Distribution of polyamine oxidase activity in rat tissues and subcellular fractions

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Abstract. The activity of polyamine oxidase (PAO) in rat tissues, and its subcellular distribution, were assayed using a simple polarographic method. The highest PAO activity was measured in the liver and the lowest in the skeletal muscle. In liver, kidney and uterus the highest specific PAO activity was found in the light mitochondrial fraction. PAO was not found in the microsomal fraction except in the kidney.
Key words. Polyamine oxidase; oxygen electrode; rat tissues; subcellular distribution; subcellular fractions; polyamines; N^1-monoacetylspermine.

Polyamine oxidase (PAO) was discovered in rat liver by Hölttä[1]. The enzyme (flavin-containing) is capable of transforming spermine into spermidine and spermidine into putrescine in the presence of benzaldehyde[1]. However, Seiler[2] demonstrated that the preferred substrates for PAO are not spermine and spermidine themselves, but the N^1-acetyl derivatives of these polyamines. It was presumed that the acetylated polyamines are natural substrates of this enzyme[2,3]. PAO attacks an imino group inside the chain of an acetylpolyamine, splitting it into a shorter polyamine, 3-acetamidopropanal and hydrogen peroxide[3,4]. Oxygen appears to be the sole electron acceptor[1,5].

Polyamine oxidase activity is found in most rat[6] and human[7] tissues. Its subcellular distribution has been established in rat liver[1], but not in other organs. In the present paper, PAO activity was measured in various rat tissues using an oxygen electrode, and the subcellular distribution of the specific enzyme activity was assayed in rat liver, kidney and uterus.

Methods

Female Wistar rats, weighing 150–180 g were decapitated and the various organs removed within 1 min. The organs were homogenized in 9 parts of ice-cold 0.25 M sucrose, 10 mM Tris buffer (pH 7.4). The supernatant of the crude homogenate after centrifugation at 600 × g for 10 min was used as the enzyme source for the investigation of PAO in the rat tissues.

Preparation of subcellular fractions was made by the procedure of Hölttä[1]. Ten percent (w/v) homogenates from rat liver, kidney and uterus were first centrifuged at 600 × g for 10 min, to sediment the cell debris and nuclei, and then at 3500 × g for 10 min to pellet the heavy mitochondria. The resultant supernatant fraction was centrifuged at 20,000 × g for 15 min to sediment the light mitochondria, peroxisomes and lysosomes, collectively designated as the light mitochondrial fraction. The final supernatant fraction was centrifuged at 105,000 × g in an MSE ultracentrifuge (England) for 60 min, to obtain the microsomal and cytosolic fractions. The pellet from each centrifugation was suspended in 10 mM Tris, 0.1 mM EDTA, 0.1 mM dithiothreitol buffer (pH 7.8) and sonicated for 10 min, using MSE Soniprep 150 (England) to solubilize the enzyme. Thereafter the fractions were centrifuged at 105,000 × g for 60 min and the supernatants used for the assay of PAO activity.

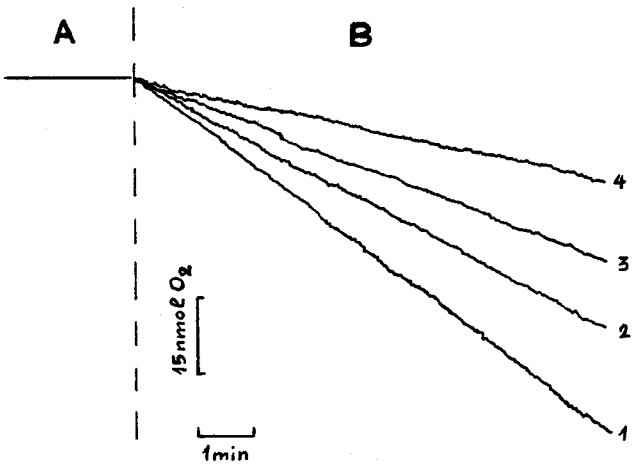
The present method for PAO assay is based in principle on the method of Tipton[8] for a monoamine oxidase assay. Polyamine oxidase activity was estimated using an oxygen electrode of the type designed by Clark, attached to a Universal polarograph, type OH-105 (Hungary). The standard reaction mixture (final volume 1.0 ml) contained 10 mM Tris, 0.1 mM EDTA, 0.1 mM pargyline, 1 mM semicarbazide, 75 units of catalase, 5 mM substrate (various polyamines were tested) and enzyme preparation. The final pH value of the reaction mixture was 9.0. The reaction was carried out at 25 °C, using air as the gaseous phase, and activity was calculated from the initial linear portion of the curve. Separate controls were determined in the absence of enzyme. Protein was determined by the method of Lowry[9], with bovine serum albumin as a standard.

