetaldehyde lost from the medium. No phenylacetaldehyde was lost provided the Thunberg cuvettes were kept in an ice bath during evacuation and aeration.

Monoamine oxidase activity was also determined manometrically using a Gilson Differential Respirometer. The main compartment of each flask contained 33.3 mM phosphate buffer (pH 7.4), 6.6 mM semi carbazide, 20 μg of catalase, and monoamine oxidase in a volume of 2.8 ml. After a 10-min equilibration period, 20 μmoles of tyramine in 0.2 ml were added from a side-arm. The center well contained filter paper saturated with 0.2 ml 10% KOH. The second side-arm contained 0.2 ml saturated boric acid to absorb ammonia.

RESULTS AND DISCUSSION

Purification of the enzyme. Solubilization of liver mitochondrial monoamine oxidase has been achieved by sonication[4] and by treatment with detergents[5]. True solubilization of mitochondrial monoamine oxidase has not been demonstrated. The criterion which has been applied is the failure of the preparation to sediment during high-speed centrifugation after sonication or treatment with detergent. The "solubilized" enzyme may be a polydispersed particulate system[6,7]. Attempts to solubilize thyroid mitochondrial monoamine oxidase by sonication with a Bronwill Bio-sonik (Model U-20) resulted in almost complete loss of activity. Of the detergents used to solubilize the enzyme, only the non-ionic detergents, Triton X-100 and Tergitol-NPX, solubilized the enzyme without appreciable loss of activity. The highest recovery of activity was consistently observed when Tergitol-NPX was used. All operations were performed at 0–5° unless otherwise specified.

The mitochondria used in this experiment were isolated by density gradient centrifugation. The mitochondrial suspension consisting of 576 mg of protein in 43 ml of approx. 1 M sucrose was treated with 10% Tergitol-NPX (adjusted to pH 7.2) to give a final concentration of 0.5% detergent. The mixture was immediately adjusted to pH 7.2 and centrifuged at 165 000 $\times$ g for 1 h. The clear yellow supernatant was decanted and dithiothreitol was added to give a final concentration of 0.5 mM. Saturated ammonium sulfate was added in a dropwise manner to the solubilized preparation which was stirred continuously. Ammonium sulfate was added to give a 25% saturated solution, and the resulting suspension was centrifuged at 27 000 $\times$ g for 15 min. The aqueous phase was removed from the precipitate which floated on the top of the solution, with a syringe and hypodermic needle. An additional 26 ml of saturated ammonium sulfate was added to the aqueous phase, and the resulting suspension was centrifuged. The aqueous phase was once again withdrawn from the floating precipitate and was redissolved in 9.0 ml of 0.1 M phosphate buffer containing 0.5% Tergitol-NPX and 0.1 mM dithiothreitol (pH 7.5). The resulting solution was dialyzed overnight against 2 l of the buffer solution in which the precipitate had been dissolved. The slightly cloudy dialyzed solution was centrifuged at 27 000 $\times$ g.

Calcium phosphate gel (17 mg/ml) was added to the enzyme solution at a ratio of 4 mg of gel to 1 mg of protein. The suspension was centrifuged and the pale yellow

TABLE I

SUMMARY OF RESULTS FOR THE PURIFICATION OF THYROID MITOCHONDRIAL MONOAMINE OXIDASE

Fraction	*Volume (ml)*	*Total units**	*Total protein (mg)*	*Specific activity***	*Yield (%)*	*Purification*
Mitochondrial suspension	43	26.28	576	0.046	100	—
Tergitol extract	40	24.35	335	0.073	93.1	
Fraction I	6.5	8.93	42.5	0.210	33.9	1.58
Fraction II	9.0	8.84	27.8	0.318	33.6	7.23
Calcium phosphate gel supernatant	13.0	8.31	10.5	0.807	31.5	17.5

* A unit of enzymic activity was defined as the number of μmoles of hydrogen peroxide generated in 5 min in a medium which consisted of: 40 mM Tris (pH 7.5), 0.64 mM *o*-dianisidine, 1 mM tyramine, 50 μg horseradish peroxidase and enzyme in a total volume of 5 ml. The foregoing reaction mixture *minus* the tyramine was pre-incubated 10 min at 32°. The reaction was initiated by the addition of tyramine. Under these conditions, the enzyme reaction was linear for 15 to 20 min, but a 5-min reaction interval was routinely used. The reaction was measured by the change in absorbance at 450 mμ.

