Ornithine decarboxylase in *Thermus thermophilus*: An RNA-associated enzyme

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Summary. Ornithine decarboxylase (ODC) of *Thermus thermophilus* is associated with the nucleoid protein fraction. Analysis of this fraction by agarose gel electrophoresis and immunostaining revealed that ODC was bound to two groups of RNA-protein complexes. These two complexes of 1.5 and 0.6 kb in size disappeared from the gel by RNase A treatment or migrated to small molecular weight complexes by proteinase K treatment. Phenol extraction of either the nucleoid fraction or the eluted RNA-protein complexes from the agarose gel, shows that both contain the 0.56 kb RNA. Both RNA-protein complexes contain the ODC protein (55 kDa) but their protein composition differs in at least six proteins. Extraction of the nucleoid fraction with H₂SO₄, indicates that ODC was present in the acid-soluble fraction, showing that it is a non-histone protein tightly bound to 0.56 kb RNA. The purified ODC by various columns (~140-fold), is close to homogeneity and still carries the 0.56 kb RNA further explaining all the difficulties in the purification of this enzyme.

Keywords: Amino acids – Ornithine decarboxylase – *Thermus thermophilus* – RNA complex

Introduction

The existence of polyamines as well as some novel polyamines such as thermine and thermospermine in the archaeabacterium *Thermus thermophilus* has been reported (Oshima, 1983), but nothing is known about the biosynthetic pathway of these molecules. In the thermophilic organisms these novel polyamines thermine and thermospermine are in higher amounts than the normal polyamines. In the extremely thermophilic bacterium *Clostridium thermohydrodsulfuricum* only the enzyme which converts ornithine to putrescine, ornithine decarboxylase (EC 4.1.1.17, ODC), has been studied so far (Poso and Paulin, 1983; Paulin and Poso, 1983).
ODC has been purified to homogeneity from different sources, its gene has been cloned (Hayashi and Canellakis, 1989) and comparison studies either on the protein or the gene level have been reported (Hayashi and Canellakis, 1989). In some eucaryotic cells, such as normal rat liver (Murphy and Brosman, 1976; Bartholens, 1983), chick embryos (Snyder and Kreuz, 1970), and \textit{T. pyriformis} (Sklaviadis et al., 1985) ODC has been found in the nucleus. In germinated barley, corn, pea and bean seeds ODC is located both in the cytosol and in the nucleus tightly bound to chromatin (Panagiotidis et al., 1982; Foudouli and Kyriakidis, 1989).

In this paper we report that the ODC of \textit{T. thermophilus} is associated with the nucleoid fraction tightly bound to RNA. The size of RNA was determined and it was found that after many steps of purification of ODC, leading almost to homogeneity, ODC still carries the 0.56kb RNA.

**Materials and methods**

**Materials**

L-\((1-^{14} \text{C})\) Ornithine (sp. activity 50mCi/mmol was purchased from Moravek Biochemicals, Inc., California (Dr E. S. Canellakis’s gift). Bacto-tryptone and yeast extract were obtained from Difco (Detroit, MI). Agarose was purchased from Bio-Rad Laboratories (California, USA). ODC antibody was a generous gift from Dr C. Panagiotidis.

**Growth of cells**

\textit{Th. thermophilus}, strain HB8, was used in all experiments. Microorganisms were grown at 75°C in a medium containing 0.3\%(w/v) yeast extract, 0.5\%(w/v) bactotryptone, 0.2\%(w/v) NaCl, 0.1\%(w/v) D-glucose, 2\(\mu\)M FeCl\textsubscript{3}, 0.2 mM CaCl\textsubscript{2}, and 1 mM MgCl\textsubscript{2}. The pH was adjusted to 7.0 by concentrated KOH. Growth was monitored by measuring the absorbance at 600nm in a Perkin – Elmer spectrophotometer. The bacteria were harvested at the end of the logarithmic phase by centrifugation at 6,000 g for 10 min. Cells were washed twice with 0.9\%(w/v) NaCl. The final yield was about 5 g of wet cells per liter of culture medium.

**Disruption of cells**

Cells (10 g) were suspended in 4 ml of 10 mM Tris-HCl buffer (pH 8.1), 20% sucrose (w/v) and 100 mM NaCl. The suspension was incubated with lysozyme (10 mg/ml) at room temperature for 3 min. Then 5 ml of a solution containing 10% Brij-58, 0.4% deoxycholate, 10 mM EDTA was added and the mixture was kept at 0°C for 15 min. The suspension of lysed cells was centrifuged at 10,000g for 10 min as described (Yamazaki et al., 1984).

**Isolation of nucleoids and nucleoid proteins**

Nucleoids were isolated by sucrose density gradient (10%–50% w/v) described by Yamazaki et al. (1984).

Nucleoid proteins, which are the acid-soluble proteins, were extracted with 0.4 M H\textsubscript{2}SO\textsubscript{4}. The acid-soluble proteins were further precipitated by two volumes of cold ethanol, whereas the acid-insoluble proteins were suspended in distilled water, neutralised by NaOH and dialysed extensively against water.