MULTIPLICITY OF MITOCHONDRIAL MONOAMINE OXIDASES

James B. Ragland

Department of Biochemistry and Clinical Research Center,[1]
University of Tennessee Medical Units, Memphis, Tennessee

Received March 13, 1968

There have been only two brief reports which presented direct evidence of the existence of more than one monoamine oxidase in mitochondria although this has been suggested by many workers. Gorkin (1963) partially separated p-nitrophenylethylamine oxidase activity from m-nitro-p-hydroxybenzylamine oxidase activity by ion exchange chromatography of mitochondrial extracts in the presence of nonionic detergent (OP-10). Youdim and Sandler (1967) report the appearance of two or three bands of enzyme activity upon polyacrylamide gel electrophoresis of partially purified monoamine oxidase from rat liver or human placental mitochondria.

This paper describes the separation of solubilized liver mitochondrial monoamine oxidases from three species by gel filtration in the presence of detergent. Based on substrate specificity there appear to be at least three different monoamine oxidases present in rat and rabbit liver and at least two in beef liver.

MATERIALS AND METHODS

Beef liver was obtained fresh from the slaughter house. Rabbit livers, Type I, were obtained frozen from Pel-Freez Biologicals, Inc., Rogers, Arkansas. Rat livers were removed fresh from decapitated Sprague-Dawley rats. Heavy liver

---

[1] The initial stages of this investigation were carried out at Houston State Psychiatric Institute, Houston, Texas.

mitochondria were isolated from all three types of liver by the method of Green and Ziegler (1963).

Beef liver submitochondrial particles were prepared by the method of Green and Ziegler for preparing ETP_H. The residue was resuspended in 0.25 M sucrose to give a final protein concentration of approximately 5 mg. per ml. Cutscum[2] was then added to a final concentration of 2%. The preparation was allowed to stand for 30 minutes, then centrifuged at 144,000 x G for 2 hours. The supernatant was removed and a sample applied to a 2.5 x 45 cm. column of Sephadex G-200 soaked, equilibrated, and eluted with 0.1 M sodium phosphate buffer pH 7.4 containing 2% Cutscum. The column was run in the upflow mode. Fractions were collected in a refrigerated collector.

Rat and rabbit liver mitochondria were suspended in 0.05 M sodium phosphate pH 7.4 to give a final protein concentration of approximately 20 mg./ml. The suspension was combined with an equal volume of 0.4% sodium lauryl sulfate in 0.05 M phosphate buffer pH 7.4 and allowed to stand for 20 minutes. This mixture was centrifuged for 15 minutes at 78,000 x G. The supernatant was removed by suction, and the fluffy layer atop the hard packed precipitate was suspended in buffer by gentle swirling. The suspension of fluffy material was mixed with an equal volume of 2% Cutscum in 0.05 M phosphate buffer pH 7.4 and allowed to stand for 30 minutes, then centrifuged at 150,000 x G for 2 hours. The resulting precipitate was suspended in 2% Cutscum in buffer, allowed to stand for 30 minutes and again centrifuged for 2 hours at 150,000 x G. The supernatant from the final centrifugation was separated on a column of Sephadex G-200 equilibrated and eluted with 0.05 M sodium phosphate pH 7.4 containing 0.5% Cutscum.

Protein in column fractions was determined by a modification of the method of Lowry et al. (1951). In the presence of Cutscum a precipitate appeared during the

---

[2] An octyl phenoxy polyethoxyethanol supplied by Fisher Scientific Company

final color reaction. Therefore, Cutscum was also added to standards and the precipitate was removed by centrifugation.

Monoamine oxidase activity towards kynuramine (MO-K) was determined by the spectrophotometric method of Weissbach et al. (1960). Monoamine oxidase activity towards tyramine (MO-T) and serotonin (MO-S) was determined by the method of Otsuka and Kabayashi (1964) using either tyramine-1-C^{14} or serotonin-2-C^{14} [3] as substrate. All enzyme incubations were carried out at 30° C.

RESULTS AND DISCUSSION:

The results of gel filtration chromatography of the solubilized monoamine oxidases for all three species are shown in Figure 1. To facilitate comparison of the elution profiles, monoamine oxidase activities for each substrate have been expressed as a percentage of the activity of the most active column fraction for that substrate.