** Specific activity of the enzyme was expressed as units per mg of protein.

supernatant contained essentially all of the activity present in the dialyzed preparation (Table I). Attempts to purify the enzyme beyond this point have not been particularly successful because of large losses in activity. Treatment of the enzyme with alumina Cγ, and also chromatography on DEAE- or CM-cellulose resulted in the loss of over 75% of the enzyme activity although nearly all of the protein could be eluted. A rather similar situation has been observed by NARA, GOMES AND YASUNOBU[6] with liver mitochondrial monoamine oxidase. These workers were able to obtain only a two-fold purification of the enzyme by chromatography on DEAE-cellulose because of the loss of enzyme activity. Attempts to restore enzyme activity by preincubation with Co^{2+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Mn^{2+}, or Zn^{2+} were unsuccessful, as were attempts to restore activity by preincubation with FAD, FMN or various lipid fractions extracted from the thyroid.

The purification achieved with the thyroid mitochondrial enzyme is slightly less than that obtained with the bovine liver mitochondrial enzyme[6] or the rat kidney mitochondrial enzyme[7]. GUHA AND MURTI have reported a much higher purification with rat liver mitochondrial enzyme[4], but a subsequent publication indicates that these investigators calculated purification using the total liver homogenate as the starting material[8].

Although this enzyme preparation is not a homogeneous protein, the following evidence suggests that it is free from impurities which would invalidate the kinetic studies which will be reported. No hydrogen peroxide was formed in the absence of substrate. Stoichiometric amounts of oxygen were consumed when the enzyme was incubated with tyramine. In this experiment it was necessary to add catalase to prevent inactivation of the enzyme by hydrogen peroxide. It was also necessary to trap the aldehyde by the addition of semicarbazide, and thus prevent the auto-oxidation of the *p*-hydroxyphenylacetaldehyde. Lastly, the K_m and V values did not vary significantly when parallel substrate-saturation experiments were conducted with different concentrations of enzyme. In these experiments, V was calculated on the basis of velocity per mg protein. The experiments were conducted over a 4-fold variation in protein concentration.

TABLE II

OXIDATION OF VARIOUS SUBSTRATES BY MITOCHONDRIAL MONOAMINE OXIDASE

Initial rates of hydrogen peroxide production were determined photometrically using the assay system described with Table I.

Substrate	*Concentration (mM)*	*H_2O_2 production (μmoles /10 min per 100 mg protein)*
Tyramine	1.0	20.7
Monoiodotyramine	1.0	—
Phenylethylamine	1.0	16.1
Benzylamine	5.0	—
Adrenalin	5.0	—
Noradrenalin	5.0	—
Histamine	5.0	—
Spermine	5.0	—
Butylamine	10.0	3.84
N-Methylbutylamine	10.0	4.61
sec.-Butylamine	10.0	—

Substrate specificity. Thyroid mitochondrial monoamine oxidase appears to be rather unique with regard to its substrate specificity (Tables II and III). The thyroid enzyme is rather specific for aromatic amines, however, benzylamine as well as 3-iodotyramine were not oxidized. This substrate specificity is distinctly different from that exhibited by mammalian plasma monoamine oxidases[9], and it also differs from that reported from mitochondrial enzyme from other mammalian tissues[10]. The substrates listed in Table II were tested at various concentrations.

It is of interest to note that ZELLER[11] found *m*-iodobenzylamine to be a better substrate than the unsubstituted benzylamine in experiments with bovine liver monoamine oxidase. The thyroid monoamine oxidase does not oxidize 3-iodotyramine.

Because of the possibility of anomalous color development with *o*-dianisidine where different substrates are tested[12], the activity of the enzyme in the presence of a number of substrates was determined by measuring oxygen uptake with a vibrating platinum electrode (Table III). The results were consistent with those presented in Table III.

