STUDIES ON ORNITHINE DECARBOXYLASE ACTIVITY IN NORMAL AND T2-INFECTED *ESCHERICHIA COLI*

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1. Introduction

Although diamines and polyamines are widespread in biological material [1, 2] little is known about their physiological role. Numerous studies have established the metabolic relationship of the putrescine (1, 4-diaminobutane) and the polyamine spermidine. In various systems, putrescine serves as a precursor for spermidine, which is synthesized by the condensation of the diamine with *S*-adenosyl-L-methionine [1, 2]. The biosynthesis of putrescine, on the other hand, is catalyzed by ornithine decarboxylase (L-ornithine carboxy-lyase, EC 4.1.1.17) which converts ornithine to putrescine [2]. Coliphages of the T-even series contain considerable amounts of polyamines [3–5]. According to Ames and Dubin [5], 8×10^{11} *Escherichia coli* B cells, contain 11.7 μmoles putrescine and 1.6 μmole spermidine. These cells give rise to 5×10^{13} T4 phages which contain 12.5 μmoles putrescine and 3.75 μmoles spermidine. It thus appears that phage-infected bacteria should synthesize polyamines at a fast rate, to supply the polycations required for the phages and to make up for the polyamines (up to 50% of the putrescine) which leak from the cells soon after infection [6–8].

In the present paper we demonstrate the stimulation of ornithine decarboxylase activity after infecting *E. coli* with T2 phage. It will be shown that the activity is maximal 6 to 9 min post-infection.

2. Materials and methods

E. coli B was grown in glycerol-lactate medium [9] to a density of 5×10^8 cells/ml and infected with T2 phage at a multiplicity of 5.0. Growth was arrested at desired times, by adding chloramphenicol (at a final concentration of 30 μg/ml) and chilling the culture. Aliquots of infected or uninfected bacterial suspensions (50 to 100 ml) were centrifuged at 5,000 rpm for 10 min and sedimented bacteria suspended in 4.0 ml quantities of EDTA phosphate buffer (10 mM sodium phosphate buffer, pH 7.2 containing 0.1 mM EDTA, 1.2 mM pyridoxal phosphate and 0.12 mM mercaptoethanol). After sonication at 4° for 4 min, bacterial debris were removed by centrifugation (10,000 rpm for 20 min) and the protein content of the supernatant fluid determined by colorimetric method [10].

Unless otherwise mentioned, ornithine decarboxylase activity was determined by the method described by Russell and Snyder [11] as follows: bacterial extracts, (0.1 ml, 0.1 to 0.4 mg protein) were incubated with 0.4 ml 10 mM sodium phosphate buffer (pH 7.2), 0.05 ml pyridoxal phosphate (0.6 μmole) in a 10 ml Erlenmeyer flask equipped with a rubber stopper supporting a polyethylene center well (Kontex Galss Co., no. 88230) that contained 0.2 ml hyamine (Packard Instrument Company Inc). Experiment was started by injecting 0.1 μCi uniformly ^{14}C-labelled-L-ornithine (New England Nuclear Corp., 240 mCi/μmole) through the rubber stopper and shaking the flasks in a 37° water bath. The reaction was terminated by injecting 0.2 ml 1 N HCl into the reaction mixture. After shaking for an additional 30 min, to allow for complete absorption of evolved CO_2, the center well was removed placed in a vial containing 10 ml scintillation fluid [12] and assayed for radioactivity in a liquid scintillation spectrometer.

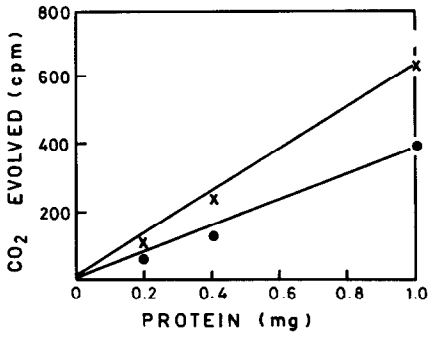

Fig. 1. Decarboxylation of ^{14}C-ornithine by bacterial extracts. Extracts from phage-infected bacteria, x——x; or from uninfected controls, •——•; were incubated with ^{14}C-ornithine (0.1 μCi) for 5 min and the radioactivity of the evolved CO_2 determined.