Results

Substrate specificity of PAO was assayed under optimal standard conditions (see Methods), using rat liver homogenate as an enzyme source. The enzyme activity was highest with N^1-monoacetylspermine. With N^1,N^{12}-diacetylspermine, N^1-monoacetylspermidine and spermine the activity was 71%, 52.5% and 27.4% respectively of the activity with N^1-monoacetylspermine (fig. 1). With spermidine there was almost no activity under these conditions beyond the endogeneous oxygen uptake. On the basis of these data, N^1-monoacetylspermine was used as the substrate in subsequent experiments.

Polyamine oxidase activity was measured in homogenates of a number of rat tissues. The results are summarized in table 1. All tissues assayed showed relatively high enzyme activity. The highest activity was in liver and the lowest in the skeletal muscle.

Subcellular distribution of specific PAO activity was assayed in the rat liver, kidney and uterus, after differential centrifugation of 10% homogenates from these organs



Substrate specificity of polyamine oxidase. The reaction was followed for 10–12 min as described in Methods. 10% rat liver homogenate (50 µl), containing approx. 5 mg wet tissue was used as an enzyme preparation. A-initial linear portion of the curve. B-progress of the reaction, depending on the substrate at concentration 5 mM (tracings of oxygen uptake): 1 – N^1-monoacetylspermine; 2 – N^1, N^{12}-diacetylspermine; 3 – N^1-monoacetylspermidine; 4 – spermine.

Table 1. Polyamine oxidase activity in rat tissues*

Organ	PAO activity** nmol O_2/g tissue/min	Number of animals
Liver	1681 ± 210	5
Kidney	1164 ± 149	5
Uterus	966 ± 167	12
Brain	843 ± 138	5
Lung	751 ± 109	5
Skeletal muscle	333 ± 86	3

* 10% homogenates (50 µl), containing 4–5 mg wet tissue were used as an enzyme source (see Methods). N^1-monoacetylspermine (5 mM) was used as a substrate. ** mean values ± SD are given.

Table 2. Specific activity of polyamine oxidase in subcellular fractions of liver, kidney and uterus*

Fraction	Specific PAO activity** nmol O_2/mg protein/min Liver	Kidney	Uterus
Crude nuclear	5.54 ± 1.57	3.80 ± 1.18	1.62 ± 0.78
Heavy mitochondrial	4.81 ± 1.46	2.73 ± 0.58	1.90 ± 0.87
Light mitochondrial	15.18 ± 1.88	6.21 ± 1.09	4.68 ± 1.08
Microsomal	–	4.41 ± 0.97	–
Cytosol	5.41 ± 1.52	4.64 ± 0.69	4.53 ± 1.69

* The subcellular fractions, obtained by differential centrifugation were sonicated to solubilize the enzyme (see Methods). Thereafter the fractions were centrifuged at 105,000 × g for 60 min and the supernatants (100 µl) were used for PAO assay. N^1-monoacetylspermine (5 mM) was used as a substrate. ** Each value is the mean ± SD of 2 experiments with 3 data points each.

(see Methods). Table 2 shows the data from this assay. The highest specific PAO activity was measured in light mitochondrial fractions of all organs assayed. No PAO activity was established in the microsomal fraction of the liver or of the uterus. However, in kidney, in contrast to the above-mentioned organs, relatively high specific PAO activity was measured in the microsomal fraction, and it was almost equal to the specific enzyme activity in the cytosol fraction of the same organ. Specific PAO activity was also found in the crude nuclear and heavy mitochondrial fractions of the three organs.

Discussion

At present, few methods for the assay of PAO in mammalian tissues have been described. Hölttä[1] employed radioactive spermine and spermidine as substrates for PAO investigation and separated their products by paper electrophoresis; Seiler et al.[6] measured PAO activity in rat tissues by isolating dansyl derivatives of the reaction products by thin layer chromatography, followed by a fluorometric analysis; and Suzuki et al.[7] assayed PAO activity in human tissues, using fluorometric measurement of hydrogen peroxide formed in the oxidase reaction.