TABLE III

OXYGEN UPTAKE WITH VARIOUS SUBSTRATES

The reaction media contained enzyme 5 mM substrates and 0.05 M Tris (pH 7.45). The total volume was 2 ml and the temperature was 30°. The reaction was initiated by the addition of substrate. The initial rate of oxygen uptake was measured with a vibrating platinum electrode

Substrate	*O_2 uptake (μatoms/min per 100 mg protein)*
Tyramine	4.16
Spermine	0.20
Histamine	—
3,4-Dimethoxybenzylamine	—
m-Iodobenzylamine	—
Benzylamine	—

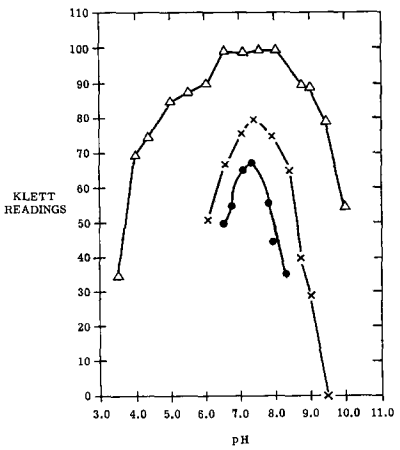

Fig. 1. Effect of pH and buffers on thyroid mitochondrial monoamine oxidase stability and activity. Each tube contained 1 mM tyramine, 0.48 mM *o*-dianisidine and 50 μg horseradish peroxidase. The curve (△—△) indicates enzyme stability. The curve (×—×) represents enzyme activity in 40 mM Tris. The curve (●—●) represents enzyme activity in 40 mM phosphate buffer. Temperature, 32°.

Effect of pH on enzyme activity. The effect of pH on the stability of the enzyme and also on enzymic activity with tyramine as the substrate is illustrated in Fig. 1 The apparent pH optimum is 7.4. In view of the excellent studies of McEwen and coll.[9,13] on the specificities of plasma monoamine oxidase of different species for the protonated and unprotonated amines, an investigation was conducted to determine the specificity of the thyroidal enzyme. The Michaelis constant for tyramine was determined at several pH values. The results at two pH values are presented in Fig. 2a and b. At pH 7.4 (Fig. 2a) substrate inhibition was not observed, however, at pH 8.75

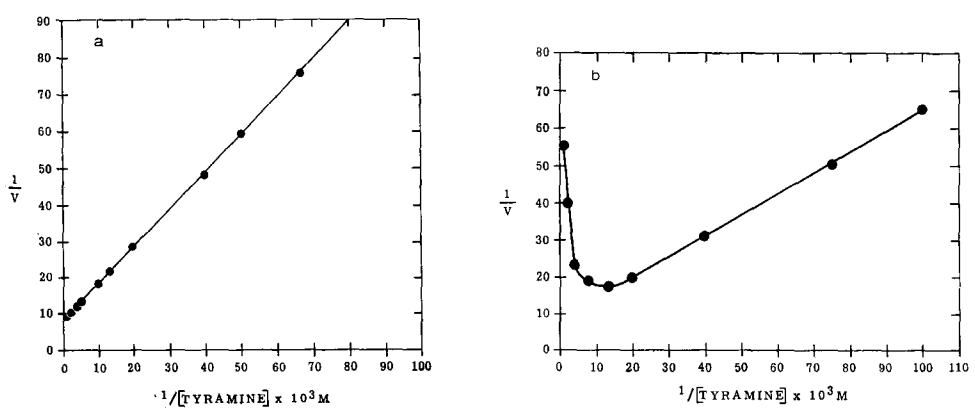

Fig. 2. a. Plot of reciprocal initial reaction velocity against reciprocal molar concentration of tyramine. Assay conditions were the same as in Fig. 1 except the media contained 0.67 mM *o*-dianisidine. The media contained 0.05 M Tris (pH 7.4). The oxygen concentration was $1.3 \cdot 10^{-3}$ M. Velocities are expressed as μmoles H_2O_2 produced per 5 min, but the velocity was measured as the initial velocity as determined with a Cary Model 15 spectrophotometer. b. Conditions were the same as those in (a) except the media contained 0.05 M borate buffer (pH 8.75) and velocities are expressed as μmoles H_2O_2 produced per 10 min.

TABLE IV

EFFECT OF VARIOUS ADDITIVES ON MITOCHONDRIAL MONOAMINE OXIDASE ACTIVITY

Enzyme activity, unless otherwise specified, was determined photometrically as described with Table I.

