HISTIDINE-DEPENDENT ACTIVATION OF ARGININE DEIMINASE IN *CLOSTRIDIUM SPOROGENES*: KINETIC EVIDENCE ON IN VIVO ALLOSTERIC INTERACTIONS

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1. Introduction

Allosteric behaviour of regulatory enzymes resulting from cooperative interactions of the substrate and effector molecules has been studied using mostly cell free and highly purified preparations [1–4]. These mechanisms, to be operative at the cellular level, would require favourable conditions such as the permeability of the substrate and effector, availability of the binding sites on the enzyme and spatial arrangement of the enzyme to permit modulations. Paucity of information on the metabolic factors regulating the activity of arginine deiminase (EC 3.5.3.6.) in the cells of *Clostridium sporogenes* during germination and sporulation cycles, led us to investigate the modulations of the enzyme activity under in vivo conditions. This communication provides kinetic data indicating the allosteric nature of arginine deiminase in cells of *Cl. sporogenes* with histidine acting as a positive effector.

2. Materials and methods

2.1. *Materials*

L-arginine HCl was obtained from V. P. Chest Institute, Delhi, India; L-histidine HCl and DL-citrulline were supplied by Sigma Chemical Co (Mo, USA) and diacetyl monoxime was the product of E. Merck, Germany.

2.2. *Growth and harvesting of cells*

Cl. sporogenes (ATCC 19494) stock culture was stored in cooked meat medium (Difco). The organism was grown in a medium consisting of tryptone 3%, ammonium sulphate 1%, yeast extract 0.1%, and 0.1% sodium thioglycollate. Sixteen hour old vegetative cells were transferred to a sterile medium of the same composition at 20% level and incubated further for a period of 4 hr at 37°C. Exponentially growing cells were harvested, washed once with 0.05 M Tris–HCl buffer, pH 7.0 and the pellet was suspended in the same buffer. Cell suspensions adjusted to an optical density of 1.0 at 600 nm were used for enzyme studies.

2.3. *Enzyme assay*

Arginine deiminase activity was estimated according to the method of Oginsky [5]. The assay mixture consisted of 0.45 ml cell suspension preincubated at 37°C for 10 min and arginine HCl (final concentration, 1–100 mM) dissolved in 0.05 M Tris–HCl buffer pH 7.0 in a total vol of 1.0 ml. An incubation period of 1 hr at 37°C was used for enzyme assay. The reaction was terminated by the addition of 1.0 ml 10% trichloroacetic acid and the citrulline liberated was estimated [5]. Protein content of the cell suspension was calculated after digesting the cells and estimating nitrogen by Nesslerization [6].

For activation experiments, the cells were first incubated with histidine (0–40 mM, pH of aqueous solution adjusted to 7.0 by 2 N NaOH) for 10 min at 37°C and later incubated for 1 hr in presence of arginine (1–100 mM) to determine the enzyme activity. Appropriate blanks were simultaneously run to determine interference, if any, in citrulline estimation.

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3. Results and discussion

3.1. *Arginine deiminase activity in whole cells*

When sporulating cells from stationary phase or spores were used in the assay system, arginine deiminase activity could not be measured unless the cells were disrupted by sonication. Therefore, in all the experiments reported in this paper, only the cells which are in the exponential phase of growth were used. It was observed that the enzyme activity in the cells showed a linear function with respect to time of incubation, at different concentrations of arginine (1–100 mM) as well as in presence of histidine (0–40 mM). Typical results are shown in fig.1. The activation effect of histidine was not repressed by actinomycin D or chloramphenicol, thereby ruling out the possibility of de novo synthesis of the enzyme (unpublished data).

3.2. *Kinetics with respect to substrate and activator*

Data on the effect of varying concentrations of substrate in presence of 0–40 mM histidine are given in fig.2. The binding of arginine to the enzyme in the absence of histidine is highly co-operative as evidenced by the sigmoidal nature of the graph. It thus appears that the enzyme interacts some way with more than one molecule of the substrate. Activation of arginine deiminase by histidine at lower concentrations of arginine suggests that in the cells of *Cl. sporogenes* permeability of both substrate and effector molecules does not become rate limiting, but they are indeed available for interaction with the enzyme. Atkinson et al. [7] have suggested that co-operative interactions of the substrate may be explained on the basis of interacting sites in the enzyme molecule. Under conditions of our assay, the interactions with the enzyme may be brought about either by arginine per se or by any of its metabolites. Nevertheless, the allosteric behaviour of arginine deiminase is evident from the sigmoid kinetics (fig.2) which is a characteristic feature of the enzymes showing co-operative interactions [8,9]. The gradual reversal of sigmoidal nature of velocity versus substrate plot with increase in concentration of effector (fig.2) is typical of an activator for allosteric enzymes [1,7]. The activity curve of arginine deiminase shifts from sigmoid to normal hyperbolic relation with 40 mM of histidine. It may be assumed as suggested by Atkinson et al. for a positive effector [7], that histidine may reduce intersite interactions, either by causing the enzyme to dissociate into subunits or by somehow blocking the sites from each other. These phenomena have been explained in terms of the conformational changes in the quarternary structure of the enzyme molecule [1,2,10].

