Superinduction of cytosolic and chromatin-bound ornithine decarboxylase activities of germinating barley seeds by actinomycin D

Christos A. Panagiotidis, John G. Georgatsos and Dimitrios A. Kyriakidis

Laboratory of Biochemistry, Aristotelian University of Thessaloniki, School of Science, Thessaloniki, Greece

Received 8 July 1982

Ornithine decarboxylase  Actinomycin D  Gibberellic acid  Barley seed

1. INTRODUCTION

Putrescine in plant cells is formed either from L-arginine by L-arginine decarboxylase (EC 4.11.19, ADC) or directly from L-ornithine by L-ornithine decarboxylase (EC 4.11.17, ODC) [1–5]. The contribution of ODC to the formation of polyamines in plants was claimed to be insignificant, since ODC activity in most plant tissues was found to be much lower than that of ADC [6]. The only well-documented work on ODC activity in plant cells is that in [4] on rapidly proliferating plant cells.

Investigations to now on plant ODC were performed in the 10000 × g supernatant of plant tissue homogenates based on the assumption that ODC is a cytosolic enzyme.

Here, evidence is presented that in barley seeds germinated for > 90 h, ODC activity is located mainly in the nucleus, tightly bound to chromatin, although the cytosol also possesses considerable activity. Both activities are superinduced when seed germination takes place in the presence of gibberellic acid and actinomycin D.

2. MATERIALS AND METHODS

D,L-[1-14C]Ornithine was purchased from Amersham Searle; putrescine and spermidine were obtained from Aldrich (Milwaukee WI); all other chemicals were obtained from Sigma (St Louis MO).

2.1. Sterilization and germination of barley seeds

Seeds of Hordeum vulgare cv. Beca were used. Sterilization of the seeds was done as in [7]. Seeds were germinated in 9 cm Petri dishes, in the dark at 26°C on filter papers which were kept moist by underlying cotton soaked in distilled water. The barley seedlings were collected at various times following germination and triturated with mortar and pestle in the presence of glass beads, in 4 vol. buffer A (50mM Tris-HCl (pH 8.5), 0.3 mM EDTA, 50 µM pyridoxal phosphate and 5 mM dithiothreitol).

2.2. Preparation of chromatin and cytosolic fraction

Bonner’s method was employed for the isolation of chromatin and the separation of the cytosolic fraction [8]. Briefly, barley seeds germinated for 100 h were homogenized in a Waring Blender for 1 min at low speed and 2 min more at high speed in 2 vol isotonic grinding medium (0.25 M sucrose in buffer A). The homogenate was filtered through 4 layers of gauze and the filtrate was centrifuged at 4000 × g for 30 min. The pellet was used for the isolation of chromatin while the supernatant was centrifuged at 100 000 × g for 1 h and the 100 000 × g supernatant was used as a source of cytosolic ODC activity.

2.3. Isolation of nuclei

Nuclei of barley seeds germinated for 100 h were prepared according to [9] as described [10] except that buffer K was modified by adding 50 µM pyridoxal phosphate, 5 mM β-mercaptoethanol and gummi arabicum instead of acacia. The crude nuclei were further purified by passage through 2.3 M sucrose as in [11].
2.4. ODC assay

Enzyme assay was performed in 50 µl final vol. as in [12]. Optimal conditions for assaying ODC activity from extracts of germinating barley seeds were found to be those of buffer A. One unit of ODC activity is the amount of enzyme that releases 1 nmol \(^{14}\text{CO}_2\) in 1 h under the conditions of the experiment. Specific activity is defined either as units/mg protein or as units/seeds.

2.5. Protein determination

Protein was determined according to [13] as modified [14] using bovine serum albumin as a standard.