Addition	*Concentration (mM)*	*Inhibition (%)*
3-Iodotyramine	0.007	50
m-Iodophenylethylamine	10.0	—
3-Iodotyrosine	5.0	—
Diiodotyrosine	5.0	—
Harmine	0.00001	42
p-Hydroxymercuribenzoate	0.05	50
Sodium arsenite	50.0	—
Hydroxylamine	2.0	—
Semicarbazide	6.6	—
Parnate	0.0025	50
Marsilid phosphate	0.07	50
Marplan	0.017	50
Diethyldithiocarbamate	0.33	27
Cuprizone	1.0	43
Cupferron	1.0	—
2,2'-Bipyridine	1.0	40
Chlorpromazine*	0.225	50
Acriflavin*	25/μg/ml	50
Tapazole*	50.0	—

* The inhibitor effect was determined in the manometric assay of monoamine oxidase activity. The manometric assay is described in the text.

the apparent K_m decreased from 0.15 mM to 0.056 mM and pronounced substrate inhibition was observed. These results suggested that the enzyme was susceptible to substrate inhibition, and that the true substrate for the enzyme was the unprotonated amine. However, when phenylethylamine was employed as the substrate, no substrate inhibition was observed at pH 8.75. This observation suggests that substrate inhibition is related to ionization of the phenolic group.

Effect of various additives on enzyme activity. Table IV shows the effect of various additions on the activity of thyroid enzyme. Of the first group of iodinated aromatic compounds, only iodotyramine was inhibitory. The inhibition of this enzyme by monoiodotyramine is of particular interest because of the role that this inhibition may play in the control of thyroid activity. Since *m*-iodophenylethylamine is not inhibitory, the inhibition may be related to the phenolic group.

The inhibition by *p*-hydroxymercuribenzoate suggests that readily available sulfhydryl groups are essential for enzyme activity or structure. The lack of inhibition by arsenite suggests that vicinal sulfhydryl groups are not involved. In contrast to plasma monoamine oxidases[14,15], but in agreement with monoamine oxidases from the mitochondria of other tissues, carbonyl reagents such as hydroxylamine and semicarbazide were not inhibitory.

The group of known inhibitors of monoamine oxidase, namely parnate (phenylcyclopropylamine), marsilid (1-isonicotinyl-2-isopropylhydrazine), and marplan (1-benzyl-2-(5-methyl-3-isoxazolylcarbonyl) hydrazine) are also potent inhibitors of the

thyroid enzyme. Since chlorpromazine and acriflavin are inhibitory, the enzyme may be a flavoprotein.

The effect of chelating compounds on the activity of the thyroid enzyme is similar to the effect of these compounds on plasma[16,17] and brain mitochondria[5] monoamine oxidase.

It was experimentally established that the inhibitors either did not significantly decrease peroxidase activity under the conditions imposed, or, in those cases where peroxidase was inhibited, the photometric assay was replaced by a manometric assay.

The effect of monoiodotyramine. Table III indicates that monoiodotyramine is a potent inhibitor of the enzyme. Fig. 3 indicates that monoiodotyramine is a non-

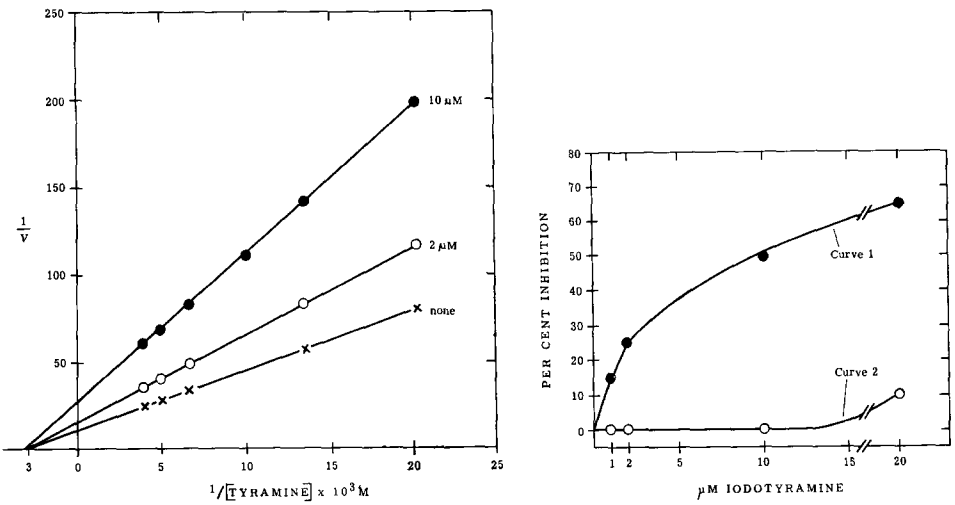

Fig. 3. Effect of 3-iodotyramine on thyroid monoamine oxidase. Reaction conditions are the same as in Fig. 2a but velocity is expressed as increase in absorbance at 450 mμ per 100 sec.

