THE RESPONSE OF SOY BEAN ARGININOSUCCINATE SYNTHETASE TO DIFFERENT ENERGY CHARGE VALUES

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1. Introduction

The production of argininosuccinic acid is an important step in the biosynthesis of arginine in all organisms. The reaction catalysed by argininosuccinate synthetase isolated from bovine liver and porcine kidney has been well studied with regard to possible reaction mechanisms [1]. No attempt appears to have been made however, to establish control mechanisms likely to operate *in vivo*, on the complex reaction catalysed by this enzyme.

The data prescribed in this communication show that argininosuccinate synthetase isolated from higher plant tissues appears to be subject to inhibition by ADP, AMP and arginine. Furthermore, the activity of the enzyme is regulated by the energy charge ratio of the nucleotide pool present in reaction mixtures. In this context, arginine performs the role of an indicator metabolite [2].

2. Methods

Soy bean (*Glycine max.* L.) cells were grown in the B5 medium described by Gamborg [3]. The cells were harvested after 96 hr, of growth at 28°C by filtration on a miracloth disc, over a buchner funnel. They were homogenized for 90 sec using an MSK homogenizer, and a 1:2:4 (w:v:w) ratio of cells: tricine buffer (0.1 M, pH 7.9): glass beads (1.05 mm), the homogenate was filtered through miracloth, and then spun at 14,600 *g* in a Sorvall centrifuge. The supernatant was treated with ammonium sulfate, to obtain a 30–70% saturation. This was dissolved in tricine buffer, and then passed through Sephadex G-50 into 0.01 M, pH 7.9 tricine buffer. The extract was now treated with calcium phosphate gel, using 0.4 g per 2 ml of extract. After sedimenting at 4,000 *g*, the gel from this step was washed in sequence with 0.1 M tricine buffer, 0.005 M phosphate buffer, and 0.1 M phosphate buffer, all pH 7.9. The final wash obtained argininosuccinate synthetase activity that was free of either argininosuccinate lyase or ATPase activities.

The system used for the assay of argininosuccinate synthetase utilized [^{14}C]carbamyl citrulline and has been described previously [4]. Lines were fitted to all kinetic data using the least squares method. Estimation of the arginine content of the soluble fraction of the cells at various stages of growth, was carried out on picric acid treated extracts [6], using an automatic amino acid analyser.

The role of the energy charge value in the control of enzyme activity was investigated by preparing mixtures of ATP and AMP giving energy charge ratios from 0 to 1, and then adding adenylate kinase, as described by Atkinson [5].

3. Results

Both AMP and ADP were found to inhibit the activity of the enzyme. This inhibition is competitive with respect to ATP (fig. 1). Arginine is also a competitive inhibitor of the enzyme with respect to citrulline (fig. 2).

From fig. 3, it is evident that the enzyme responds to the energy charge value of the adenine nucleotides in the reaction mixtures. It is also evident that the

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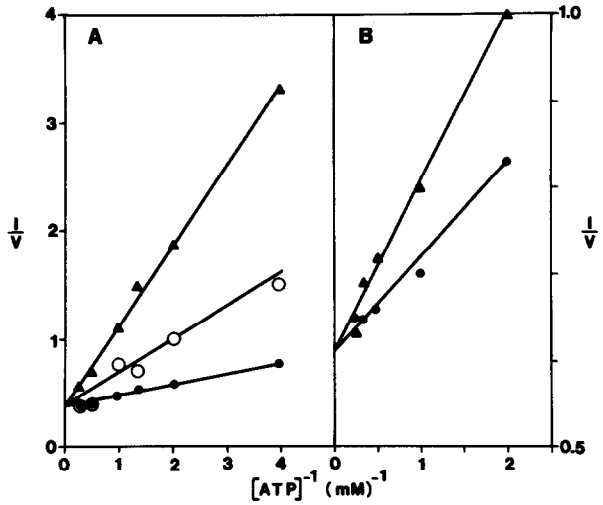

Fig. 1. The effect of AMP and ADP on the activity of argininosuccinate synthetase. v is expressed in μmol/min $\times 10^2$. A) (▲—▲—▲) 2.0 mM AMP; (○—○—○) 0.5 mM AMP; (●—●—●) no AMP. B) (▲—▲—▲) 2.0 mM ADP; (●—●—●) no ADP.

