MONOAMINE OXIDASE ACTIVITY OF CELL NUCLEI AND NUCLEAR FRACTIONS OF MOUSE LIVER UNDER NORMAL CONDITIONS AND DURING DEVELOPMENT OF ASCITES SARCOMA 37

M. Khuzhamberdiev, S. N. Kuz'mina, V. Z. Gorkin, and I. B. Zbarskii

UDC 612.351.11:577.158.47+616-006.3.04-07:616.36-008.931:577.158.47

Monoamine oxidase (MAO) activity in a homogenate and in isolated nuclei and nuclear fractions of the liver of normal mice and of mice with tumors was investigated by the use of p-nitrophenylethylamine hydrochloride as the substrate. Normally MAO activity of the nuclei was concentrated just as in the rat liver, in the nuclear membrane, but the specific MAO activity was only 25-50% of that found in rats. MAO activity was reduced in the liver of mice with tumors, and the decrease was especially marked in the nuclei and nuclear membranes; meanwhile, activity was found in the deoxyribonucleoprotein fraction, in which none is normally found. MAO activity was not found in the tissues of mouse tumors (sarcoma 37 and Ehrlich's ascites carcinoma).

Isolated nuclear membranes of the rat liver possess high monoamine oxidase (MAO) activity, even higher than the activity of this enzyme in other cell structures. Comparison of the specific MAO activity of isolated nuclear membranes and nuclei suggests that all the MAO activity of the nucleus is concentrated in the nuclear membranes. On the other hand it has been found that the nuclei and nuclear membranes of Zajdela's ascites hepatoma, and a homogenate of this hepatoma, of Jensen's rat sarcoma, and of sarcoma 37 of mice do not possess MAO activity [2].

With these facts and also the high content of certain oxidative and other enzymes in nuclear membranes [5, 7] in mind, the present investigation was undertaken to study MAO activity in isolated nuclei and their fractions, and, in particular, in fractions rich in nuclear membranes, in the liver of normal mice, and in the liver of mice with transplanted sarcoma 37.

EXPERIMENTAL METHOD

Noninbred albino mice of both sexes weighing 20-22 g were used. The liver was taken from the mice with tumors (ascites sarcoma 37 was transplanted by intraperitoneal injection of a suspension of the tumor) on the 8th day after transplantation.

Isolated nuclei were obtained from the liver taken from ten mice by a two-stage method in concentrated sucrose [8]. The nuclei were fractionated in two ways.

The first method (salt fractionation) consisted of successive extraction of the isolated nuclei with 0.14 M and 1.5 M NaCl solutions, thereby yielding fractions of nuclear sap and deoxyribonucleoprotein (DNP) of the chromatin [3] respectively. The residue after extraction of the DNP, containing material

Laboratory of Biochemistry, Institute of Biology of Development, Academy of Sciences of the USSR. Laboratory of Biochemistry of Amines and Other Nitrogenous Bases, Institute of Biological and Medical Chemistry, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR, V. N. Orekhovich.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 73, No. 4, pp. 37-39, April, 1972. Original article submitted June 14, 1971.

TABLE 1. Specific MAO Activity (in nmoles deaminated substrate/mg protein/min at 37°C) of Nuclei and Nuclear Fractions of Liver from Normal Mice and Mice with Transplanted Sarcoma 37 (M±m)

Preparation	Mice	
	normal	with tumors
Homogenate	0,88±0,03 (5)	0,62±0,01 (6)
Nuclei	0,50±0,04 (5)	0,18±0,008 (6)
Salt fractionation		
Residue after extraction of nuclear sap	0,69±0,03 (4)	0,29±0,01 (3)
Residue after extraction of DNP	1,21±0,20 (4)	0,63±0,09 (3)
DNP	0,00 (4)	0,18±0,03 (3)
Deoxycholate extract	3,19±0,41 (4)	0,55±0,09 (3)
Residue after extraction with deoxycholate	0,38±0,03 (4)	0,00 (3)
Osmotic shock		
Unpurified membrane fraction	2,9±0,11 (3)	0,8±0,05 (3)
Purified nuclear membranes	4,3±0,05 (3)	

Note: Samples contained from 0.5 to 3 mg protein in 3 ml medium including p-nitrophenylethylamine-HCl and detergent OP-10 or Triton X-100 in concentration of 1.6 M, 5%, and 1% respectively. Incubation for 60 min at pH 7.4 (number of experiments given in parenthesis).

of the nucleoli and nuclear membranes, was treated with 1% sodium deoxycholate solution and centrifuged [6]. The supernatant contained material of the nuclear membranes; the residue consisted mainly of material from the nucleoli.

The second method (osmotic shock) consisted of obtaining nuclear membranes by destruction of the nuclei in hypotonic phosphate buffer [4]. In some experiments an unpurified fraction of nuclear membranes was obtained by removal of nuclear fragments from the membrane fraction after osmotic shock by centrifugation at 300 g for 5 min, and by sedimentation at 50,000-60,000 g for 15 min without passage through a sucrose gradient.

Protein [9] and MAO activity, using p-nitrophenylethylamine as the substrate [1, 10], were determined in the resulting fractions.

EXPERIMENTAL RESULTS

The results (Table 1) show that specific MAO activity in the mouse liver was much lower than in the corresponding structures of the rat liver [2]. The decrease was particularly marked in the nuclear fractions; activity in the mouse homogenate, for instance, was only 67%, and in the nuclei and nuclear membranes 25-33% of that found in rats. On fractionation of the cell nuclei of normal mouse liver no MAO activity was found either in the nuclear juice or in the DNP fraction. During salt extraction the specific MAO activity increased by 40% after extraction of the nuclear sap, and was again doubled after extraction of DNP from the residue. The greater part of the MAO activity could be extracted from this last residue with 1% deoxycholate solution, and the specific activity of this extract was 2.6 times greater than the activity of the original residue and 6.5 times greater than in the original nuclei.

The unpurified membrane fraction obtained by the osmotic shock method was almost six times richer in MAO than the original nuclei, while the specific MAO activity of the purified nuclear membranes was 8.6 times higher than that of the whole nuclei.

MAO activity in liver homogenate of mice with tumors was 43% lower than in normal mice, whereas MAO activity in the isolated nuclei was reduced by 64%. Approximately the same ratio was found for the nuclear fractions after salt extractions. However, MAO activity in the membrane fraction obtained after osmotic shock was 73% lower, i.e., a decrease of 3.6 times, while in the membrane fraction obtained by deoxycholate extraction it was 83% lower, a decrease of 5.8 times. Meanwhile, some MAO activity in the

liver of the mice with tumors was extracted with the DNP fraction, which normally does not contain this activity.

On repeated determinations of MAO activity in the homogenate and isolated nuclei of mice with tumors (sarcoma 37 and Ehrlich's ascites carcinoma) this activity was not found.

The investigation thus confirmed previous findings indicating high MAO activity in the nuclear membranes of the liver and its absence in tumors [2]. The redistribution of MAO activity in the nuclear fractions of the mice with tumors, namely its appearance in the DNP fraction while MAO activity was particularly sharply reduced in the nuclear membranes and nuclei, is especially interesting. The mechanism of this decrease will be the subject of a special investigation.

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