Fig. 4. Desensitization of thyroid monoamine oxidase to monoiodotyramine by urea. Curve 1 represents the effect of monoiodotyramine on enzymic activity in the absence of urea. Curve 2 represents the effect of monoiodotyramine on enzymic activity in 0.5 M urea. The enzymic activity was determined as described with Table I.

competitive inhibitor when tyramine is the variable substrate. Urea concentrations up to 1 M had no effect on enzyme activity, but as indicated in Fig. 4, 0.5 M urea appears to desensitize the enzyme to monoiodotyramine inhibition. This is suggestive of allosterism, but this suggestion must remain tentative until the enzyme is purified further.

Interaction of thyroid mitochondrial monoamine oxidase with oxygen. The Lineweaver–Burk plot resulted in a series of parallel lines when tyramine was the variable substrate at various concentrations of oxygen (Fig. 5). This observation suggests that the substrates bind to the enzyme in an ordered sequence, and that either (a) one or more products dissociates from the enzyme before the addition of the second substrate, or, (b) one of the steps between the addition of the first and second substrate is kinetically irreversible, or, (c) both of the foregoing possibilities might exist. The computer

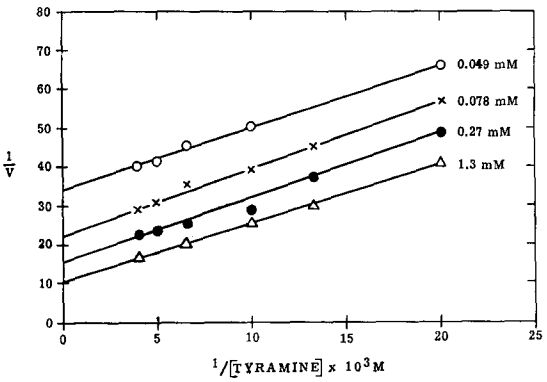

Fig. 5. Plot of reciprocal initial velocity against reciprocal molar concentration of tyramine. The reaction conditions were those described in Fig. 2a except the oxygen concentrations are those indicated. Velocities are reported as in Fig. 3.

program employed in these studies provides for a secondary analysis of the effect of a recorded substrate on both the slope and the intercept of the Lineweaver–Burk plot. The Michaelis constant for oxygen as determined from this secondary plot was 0.12 mM. The Michaelis constant for tyramine in a medium which has been equilibrated with 100% oxygen (1.3 mM oxygen) was 0.16 mM.

Although no hydrogen peroxide was formed in the absence of oxygen, after the incubation of a large amount of enzyme with tyramine in a medium equilibrated with nitrogen, aldehyde formation could be demonstrated. This indicates that tyramine is the first substrate to react with the enzyme. It cannot be taken as evidence for the dissociation of aldehyde prior to addition of oxygen because the addition of dinitrophenylhydrazine in strong acid would be expected to decompose any enzyme–aldehyde complex.

Product inhibition studies. Product inhibition is a useful means of investigating the reaction sequence of an enzymic reaction[18]. Ammonia and phenylacetaldehyde were

