Biochimica et Biophysica Acta, 616 (1980) 381—383

BBA Report

BBA 61382

Δ^1-PYRROLINE-5-CARBOXYLATE REDUCTASE FROM BAKER'S YEAST

FURTHER PURIFICATION BY AFFINITY CHROMATOGRAPHY WITH 5′ AMP-SEPHAROSE 4B

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(Received July 15th, 1980)

Key words: Pyrroline-5-carboxylate reductase (S. cerevisiae), Affinity chromatography, 5′ AMP-sepharose 4B

Summary

A homogeneous preparation of Δ^1-pyrroline-5-carboxylate reductase (L-proline:NAD (P)$^+$ 5-oxidoreductase, EC 1.5.1.2) was obtained from baker's yeast by an affinity chromatography, using 5′ AMP-Sepharose 4B. After the 1st DEAE-Sephadex column chromatography, the enzyme absorbed on 5′ AMP-Sepharose column was eluted with 1 mM ATP. The final preparation was homogeneous on polyacrylamide gel electrophoresis and purified 3500-fold from the crude extract. This purified enzyme was very useful as a coupling enzyme for the assays of ornithine and pyrroline-5-carboxylate in tissues, and for the rate assay of ornithine aminotransferase activity.

We have reported previously, the purification of baker's yeast pyrroline-5-carboxylate reductase to the apparently homogeneous state and the application to the enzymatic assays of pyrroline-5-carboxylate and ornithine in tissue [1], but a more effective procedure was not known at that time. Now we have found that the activity of this enzyme is slightly activated with AMP and inhibited with ATP. This finding prompted us to use affinity chromatography with 5′ AMP-Sepharose 4B for further purification. The present paper describes the further purification of baker's yeast pyrroline-5-carboxylate reductase by affinity chromatography with 5′ AMP-Sepharose to a homogeneous preparation. Adenine nucleotides were found to be ligands of this enzyme: 1 mM AMP stimulated the enzyme activity (120—160% of the control)

and 1 mM ATP inhibited markedly (35—45% of the control), but ADP had no effect. The procedure from the preparation of crude extract to the first DEAE-Sephadex A25 column chromatography, and the enzyme assay and other methods used were similar to those previously described [1]. The enzyme eluted from the first DEAE-Sephadex A25 column chromatography was concentrated and dialyzed against 0.01 M potassium phosphate buffer (pH 7.5) including $1 \cdot 10^{-4}$ M dithiothreitol (buffer 1), and then poured onto a 5′ AMP-Sepharose 4B column (1.5 × 20 cm) equilibrated with buffer 1. The column was washed with 100 ml buffer 1, by which contaminated proteins were eluted. After the absorbance of the eluate at 280 nm decreased below 0.05, the column was eluted with 1 mM ATP (in buffer 1). Fig. 1 shows an elution profile of the affinity chromatography. 1 mM ATP eluted the enzyme selectively and the specific activity attained was 280 unit/mg protein. The recovery of enzyme activity from the step of the first DEAE-Sephadex A25 column chromatography was about 60%. The DEAE-Sephadex A25 (or DEAE-Sepharose Cl-6B) column chromatography was absolutely necessary before affinity chromatography (see Fig. 2A). The final concentrated preparation showed a homogeneous protein on polyacrylamide gel electrophoresis, and its mobility indicated that it was a less negatively-charged protein (Fig. 2). A typical example of the purification is shown in Table I. The enzyme content in the yeast sometimes varied per lot. The recovery at the final step was about 20—30%. The final preparation tended to lose its enzyme activity and had to be immediately concentrated to a protein concentration of about 1 mg/ml, dissolved in 50% glycerol containing $1 \cdot 10^{-4}$ M dithiothreitol and stored at —20°C. The 5′ AMP-Sepharose column used once, was eluted with 500 ml 1 mM AMP (in buffer 1), then equilibrated

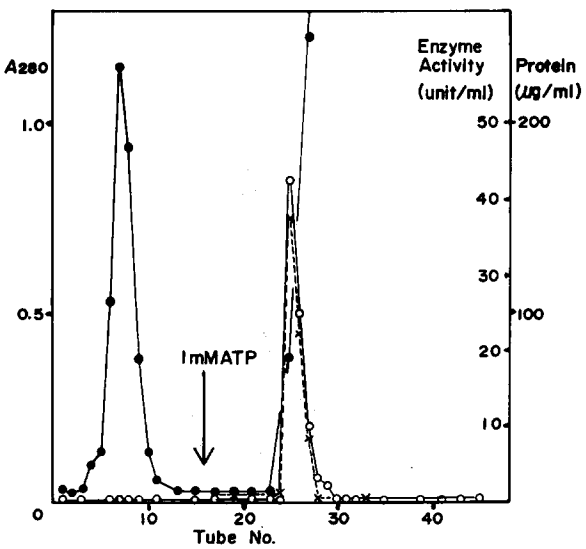

Fig. 1. Affinity chromatography of Δ^1-pyrroline-5-carboxylate reductase with 5′ AMP-Sepharose 4B. ○—○, enzyme activity; ●—●, absorbance at 280 nm; X—X, protein concentrations determined by the dye method [2]. Volume of each fraction was 3.5 ml.

TABLE I

PURIFICATION OF BAKER'S YEAST PYRROLINE-5-CARBOXYLATE REDUCTASE

Step	Volume (ml)	Total protein (mg)	Total activity (unit)	Specific activity (unit/mg protein)
Crude extract	4500	15000	1200	0.08
DE-52 cellulose	850	933	1400	1.5
$(NH_4)_2SO_4$	17	350	700	2.0
Sepharose 6B	30	87	650	7.5
DEAE-Sephadex A25	30	47	560	11.9
5′ AMP-Sepharose 4B	17	1.21	338	280

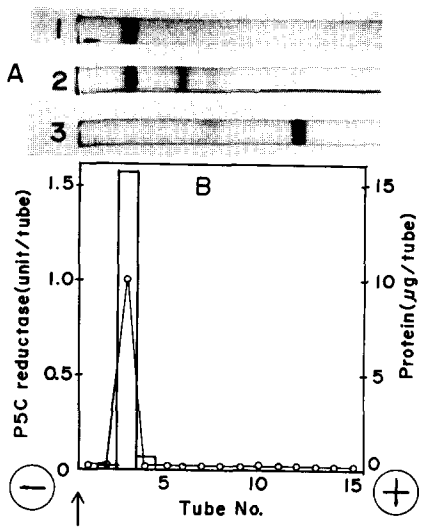

Fig. 2. Polyacrylamide gel electrophoresis of Δ^1-pyrroline-5-carboxylate reductase. In A, the final preparation of purified enzyme (gel 1), the preparation obtained with omitted step 5 (gel 2), and bovine serum albumin (gel 3) were subjected to polyacrylamide gel electrophoresis, and the gels were then stained for protein. In B, the gel (the same as gel 1) after electrophoresis was cut into 5 mm-width pieces and the extracts were made from each piece, and the enzyme activity (open bars) and protein concentration (open circles) were determined. Arrow indicates the origin.

with buffer 1, and re-utilized. This final preparation was very useful as a coupling enzyme for the enzymatic assays of ornithine and pyrroline-5-carboxylate in tissues [3], for the rate assay of ornithine aminotransferase activity [4], and for the fluorimetric microassay of blood ornithine, e.g., for the screening of hyperornithinemia [4].

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