Analysis of the data given in fig.2, by Lineweaver-Burk plot and Hill plot (fig.3 a and b) point to the

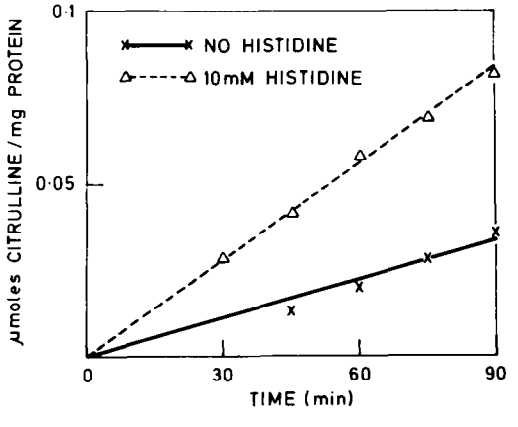

Fig.1. Activation of arginine deiminase by histidine in whole cells of *Cl. sporogenes* as a function of time. The details of enzyme assay are given in the text.

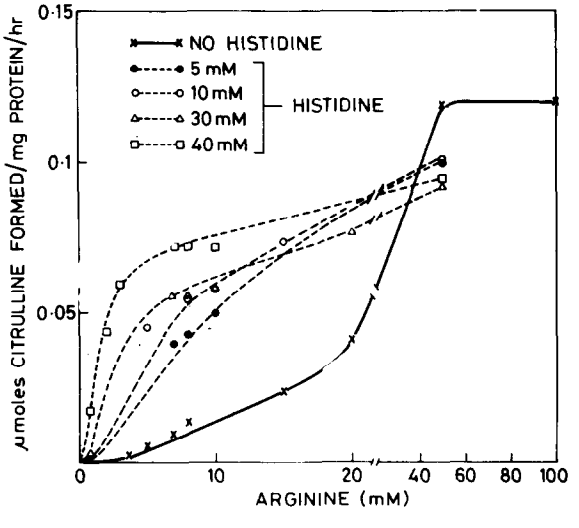

Fig.2. Activity of arginine deiminase in *Cl. sporogenes* as a function of arginine concentrations in presence of varying concentrations of histidine. The assay procedure was as described in Materials and methods.

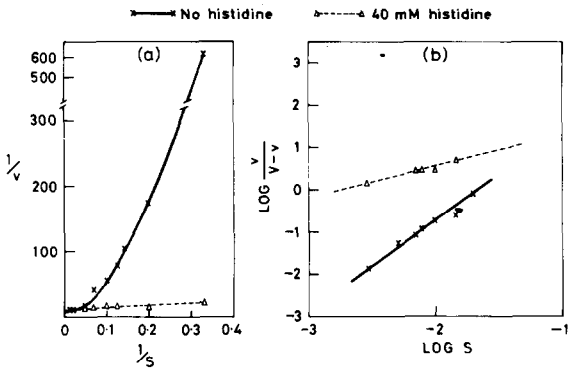

Fig.3. Kinetics of arginine deiminase activity in whole cells of *Cl. sporogenes*. (a) Lineweaver Burk plot (b) Hill plot. Data taken from fig.2.

allosteric nature of arginine deiminase. Thus, a non-linear plot for the enzyme in the absence of histidine (fig.3 a) and linear plots with increased V_{max} in the presence of histidine indicate the co-operative interactions of the enzyme with the substrate. The Hill interaction coefficient of the enzyme calculated from fig.3 b was 2 for the enzyme in the absence of histidine which was reduced to 0.8 in presence of 40 mM histidine. Hill interaction coefficient has been shown to be a function of, or the actual number of binding sites in the enzyme [9]. It would thus appear that arginine deiminase in *Cl. sporogenes* contains at least two binding sites for arginine.

Reviews of Atkinson [11] and Kirschner [2] have focussed attention on the in vivo regulation of allosteric enzymes when integrated into the complex network of metabolic circuitry. Recently, allosteric interactions under nearly cellular conditions have been reported using the reactants approximating cellular concentrations [12] and by increasing the permeability of cells [13,14]. The kinetic evidence presented in our studies is perhaps the first instance demonstrating allosteric interactions for arginine deiminase in the whole cells of *Cl. sporogenes*. We are now attempting to purify arginine deiminase to understand the structural alterations associated with histidine dependent activation of the enzyme.

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