A Rapid Spectrophotometric Assay of Monoamine Oxidase Based on the Rate of Disappearance of Kynuramine

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Studies in this laboratory (1-3) have dealt with the metabolism of amines derived from tryptophan, such as tryptamine and serotonin. Tryptophan may give rise to additional amines through decarboxylation of kynurenine and 3-hydroxykynurenine. Makino and Arai (4) were the first to suggest this pathway and later showed (5) that mouse liver homogenates convert kynuramine, the decarboxylation product of kynurenine, to 4-hydroxyquinoline which was identified by its paper chromatographic and spectral properties. The mechanism of this conversion has not been studied in further detail.

Kynuramine (1) has now been found to be rapidly oxidized by monoamine oxidase (MAO) but not by diamine oxidase (Scheme 1). The former enzyme conceivably could oxidatively deaminate kynuramine to an aldehyde II which would either condense to 4-hydroxyquinoline (4(1H)-quinolone, III) or undergo further oxidation to an acid IV or the lactam, 2,4-dihydroxyquinoline (formulated as 4-hydroxy-2(1H)-quinolone, V). Intramolecular (nonenzymatic) condensation of the aminoaldehyde proved to be faster than the further oxidation of the aldehyde to the acid or lactam. The overall reaction could be conveniently followed in situ in a self-recording spectrophotometer and has been developed into a rapid method for the assay of monoamine oxidase. The details of this procedure are presented in the present report.

EXPERIMENTAL

Materials and Methods

Kynuramine (1) was prepared in the following way by a modification of the ozonolysis procedure of Witkop (6). N-Carbobenzoyltryptamine (2.9 g) (7) was dissolved in 100 ml of acetic acid and subjected to ozonolysis with use of a Welsbach Ozonator. The yellow-brown solution was then concentrated at 40 to 50°C almost to dryness and cooled while 15 ml of 48% hydrobromic acid in acetic acid were added. After 5 hours at 5°C, the solution was concentrated to dryness under reduced pressure, water was added and the solution was shaken with ether. The aqueous layer was then passed over a Dowex 1-X8 resin (hydroxide form). To the eluant was added an excess of alcoholic hydrobromic acid. The solution was concentrated under reduced pressure to dryness and the residue crystallized from methanol-ethyl acetate after treatment with Norit. A yield of 0.5 g, 15%, of kynuramine dihydrobromide was obtained, m.p. 214-216°C decomposes. This yield compares with an over-all yield of 14% by total synthesis (8).

Various hydroxyquinoline derivatives were obtained through the courtesy of Dr. Siro Senoh and Dr. Chozo Mitoma. Spectra were obtained with a model 14 Cary recording spectrophotometer, and enzyme experiments were performed either with a Beckman model DU spectrophotometer or the model 14 Cary instrument.

Tissues were homogenized in 5 volumes of cold distilled water and passed through a layer of cheesecloth. In most of the experiments to be described below (Figs. 1 to 4), the homogenate was centrifuged at 500 r.p.m. for 15 minutes to remove cellular debris. Soluble, partially purified monoamine oxidase was prepared as described previously (9). Partially purified diamine oxidase was prepared from hog kidney according to the procedure of Tabor (10).

Incubations with kynuramine were performed in 3-ml silica cuvettes. The experimental cuvette contained tissue extract, about 0.3 mmole of kynuramine, 0.3 ml of 0.5 M phosphate buffered at pH 7.4, and water to a total volume of 3 ml. The initial absorbancy at 360 mμ is approximately 0.5. Since the incubations were run at room temperature, only the enzyme solutions were kept cold. A blank cuvette was prepared in which the kynuramine was replaced with water. After the final addition, the mixing was achieved by inversion and further readings were then recorded at suitable time intervals depending on the activity of the enzyme preparation. With crude tissue preparations, the readings observed in the first minute or two may be erratic because of settling of particles in the cuvette. However, after this period there is no difficulty, and the assay can be employed on tissue homogenates. Activity is expressed as the change in absorbancy at 360 mμ per unit of time.

For comparative purposes, monoamine oxidase activity was also assayed in another manner by measuring the rate of serotonin disappearance (2). In this procedure, the serotonin in the incubation mixture is extracted from alkaline solution into butanol and then re-extracted into dilute HCl. The dilute acid

1 Kynuramine can now be obtained from Regis Chemical Company, Chicago, Ill.
is then assayed colorimetrically. It should be noted that the rate of serotonin disappearance, when incubated with monoamine oxidase, was measured at 37°C, whereas measurement of the rate of kynuramine disappearance took place at the temperature inside the Beckman spectrophotometer (about 30°C). Kynuramine disappearance was found to be 60% faster at 37°C.