In the present study an oxygen electrode was used for the measurement of PAO activity in various rat tissues and subcellular fractions. This method is simpler and quicker than the previously reported methods. N^1-monoacetylspermine was used as the substrate for PAO, as it was found to be the substrate with which the enzyme showed the highest activity (fig.). The data of Suzuki et al.[10] suggest that N^1-monoacetylspermine, N^1,N^{12}-diacetylspermine, and N^1-monoacetylspermidine are not easily metabolized by monoamine oxidase (EC 1.4.3.4), or diamine oxidase (1.4.3.6) in mammalian tissues. Nevertheless pargyline, a specific inhibitor of mitochondrial monoamine oxidase, and semicarbazide, which is an inhibitor of pyridoxal-containing amine oxidases (such as diamine oxidase, serum monoamine oxidase and an amine oxidase found in connective tissues)[11] were added to the reaction mixture, to ensure a sufficiently specific assay of PAO. These inhibitors had almost no effect on PAO activity at the concentrations used[1,6,7].

The results of the present study of the distribution of PAO activity in rat tissues (table 1) are close to the data of Seiler et al.[6]. In addition, in female animals a relatively high PAO activity was established in the uterus.

The highest specific PAO activity, measured in the light mitochondrial fraction of rat liver (in an assay of the subcellular distribution of PAO), agrees with the finding of Hölttä[1] that PAO is located in peroxisomes, which are components of this fraction. The major portion of rat kidney and uterus polyamine oxidase is most probably also localized in peroxisomes (table 2). However, more detail assays are needed to confirm this. The PAO activity found in the crude nuclear fraction of the three organs studied is most probably due to the presence of unbroken cells after the preliminary homogenization.

The subcellular distribution of the specific PAO activity in the rat kidney and uterus differs from that in rat liver. Unlike that in the liver, the specific PAO activity in the cytosol fraction of the uterus has an almost equivalent value to that found in the light mitochondrial fraction. In the kidney, PAO activity was established in the microso-

mal fraction, but no activity was found in the microsomal fraction of the other organs. The reasons for these differences can hardly be discussed on the basis of the present brief study, but a knowledge of its distribution could be useful in further investigations of PAO.

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Decrease of mRNA levels and biosynthesis of sucrase-isomaltase but not dipeptidylpeptidase IV in forskolin or monensin-treated Caco-2 cells

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Abstract. Treatment for 48 h of differentiated, confluent Caco-2 cells with $2.5\ 10^{-5}$ M forskolin or 10^{-6} M monensin, which produces a significant decrease of the de novo biosynthesis of sucrase-isomaltase, does not change quantitatively the de novo biosynthesis of dipeptidylpeptidase IV. Western blot analysis and silver nitrate staining indicate that neither drug induces any modification in the steady state expression of these two brush border hydrolases. Northern blot analysis shows that the level of dipeptidylpeptidase IV mRNA does not change in treated as compared to control Caco-2 cells. In contrast, forskolin and monensin dramatically decrease the level of sucrase-isomaltase mRNA. These observations suggest a separate regulation of biosynthesis for sucrase-isomaltase and dipeptidylpeptidase IV in intestinal cells. The mechanisms responsible for such a difference are discussed. Among them, the role of glucose metabolism, which is perturbed by both drugs, appears to be of crucial importance.
Key words. Sucrase-isomaltase; dipeptidylpeptidase IV; glucose metabolism; human colon cancer cells.

Brush border associated hydrolases normally present in human intestine also have a polarized expression in post-confluent cultured Caco-2 cells [1, 2]. This property makes it possible to study the mechanisms by which intestinal hydrolases are synthesized, processed and vectorially transported to the brush border membrane [3, 4]. Several studies have compared the fate of peptidases and disaccharidases and demonstrated asynchronous transport of these proteins, with peptidases being rapidly transported to the cell surface, whereas disaccharidases make the transit much more slowly [5, 6].

Previous reports from this laboratory have also suggested differences in the regulation of the biosynthesis of these brush border enzymes in cultured human colon cancer cells. Most of these studies have focused on the relationships between glucose metabolism and brush border enzyme biosynthesis [7–10], as it is well known that glucose metabolism is severely impaired in cancer cells [11]. Therefore drugs such as forskolin and monensin have been used in order to perturb the intracellular utilization of glucose in Caco-2 cells [2, 7, 8, 9]. For example, forskolin, a potent activator of adenylate cyclase [12], strongly inhibits the biosynthesis of sucrase-isomaltase (EC 3.2.1.10, EC 3.2.1.48.) (SI)[2, 7]. Monensin, an ionophore known to alter distal Golgi functions [13], also decreases the biosynthesis of SI, without affecting the biosynthesis of dipeptidylpeptidase IV (EC 3.4.14.5.) (DPP IV)[8]. The decreased biosynthesis of SI has been correlated with a net reduction in the amount of its specific mRNA [7, 9].

Most of these studies have focused on SI, for which all the necessary tools were available, including antibodies [10] and a cDNA probe [14]. However, DPP IV could be thoroughly investigated in these systems only recently, as both a cDNA for human DPP IV [15] and a monoclonal