INTRACELLULAR LOCALIZATION OF PYRIDOXAMINE-5-PHOSPHATE OXIDASE IN RABBIT LIVER

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1. Introduction

Pyridoxine-P (pyridoxamine-P) oxidoreductase (deaminating, EC 1.4.3.5.) was partially purified from rabbit liver by Pogell [1] first, and further purification was obtained by Wada and Snell [2], Korytnyk et al. [3] and recently by Kazarinoff and McCormick [4].

Specificity of the enzyme with regard to other amine oxidases, such as monoamine oxidase and diamine oxidase was discussed by Pogell [1].

In the present paper evidence is given for intracellular localization of pyridoxamine-P oxidase, as compared with monoamine and diamine oxidases, and a few data are presented, confirming specific distinction among these enzymes.

2. Materials and methods

Rabbit liver tissue fractionation was performed according to De Duve et al. [5], using monoamine oxidase, acid phosphatase, and glucose-6-phosphatase as marker enzymes for mitochondria, lysosomes, and microsomes, respectively. The nuclear fraction was completely discarded, since under the phase-contrast microscope, this fraction appeared strongly contaminated by unbroken cells and cell debris, for the rabbit liver is very resistant to homogenization. On the other hand the same fraction showed a very low pyridoxamine-P-oxidase specific activity (data not shown). The recoveries are therefore calculated with respect to the cytoplasmic extract.

Diamine oxidase activity was determined by the method of Gordon and Peters [6], with the modifications described by Argento-Cerù et al. [7]. This same method was used for testing possible production of H_2O_2 during the pyridoxamine-P oxidase activity. For the same purpose was also used the method described by Hildebrandt et al. [8]; this latter test was carried out either in the presence or in the absence of 10^{-1} M sodium azide, in order to exclude failure in H_2O_2 detection due to the presence of catalase, which is inhibited by azide ion at this concentration, while pyridoxamine-P-oxidase is not.

Pyridoxamine-P-oxidase activity was determined by the method of Wada and Snell [2], with some modifications of those suggested by Korytnyk et al. [3]. Three ml of each tissue fraction were incubated for 30 min at 38°C in Tris–HCl buffer pH 8 (0.2 M in the final volume of 6 ml), using 1.5 μmoles pyridoxamine-5-phosphate as substrate. The reaction was stopped by addition of 0.6 ml of 100% (w/v) trichloroacetic acid. In the experiments combined with the *o*-dianisidine-peroxidase method [6, 7], 1 ml of 9.8 N HCl was used for stopping the reactions.

Protein concentrations were determined according both to the method of Lowry et al. [9] and to a modified biuret method [10].

3. Results and discussion

The diamine oxidase activity was proposed by Finazzi Agrò et al. [11], to be a ping-pong mechanism in which the pyridoxal-phosphate moiety [12] of the enzyme is firstly reduced by the amine substrate, yielding the aldehyde product; then the reduced enzyme would be reoxidized to the pyridoxal-phosphate form by oxygen, producing NH_3 and H_2O_2.

Under such perspective, it could be thought that pyridoxamine-P oxidase was an enzyme with an action mechanism very similar to the second phase of the above recalled one for diamine oxidase. The results of the present work led however to the opposite conclusion, thus confirming the early observations of Pogell [1].

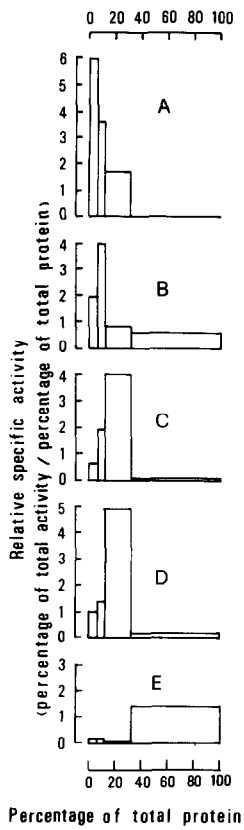

Fig. 1. Distribution patterns of diamine oxidase (D), pyridoxamine-P-oxidase (E), and marker enzymes: monoamine oxidase, (A) acid phosphatase (B), glucose-6-phosphatase (C) in rabbit liver. Fractions are represented on the abscissa scale by their relative protein content, in the order in which they were isolated from cytoplasmic extract, i.e. from left to right: mitochondria, lysosomes, microsomes, and final supernatant.

This author had already pointed out differences between diamine oxidase and pyridoxamine-P-oxidase activities. But still, as the two enzymes were found together in the Pogell's 18 000 *g* supernatant fraction, it could have been conceivable that one enzyme shows two different activities under different conditions.

The distribution patterns of diamine oxidase and pyridoxamine-P-oxidase are shown in fig. 1: pyridoxamine-P-oxidase is entirely present in the 105 000 *g* supernatant fluid, while the diamine oxidase, as was previously demonstrated [7, 13], was completely recovered in the microsome fraction.

During the pyridoxamine-P-oxidase activity, H_2O_2 production was tested, which could be expected in the above reported hypothetical supposition of a similarity of action mechanism with the diamine oxidase. Production of H_2O_2 by pyridoxamine-P-oxidase would not be surprising, since it is a flavin dependent enzyme [2]. But all our attempts in this direction gave negative results, both in the presence and in the absence of azide ions.

Monoamine oxidase was used as a marker enzyme for the mitochondrial fraction, and was also completely absent in the supernatant fluid.

Pig kidney diamine oxidase, purified according to Mondovì et al. [14], was also tested in the same conditions reported above for pyridoxamine-P-oxidase activity, but no measurable product formation was observed.

All these data of different intracellular localisation, and different action mechanisms, seem to confirm the specific distinction between pyridoxamine-P-oxidase and other maine oxidases.

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