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

From the National Heart Institute and National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland

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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 kynurenine, 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 (I) has now been found to be rapidly oxidized by monoamine oxidase (MAO) but not by diamine oxidase (Scheme IV). 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-dihydroxyquinolone (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 (I) was prepared in the following way by a modification of the ozonolysis procedure of Witkop (6). N-Carbobenzyloxytryptamine (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° almost to dryness and cooled while 15 ml of 48% hydrobromic acid in acetic acid were added. After 5 hours at 5°, the solution was converted 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) and the eluant, which contained the free base, was treated with a saturated aqueous solution of picric acid. The yellow-brown solution was then re-extracted into dilute HCl. The dilute acid 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° decomposes. This yield compares with an over-all yield of 14% by total synthesis (8).

\[ C_{11}H_{19}NO_5 \cdot 2HBr \]

Calculated: C 33.15, H 4.33, N 8.59, Br 49.02

Found: C 33.27, H 4.40, N 8.69, Br 48.81

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 300 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 buffer at pH 7.4, and water to a total volume of 3 ml. The initial absorbancy at 360 m\(\mu\) was 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 an initial reading was made at 360 m\(\mu\). 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 m\(\mu\) 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
is then assayed colorimetrically. It should be noted that the rate of serotonin disappearance, when incubated with mono-
amine 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). Kynura-
mine 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-hydroxy-
quinoine, 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 quino-
line derivatives such as 8-hydroxyquinoline, 4,8-dihydroxyquin-
oline, 2-hydroxyquinoline, and 4-hydroxyquinoline-2-carboxylic

![Scheme 1](image)

**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).
Acid were found to have completely different spectra. The oxidation product also behaved similarly to authentic 4-hydroxyquinoline in several chromatographic systems on paper.

As shown in Fig. 3, kynuramine disappearance was linear with time until the absorbancy at 360 mp fell below 0.150. Activity was also proportional to enzyme concentration as shown in Fig. 4.

The following findings indicate that kynuramine is a far better substrate of monoamine oxidase than of diamine oxidase. The reaction was extremely sensitive to iproniazid, an excellent inhibitor of monoamine oxidase (11, 12). Rat liver mitochondria, which are an excellent source of monoamine oxidase, rapidly metabolized kynuramine, as did a partially purified monoamine oxidase preparation from guinea pig liver (9). Neither of these preparations was able to oxidize histamine at a significant rate.

The ordinate values show the decrease in absorbancy at 360 mp during this incubation.

FIG. 4. Effect of tissue concentration on kynuramine disappearance. Various amounts of a rat liver homogenate were incubated for 10 minutes with kynuramine as described in the text. The ordinate values decrease in absorbancy at 360 mp.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Kynuramine</th>
<th>Serotonin</th>
<th>Ratio: kynuramine/serotonin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat liver</td>
<td>0.021</td>
<td>0.522</td>
<td>0.149</td>
</tr>
<tr>
<td>Rat brain</td>
<td>0.030</td>
<td>0.201</td>
<td>0.149</td>
</tr>
<tr>
<td>Guinea pig liver</td>
<td>0.178</td>
<td>1.31</td>
<td>0.136</td>
</tr>
<tr>
<td>Guinea pig brain</td>
<td>0.071</td>
<td>0.164</td>
<td>0.432</td>
</tr>
<tr>
<td>Rabbit liver</td>
<td>0.099</td>
<td>0.073</td>
<td>2.75</td>
</tr>
<tr>
<td>Rabbit brain</td>
<td>0.014</td>
<td>0.052</td>
<td>0.27</td>
</tr>
</tbody>
</table>

* Values are expressed as μmoles per hour per mg of protein.
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REFERENCES

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