3. RESULTS

The activity of cytosolic and chromatin-bound ODC increases during germination of barley seeds. Fig.1 shows the time course of the increase of cytosolic ODC specific activity that reaches a plateau in 90 h and remains at this level for an additional 30 h. The time necessary to reach maximal activity is somewhat variable; however, the maximal ODC activity is relatively constant (0.2 units/mg protein). Gibberellic acid added to the filter paper at zero time at \(10^{-5}\) M, more than doubles cytosolic ODC activity, while actinomycin D (2 µg/ml) added at 90 h, superinduces cytosolic ODC by 4-fold. When actinomycin D is added at zero time the activity of ODC does not increase during the germination, while actinomycin D added at 90 h gradually inhibits ODC activity.

Fig.2 shows the time course of the increase of chromatin-bound ODC activity which at 120 h of germination reaches a value of 25–30 units/mg protein. The specific activity of chromatin-bound ODC is 150-times higher than that of the cytosolic enzyme. Chromatin-bound ODC activity is also induced by gibberellic acid and super-induced by actinomycin D. When actinomycin D is added at zero time the activity of ODC remains at basal level. If actinomycin D is added at 90 h, the ODC activity remains unchanged.

The relative increase in the 2 ODC activities expressed as units/g seeds, is shown in fig.3. Cytosolic ODC activity gradually increases from the onset of germination, whereas chromatin-bound ODC activity shows a lag period of 70 h before it rapidly increases. Fig.3 also reveals that at 120 h of germination cytosolic and chromatin-bound ODC activities account for 25% and 75% of total activity, respectively.

To ensure that ODC activity is truly bound to chromatin in vivo and not merely adsorbed onto the chromatin during its preparation, nuclei from seeds germinated for 100 h were prepared as in section 2. The specific activity of ODC assayed in chromatin prepared from nuclei was 25 units/mg protein, which is very close to the specific activity of chromatin of whole homogenates.

A variety of reagents such as 0.3–2.0 M NaCl, 0.25 M Tris-HCl and 0.35 M NaCl, 0.2 M EDTA, \(10^{-3}\) M putrescine, \(10^{-3}\) M spermidine and the
Actinomycin D (2 μg/ml) was added at 90 h at the control cultures (○—○) or the cultures grown in the presence of gibberellic acid (●—●). ODC activity was measured as in fig.1.

ODC of chromatin prepared from seeds germinated in the presence of both gibberellic acid and actinomycin D, could be extracted by ~80% when a suspension of chromatin in buffer A was frozen and thawed twice (table 1). Similar treatment of chromatin isolated from seeds grown in the presence of either gibberellic acid or actinomycin D solubilized only ~20% of ODC activity, while chromatin of control seeds yielded only 3% of their ODC activity.

Actinomycin D was added at 90 h and the germination was followed for a further 10 h. The growing conditions of barley seeds and the preparation of chromatin were performed as in section 2. After freezing and thawing of chromatin the mixture was centrifuged at 10,000 × g and the released ODC activity was measured by assaying 15 μl from the supernatant. 100% of ODC activity was defined as the activity of each chromatin suspension measured before the extractions.
4. DISCUSSION

Our results show clearly that ODC activity of germinating barley seeds is present in both cytosol and the nucleus where it is tightly bound to chromatin. Both activities are induced by gibberellic acid and superinduced by actinomycin D.

Superinduction of ODC activity by actinomycin D has been reported in rat liver and in a variety of cultured mammalian cells [15-19]. It is not yet possible to interpret the effect of actinomycin D. One plausible explanation is that actinomycin D increases the levels of ODC activator, which was found to be present in rat liver, *Escherichia coli* and barley seeds [20,21].

The physiological role of ODC in the nucleus of barley seeds remains to be elucidated. An acidic phosphoprotein has been identified in nucleoli of *Physarum polycephalum*, which is tightly bound to ribosomal DNA (rDNA) and has been shown to possess ODC activity [22]. The suggestion has been put forward that the protein kinase which catalyzes the phosphorylation of this protein is polyamine-dependent, with properties similar to those of ODC antizyme [23]. Dephosphorylation of the phosphoprotein abolishes its capacity to bind to a specific region of the DNA and stimulates transcription [24].

REFERENCES