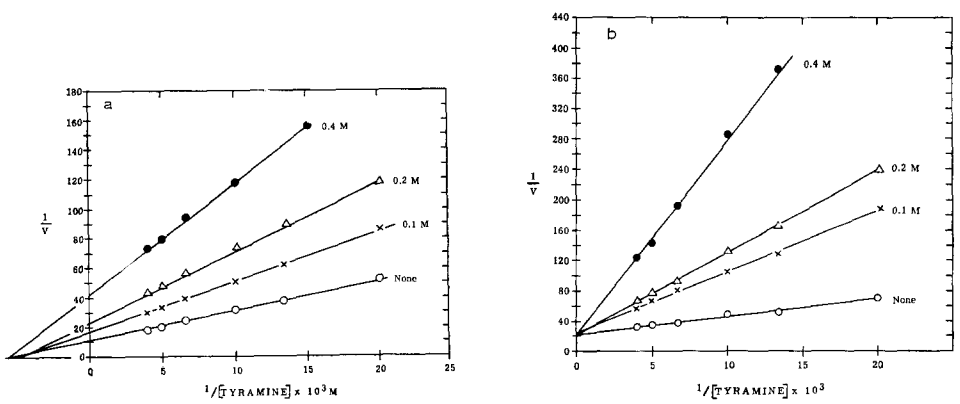

Fig. 6. a. Effect of ammonium ion on the initial velocity of thyroid monoamine oxidase. The conditions are the same as those in Fig. 3, except the oxygen concentration was $7.8 \cdot 10^{-5}$ M and the ammonium ion concentrations are those indicated. b, The same conditions as in (a) except the oxygen concentration was $1.3 \cdot 10^{-3}$ M.

employed as inhibitors. The results with ammonia as an inhibitor are presented in Fig. 6a and b. The concentration of oxygen in Fig. 6a was $7.8 \cdot 10^{-2}$ mM while in Fig. 6b the oxygen concentration was 1.3 mM. The concentrations of ammonia included in these experiments were quite high, but the effects are specific for the ammonium ion as the ionic strength of the media was adjusted with KCl. With a sub-saturating concentration of oxygen, the ammonium ion was a non-competitive inhibitor of tyramine oxidation, while the inhibition was competitive when the enzyme was saturated with oxygen. We refer to non-competitive inhibition as that type of inhibition in which both the slope and the intercept of the Lineweaver–Burk plot are increased by the presence of inhibitor. This is the terminology employed by CLELAND[18].

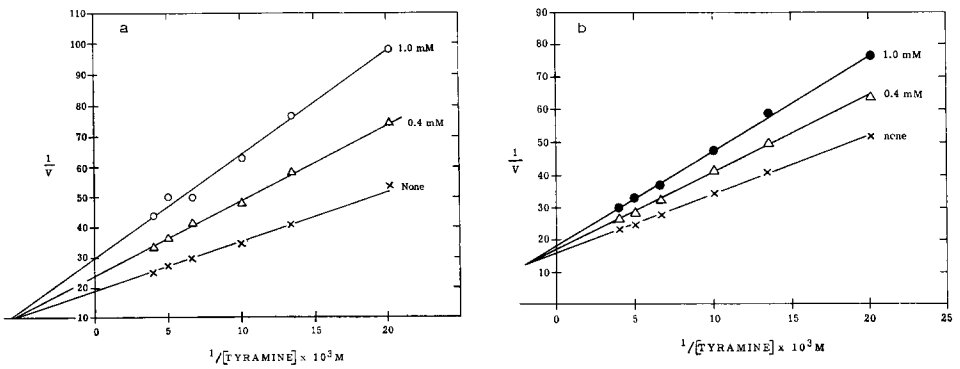

Fig. 7. a. Effect of phenylacetaldehyde on the initial velocity of thyroid monoamine oxidase. The conditions were identical to Fig. 6a except phenylacetaldehyde was employed as the product in the concentrations indicated in the figure. Oxygen concentration was $7.8 \cdot 10^{-5}$ M. b. The same conditions as in (a) except the oxygen concentration was $1.3 \cdot 10^{-3}$ M.

The results obtained with phenylacetaldehyde as the inhibitor are shown in Fig. 7a and b. Since both tyramine and phenylethylamine serve as substrates for this enzyme, phenylacetaldehyde was used as the product inhibitor because of the auto-oxidizability of *p*-hydroxyphenylacetaldehyde. Because of the low solubility of phenylacetaldehyde in water, the aldehyde was added in an alcoholic solution. A similar amount of alcohol was added to the control tube. The poor solubility of the phenylacetaldehyde prevented the use of higher concentrations of this product. Phenylacetaldehyde was a non-competitive inhibitor of tyramine oxidation when oxygen was present at a sub-saturating concentration of oxygen (Fig. 7a) while at an oxygen concentration which was 10 times the Michaelis constant for oxygen (Fig. 7b) phenylacetaldehyde approached competitive inhibition, but complete competitive inhibition was not attained.

