IDENTIFICATION OF PANTETHINASE IN HORSE KIDNEY EXTRACT *

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1. Introduction

Purification of an oxygenase oxidizing cysteamine to hypotaurine has been recently reported from this laboratory [1]. Cysteamine, however, is not a common metabolite and we have sought a possible mechanism capable of producing the cysteamine used as substrate by this enzyme. Although a direct decarboxylation of cysteine has never been reported, it is known that cysteine is decarboxylated to cysteamine when it is bound to pantothenic acid in the course of the biosynthesis of coenzyme A [2]. Moreover pantetheine and phosphopantetheine are present in considerable amounts in the free or bound form in a number of tissues [3,4]. The enzymic cleavage of these compounds could represent, therefore, a source of free cysteamine. We have used pantethine as a representative substrate for checking the occurrence of a cysteamine (cystamine) producing enzyme in horse kidney. For convenience this enzyme will be referred to as pantethinase.

2. Methods and materials

Labelled DL pantethine was prepared starting with DL^{14}C-1-pantolactone obtained from SORIN (Saluggia, Italy) by adapting the conventional procedure for the preparation of pantethine [5]. The compound was chromatographically pure with a specific activity of 22.5 μc/mmole. Fresh horse kidney cortex was homogenized with 1 vol of water in a Waring blendor for 10 minutes. After centrifugation the supernatant was brought to 70% saturation with ammonium sulfate. The precipitate was collected by centrifugation, dissolved in phosphate buffer pH 7.6 0.01M and dialyzed against water. Incubation mixtures contained: 90 to 100 mg protein; 20 μmoles of labelled pantethine; 0.1 mmoles of potassium phosphate buffer pH 7.6; water, with eventual additions, to 3 ml. At the end of the incubation at 38° the solution was deproteinized by adding 0.3 ml 40 percent perchloric acid. The latter was eliminated by alkalinization with 2 N KOH (final pH ca. 7.5) and centrifugation. To 1 ml of the final solution was added 0.5 ml 30 percent H_2O_2 and 0.5 ml 0.1 percent ammonium molybdate in order to oxidize the excess of pantethine, and any other sulfur compound, to the respective sulfone [6]. After 10 min standing, an aliquot sample was spotted on a strip of Whatman no. 1 filter paper and chromatographed overnight using n-butanol, acetic acid, water (40–10–50) as solvent. The chromatogram was then scanned for radioactivity by using a gas flow chromatoscanner (4 pi Actigraph, Nuclear Chicago).

3. Results

The incubation mixture, prepared as described above, gives the chromatographic picture in fig. 1. A large peak of unreacted pantethine (sulfonate derivative) is present at Rf = 0.17. Another radioactive peak, which increases with time of incubation, is seen at Rf = 0.75. After 4 hours of incubation the second peak represents 5 percent of the total radioactivity of the chromatogram, or 2.5 percent of the pantethine radioactivity (the difference being due to binding of part of pantethine to protein precipitated

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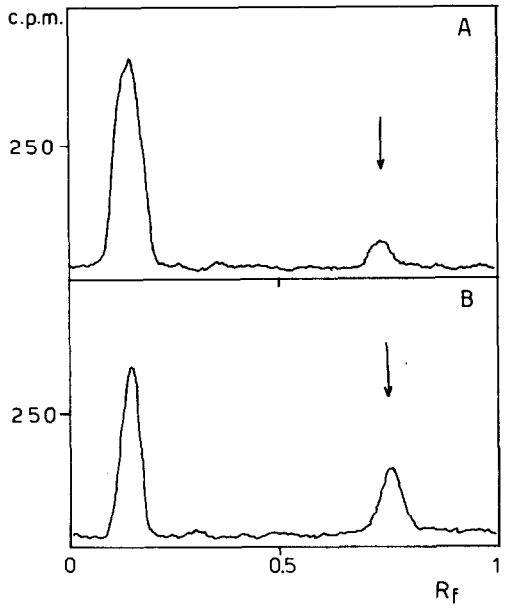

Fig. 1. Radioscanning of chromatograms of incubation mixtures containing: (A) horse kidney extract (90 mg protein); potassium phosphate buffer pH 7.6, 0.1 mmoles; ^{14}C-pantethine, 20 μmoles; water, to the final vol of 3 ml. (B) the same as in A with 30 μmoles of neutralized cysteine hydrochloride. Incubation for 4 hours at 38°. The arrow indicates the position of pantothenic acid. The large peak on the left is due to the excess of pantethine (oxidized to the sulfone derivative). Chromatographic solvent: n-butanol, acetic acid, water (40–10–50).

with perchloric acid). Incubation of pantethine in the absence of the kidney extract or in the presence of boiled kidney extract does not produce the Rf = 0.75 peak. The Rf = 0.75 peak has been identified as pantothenic acid by the following tests.

1) An authentic sample of pantothenic acid has been chromatographed under the same conditions described above. The strip was cut in pieces 3 cm long, each piece was eluted with 1 ml of 0.25 N NaOH in separate test tubes. The eluted solutions were hydrolyzed for 1 hour in boiling water. After neutralization to pH with acetate buffer the solutions were reacted with ninhydrin [7] for 20 min in boiling water. Test tubes containing the eluates coming from the area of Rf = 0.75 exhibited a strong ninhydrin color due to hydrolyzed β-alanine. This test indicates that an authentic sample of pantothenic acid exhibits the same chromatographic behaviour of the Rf = 0.75 peak.

2) A suitable number of paper chromatograms were prepared and the area of the Rf = 0.75 peak (located by radioscanning) was cut out and eluted with water. The solutions were collected, hydrolyzed with HCl and analyzed for aminoacids by ion exchange chromatography [7]. Radioactivity was also determined on an aliquot sample by scintillation counting (Nuclear Chicago 725). The ratio counts p.m./μmoles β-alanine of the solution was 0.14. The same ratio determined on the labelled pantethine used as substrate was 0.10, indicating that the compound responsible for the Rf = 0.75 peak contained both β-alanine and pantoic acid in a ratio close to that occurring in pantethine.

3) A sample of the solution eluted from the area of Rf = 0.75 was analyzed for pantothenic acid by Prof. M.Principalle, to whom we express our thanks (using the microbiological assay described by Cancellieri [8]). Pantothenic acid was found at a concentration of 20 μg/ml. This value is sufficiently close to the value of 14 μg/ml calculated from the amount of β-alanine determined on the same sample after hydrolysis.

In another experiment we have investigated whether the presence of cysteine as a reducing agent could have a favourable effect on pantethinase activity. It was actually found that the presence of 30 μmoles of cysteine stimulates enzymic activity (fig. 1B). After 4 hours of incubation 0.92 μmoles of pantothenate were produced in the absence of cysteine compared with 3.1 produced in the presence of 30 μmoles of cysteine. The activation by cysteine seems specific for this compound, since mercaptoethanol and cysteamine under comparable conditions, were without effect.

In a previous paper it was reported that cystamine is produced during the incubation of pantethine with a number of organ extracts, including horse kidney extract [9]. The result of the present work, together with those reported in the previous paper, indicate therefore that the products of the enzymic cleavage of pantethine by pantethinase are cystamine and pantothenic acid. Attempts to purify this enzyme are now in progress in our laboratory.

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