**EXPERIMENTAL**

Liver homogenates rapidly metabolized kynuramine as evidenced by a marked change in the spectrum of the incubation mixture (Fig. 1). The initial peak due to the substrate at 358 to 360 μm diminished with the concurrent appearance of new peaks at 315 and 329 μm. As shown in Fig. 2, the final spectrum obtained in a typical incubation resembled that of 4-hydroxyquinoline, the metabolite first identified by Makino et al. (5). Quantitative measurements based on the extinction coefficient of 4-hydroxyquinoline at 315 μm indicated complete conversion of the amine to 4-hydroxyquinoline. Other closely related quinoline derivatives such as 8-hydroxyquinoline, 4,8-dihydroxyquinoline, 2-hydroxyquinoline, and 4-hydroxyquinoline-2-carboxylic acid were also found in the incubation mixture.

Fig. 1. Spectral changes during incubation of kynuramine with rat liver homogenate. Details of the incubation procedure are described in the text.

Fig. 2. Final spectrum (A) of an incubation of kynuramine with 0.1 ml of rabbit liver homogenate. The incubation continued until no further change in the spectrum was observed. The spectrum of 4-hydroxyquinoline (B), the postulated product, is also shown.

Fig. 3. Decrease in absorbancy at 360 μm as a function of time during incubation of kynuramine with rat liver homogenate (0.1 ml).
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The spectral changes observed with these later monoamine oxidase preparations also showed the formation of 4-hydroxyquinoline. Partially purified diamine oxidase was shown to have very weak, but significant activity towards both kynuramine and serotonin (1/4 and 1/8 the rate observed with histamine). Since monoamines are known to be weak substrates of diamine oxidase (13), the low activity observed is understandable.

Several tissues were assayed for monoamine oxidase by both methods, serotonin disappearance and kynuramine disappearance. The results are shown in Table I. In most of the tissues tested, serotonin was oxidized at a faster rate by monoamine oxidase although the values are not comparable since the kynuramine incubations were performed at a lower temperature. Correcting for this, serotonin was generally 2 to 4 times better than kynuramine. One notable exception was rabbit liver which oxidized kynuramine at a faster rate than serotonin. An alternate pathway of kynuramine metabolism was excluded since the end product formed in the incubations with rabbit liver was 4-hydroxyquinoline (Fig. 2). In all tissues, including those of the rabbit, the bulk of enzymatic activity with both kynuramine and serotonin as substrates was in the mitochondrial fraction.

**DISCUSSION**

The generally employed procedures for measuring monoamine oxidase activity depend on either oxygen uptake (11, 14) or amine disappearance (2). In the latter case, serotonin or tyramine have been used as substrates, since both these compounds can be readily measured. However, the cumbersome analytical methods involve multiple extraction procedures (15), followed by colorimetric assay (15, 16). On the other hand, the manometric assay procedures are not sufficiently accurate or sensitive, especially so since crude tissue preparations show appreciable oxygen uptake in the absence of added substrate. With use of purified preparations of amine oxidase, oxidative deaminations have also been assayed by measuring the appearance of DPNH in the further oxidation of the aldehyde to the acid by aldehyde dehydrogenase and DPN (9, 10), and by the spectrophotometric assay of benzaldehyde formed with benzylamine as a substrate (17). These procedures are not applicable to crude tissue extracts.

The present assay for monoamine oxidase utilizes a direct spectrophotometric method which allows the rapid and exact determination of the disappearance of kynuramine (360 μμ), or of the appearance of 4-hydroxyquinoline (310 to 335 μμ). The assay is applicable to crude tissue extracts, and appears to be the method of choice for following the localization and purification of monoamine oxidase.

The use of an analogous (nonsensematic) intramolecular condensation reaction of an intermediate carbonyl derivative should be applicable to other enzymatic oxidations and is now being investigated as a means of assaying L-amino acid oxidase with L-kynurenine as substrate.

**SUMMARY**

The conversion of kynuramine to 4-hydroxyquinoline has been shown to occur readily in tissue homogenates and to be dependent upon monoamine oxidase activity. The reaction can be followed conveniently in a spectrophotometer and has been developed into a simple and rapid method for the assay of monoamine oxidase.
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REFERENCES

A Rapid Spectrophotometric Assay of Monoamine Oxidase Based on the Rate of Disappearance of Kynuramine
Herbert Weissbach, Thomas E. Smith, John W. Daly, Bernhard Witkop and Sidney Udenfriend