The results of these kinetic studies are consistent with the mechanism presented in Fig. 8. This mechanism provides for the formation of a reduced enzyme as an intermediate in the reaction sequence. In contrast to the findings with purified plasma monoamine oxidases[14,15] and kidney diamine oxidase[19], which contain pyridoxal phosphate, liver mitochondrial enzymes are not sensitive to carbonyl reagents, and possibly contain a flavin as a prosthetic group. The above scheme included dead-end complexes by reaction of the oxidized enzyme with either ammonia or the phenylacetaldehyde. The ammonia–oxidized enzyme complex would account for the com-

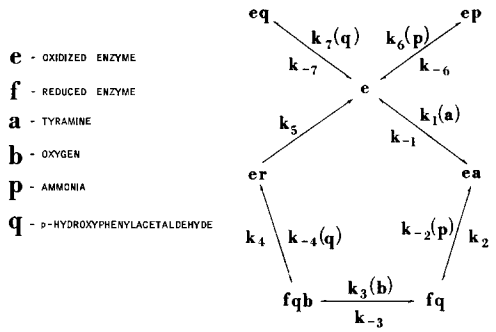

Fig. 8. Proposed reaction sequence for thyroid mitochondrial monoamine oxidase. The dissociation of er, the enzyme–H_2O_2 complex, to form free enzyme and H_2O_2 is portrayed to be irreversible because the assay employed utilizes H_2O_2 as rapidly as it is formed.

petitive inhibition by ammonia in presence of a saturating concentration of oxygen. The double reciprocal equations based on the proposed mechanism, where tyramine is the variable substrate and ammonia $[P]$ and the aldehyde $[Q]$ are inhibitors are as follows:

$$\frac{1}{v_a} = \frac{K_a}{V}\left\{1 + \frac{[P]}{K_{ip_2}} + \frac{K_{ia}K_b}{K_a[B]}\left(1 + \frac{[P]}{K_{ip_2}}\right)\frac{[P]}{K_{ip_1}}\right\}\frac{1}{[A]} + \frac{1}{V}\left\{1 + \frac{K_b}{[B]}\left(1 + \frac{[P]}{K_{ip_1}}\right)\right\} \quad (1)$$

$$\frac{1}{v_a} = \frac{K_a}{V}\left\{1 + \frac{[Q]}{K_{iq_2}}\right\}\frac{1}{[A]} + \frac{1}{V}\left\{1 + \frac{K_b}{[B]}\left(1 + \frac{[Q]}{K_{iq_1}}\right) + \frac{K_b[Q]}{K_{ib}K_{iq_1}}\right\} \quad (2)$$

The Michaelis constants and inhibition constants in the equations are defined according to the convention employed by CLELAND[18]. Eqn. 1 would predict that the apparent slope inhibition constant decreased from 208 mM in the presence of 0.078 mM oxygen to 29 mM in the presence of 1.3 mM oxygen.

Eqn. 2 predicts that the apparent intercept inhibition constant for the aldehyde should decrease with increasing oxygen concentration. This is consistent with the experimental observations illustrated by Fig. 7a en b.

The possible significance of thyroid monoamine oxidase in the biosynthesis of thyroxine has been discussed in previous communications[2,21]. The thyroid gland contains an aromatic amino acid decarboxylase which catalyzes the formation of tyramine and phenylethylamine as well as iodotyramine[21]. Thus, this decarboxylase can provide not only the substrate for monoamine oxidase, and therefore a source of H_2O_2, but also a substance which can exert a regulatory effect. NAGATAKI AND INGBAR[22] have emphasized the self-regulatory behavior of the thyroid gland; the aromatic amino acid decarboxylase and monoamine oxidase would provide such a self-regulatory system.

Some comment should be made concerning the distribution of this enzyme in the thyroid gland. The enzyme is associated with both the mitochondrial and microsomal fractions[3]. Thyroid peroxidase appears to be a microsomal enzyme[23] and one might question whether thyroid mitochondrial monoamine oxidase plays an important

role as a source of H_2O_2. However, thyroid mitochondria catalyze a H_2O_2-dependent iodination of tyrosine[24].

The enzymic reactions involved in thyroid hormone biosynthesis have been studied with a simulation model and the aid of a digital computer program using the kinetic data for other enzymic reactions and steady-state levels of various compounds obtained from the literature. While these studies are still of a preliminary nature, it is apparent that the thyroidal amino acid decarboxylase and monoamine oxidase are more than adequate to provide sufficient hydrogen peroxide to maintain the observed steady-state level of iodinated components in the thyroid. Furthermore, iodotyramine appears capable of exerting a regulatory effect under these conditions.

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