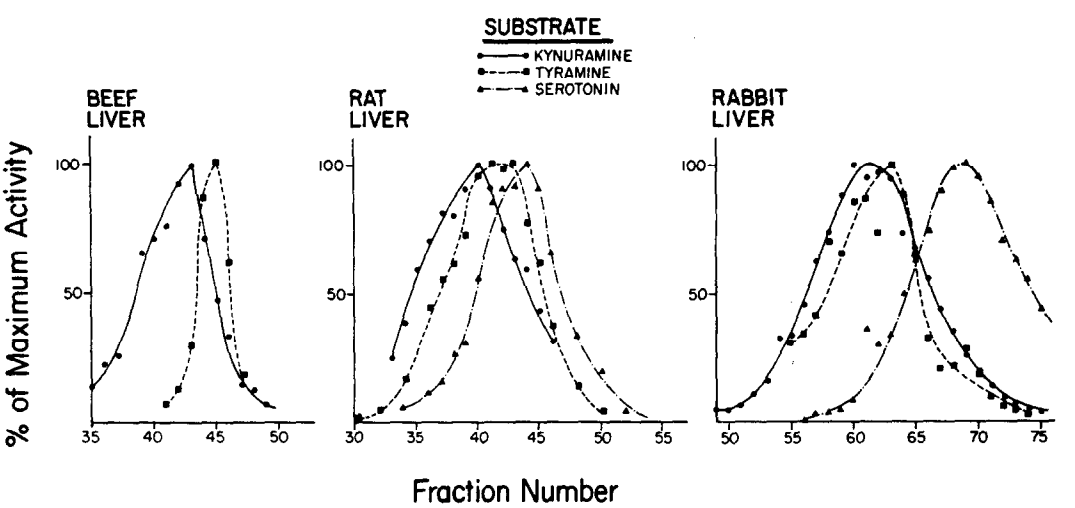

Figure 1. Separation of solubilized mitochondrial monoamine oxidases on Sephadex G-200. Activities are expressed as per cent of activity of the most active fraction for each substrate and each species.

[3] p-hydroxy phenylethyamine-1-C^{14}-hydrobromide and 5-hydroxytryptamine-2-C^{14} obtained from New England Nuclear Corporation.

Recovery of monoamine oxidase activity applied to the column varied from 40-80% with substrate and with species. Other experiments carried out in this laboratory have shown that monoamine oxidase activity is not stable in Cutscum solutions.

When beef liver submitochondrial particles are treated with 2% Cutscum both MO-K activity and MO-T activity are found in the supernatant following centrifugation at 144,000 x G for two hours. Gel filtration in the presence of 2% Cutscum separated these two activities into two closely juxtaposed peaks. There were two broad ill-defined protein peaks found in the elution pattern (not shown). The first of these showed a maximum in fraction 33, the second in fraction 44. The void volume of the column was collected between fractions 1 and 26. Thus, under the conditions of this experiment both MO-K and MO-T are retarded by the column, indicating they can enter the gel matrix. When Sephadex G-100 was used under the same conditions all monoamine oxidase activity emerged immediately after the void volume. If Cutscum was omitted from the buffer used to pack and elute a column of Sephadex G-200, all monoamine oxidase activity was eluted immediately after the void volume. These findings taken together indicate that solubilized mitochondrial monoamine oxidases have smaller particle size than they do in the absence of detergent. One explanation for this behavior could be that mitochondrial monoamine oxidases are constituents of repeating units of mitochondrial membranes that recombine in the absence of detergent, Green, et al., 1967.

The elution patterns are somewhat different for rat and rabbit liver, but in both cases, there appear to be three different monoamine oxidases on the basis of substrate specificity. The best separation of MO-K and MO-T activity was obtained with beef liver. The order of elution for MO-K and MO-T is the same for all three species. MO-S is most clearly separated in the rabbit liver mitochondria experiment.

The most apparent explanation for the results obtained in these experiments is

that there is not a single monoamine oxidase, at least for the substrates tested, but that there are separate and distinct oxidases for each of the amines. If this is the case, then the name, monoamine oxidase, is ambiguous and would best be replaced by the more specific terms, tyramine oxidase, serotonin oxidase, etc. Another explanation is that the elution profiles do not actually represent three different monoamine oxidase activities per se but represent the separation of other factors differentially influencing activity with the three substrates. Such factors could be other enzymes acting upon the specific substrates or products, specific inhibitors, or specific activators.

If there is indeed a multiplicity of specific amine oxidases present in liver mitochondria, then preparations of purified monoamine oxidase that show wide substrate specificity (Erwin and Hellerman, 1967, Nara et al., 1966) must be reexamined. Furthermore, if a monoamine oxidase is to be used as a marker for sub mitochondrial particles and membrane fractions, as has been proposed by Schnaitman et al. (1967), then it must be determined whether or not all monoamine oxidases have the same submitochondrial localization. This question is now being investigated in this laboratory. In addition, the existence of multiple enzymes raises the possibility that there may be specific monoamine oxidase inhibitors that could inhibit one type of monoamine oxidase but not another.

In conclusion, three different mitochondrial monoamine oxidase activities have been separated by gel filtration chromatography in the presence of detergent. The assumption that these results represent the separation of three distinct enzymes must be confirmed by future experiments. If such confirmation is obtained, then monoamine oxidases should not be considered a series of isoenzymes with multiple substrate specificity but rather a series of homologous specific amine oxidases with closely related structural, coenzyme, and purification characteristics.

REFERENCES

Erwin, V. G. and Hellerman, L., J. Biol. Chem., 242, 4230 (1967).
Gorkin, V. Z., Nature, 200, 77 (1963).
Green, D. E., et al., Archives of Biochem. and Biophys., 119, 312 (1967).
Green, D. E. and Ziegler, D. M. in Methods in Enzymology (S. P. Colowick and N. O. Kaplan eds.) VI, 416, Academic Press (1963).
Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem., 193, 265 (1951).
Nara, S., Gomes, B., and Yasunobu, K. T., J. Biol. Chem., 241, 2774 (1966).
Otsuka, S. and Kobayashi, Y., Biochem. Pharmacol., 13, 995 (1964).
Schnaitman, C., Erwin, V. G., and Greenawalt, J. W., J. Cell. Biol., 32, 719 (1967).
Weissbach, H., Smith, T. E., Daly, J. W., Witkop, B., and Udenfriend, S., J. Biol. Chem., 235, 1160 (1960).
Youdim, M. B. H. and Sandler, M., Biochem. J., 105, 43P (1967).