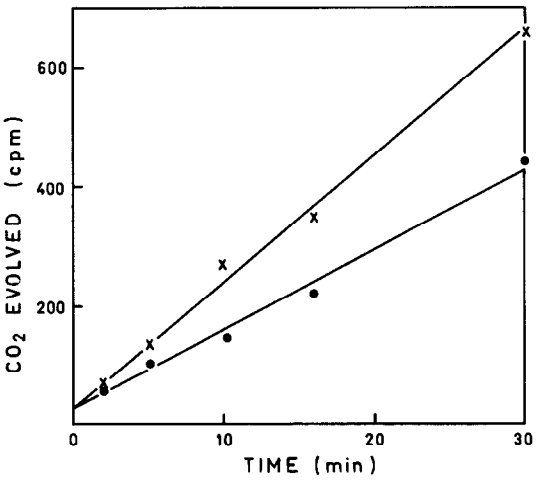

Fig. 2. Kinetics of ornithine decarboxylase. Extracts (0.4 mg of protein) from phage-infected bacteria, x——x; extracts prepared from uninfected controls, •——•; were incubated with ^{14}C-ornithine (0.1 μC) for different times and the radioactivity of the evolved CO_2 determined.

3. Results and discussion

Preliminary experiments indicated the presence of ornithine decarboxylase in extracts of infected and uninfected *E. coli*. It may be seen (fig. 1) that extracts prepared from T2-infected bacteria (6 min post infection) were more active in decarboxylation compared to extracts from uninfected controls. It may also be seen that evolution of $^{14}CO_2$ was proportional to the amount of protein in the extracts. The formation of ^{14}C-putrescine from ^{14}C-ornithine was confirmed by paper chromatography (methanol–pyridine–acetic–H_2O, 6:6:1:4) and by paper electrophoresis [13]. These semi-quantitative analyses also corroborated the above mentioned results that infecting *E. coli* with T2 phage stimulated ornithine decarboxylase activity.

A linear rate of $^{14}CO_2$ formation was also observed when extracts were incubated with ^{14}C-ornithine for different times (fig. 2). It may also be seen that the extracts from infected *E. coli* cells were more active than those obtained from uninfected bacteria (fig. 2). In these experiments chloramphenicol was added to the cells 6 min after the onset of infection. Similar results were obtained when phage maturation was arrested by rapid chilling of bacterial suspensions prior to their sonication. Bacterial extracts retained most of their ornithine decarboxylase activity after storage at 4° for 24 hr. Best results were obtained when sonication was performed in the presence of EDTA and mercaptoethanol. Even with crude extracts, the addition of EDTA, mercaptoethanol and pyridoxal phosphate was required to retain activity.

The following experiment was next designed to find out the time course of the stimulation of ornithine decarboxylase activity in infected cells. Fig. 3 shows that enzyme activity was maximal if extracts were prepared 6 min post infection. A similar stimulation was observed when extracts were incubated with ^{14}C-ornithine for 5 or 10 min (fig. 3).

Recent work has shown the stimulation of ornithine decarboxylase activity in extracts of regenerating liver extracts [11, 13, 14], after injection of epidermal growth hormone (EGF) into mice [15], or after administration of either growth hormone [16–18], or testosterone [19, 20] into rats. In all of these cases stimulation of ornithine decarboxylase activity could be correletated with the accumulation of ribonucleic acid (RNA). In phage-infected bacteria no significant stimulation of RNA synthesis has been detected as yet. On the other hand synthesis of phage DNA commences in the infected bacteria, 6 to 8 min post-infection. It is remarkable that this is the time when ornithine decarboxylase reaches highest activity and when putrescine leakage

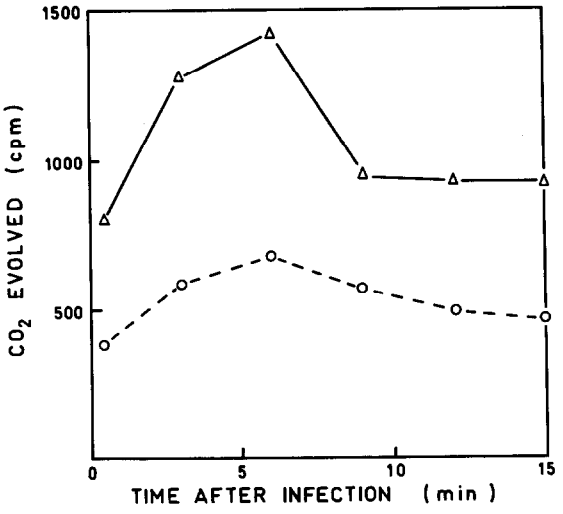

Fig. 3. Stimulation of ornithine decarboxylase activity after infection with T2 coliphage. *E. coli* was infected with T2 coliphages and sonicated at various times after infection. Extracts (0.4 mg of protein) were then assayed for ornithine decarboxylase activity by measuring the evolution of radioactive CO_2 after incubation with ^{14}C-ornithine for 5 min ○- - -○, or 10 min △——△.

is arrested [6, 8]. It is therefore tempting to speculate that the availability of polyamines, affects the course of viral DNA synthesis as it does for the synthesis of ribosomal RNA [21]. This hypothesis, which was first put forward by Cohen [22], may thus tie DNA synthesis to polyamine accumulation and would generalize the stimulatory action of polyamines for all classes of nucleic acids.

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