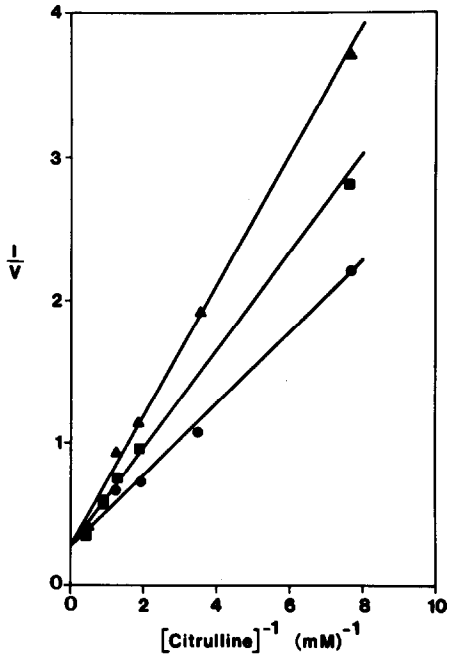

Fig. 2. The effect of arginine on the activity of argininosuccinate synthetase. v is expressed in μmol/min $\times 10^2$. (▲—▲—▲) 1.0 mM arginine; (■—■—■) 0.5 mM arginine; (●—●—●) no arginine.

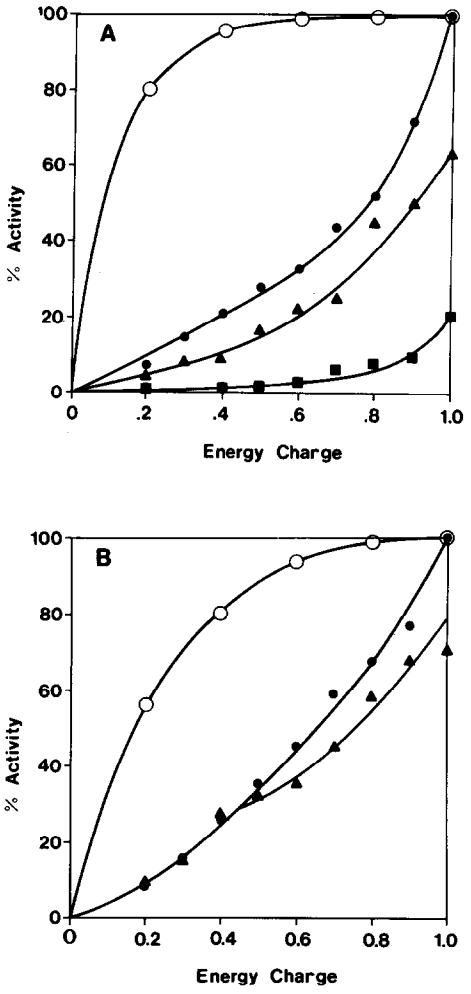

Fig. 3. The response of argininosuccinate synthetase to energy charge. The ordinate legend refers to % activity, using the 1.0 energy charge activity as 100%. A) Using a total nucleotide concentration of 4.0 mM. (○—○—○) ATP alone (equivalent to ATP in energy charge experiments); (●—●—●) energy charge; (▲—▲—▲) energy charge + 0.1 mM arginine; (■—■—■) energy charge + 2 mM arginine. B) Using a total nucleotide concentration of 1.0 mM. (○—○—○) ATP alone (equivalent to ATP in energy charge experiments); (●—●—●) energy charge; (▲—▲—▲) energy charge + 0.1 mM arginine.

activity of the enzyme at various energy charge values is affected by the presence of arginine. This response is seen to occur at both high (fig. 3A) and low (fig. 3B) levels of the adenine nucleotide pool. It is plain from fig. 3B however, that the response to arginine vanishes

Table 1
The arginine levels found in the soluble fraction of soy bean cells at various growth stages.

Growth stage (hr)	Arginine (μmoles/g fresh wt.)
0	0.1110
24	0.0538
48	0.1804
72	0.0868
96	0.1100

when the level of the nucleotide pool is approaching zero.

At this point it is important to note that the amount of enzyme used in each experiment shown in fig. 3 corresponds to that recovered from 1 g (wet wt) of cells, the yield of enzyme from the gel treatment being between 50 and 60% of that present in the crude extract. In addition, the 0.1 M arginine concentration used in fig. 3 is equivalent to the average concentration of arginine found per g (wet wt) of cells, over the growth period of 96 hr (table 1).

4. Discussion

Since the activity of argininosuccinate synthetase is increased at high energy charge values, and decreased at low energy charge values, we are able to classify it as a U type enzyme [2]. Arginine appears to play the role of an indicator metabolite, increasing or decreasing the energy charge effect, as its level rises or falls.

It would seem likely that this type of control mechanism is also applicable to the argininosuccinate synthetase from animal cells, which is known to be competitively inhibited by AMP, and arginine, although the effect of ADP does not seem to have been studied [1].

The findings presented in this communication have particular importance for the consideration of arginine biosynthesis in plants, where so far, the only control mechanism discovered is the stimulation of the plant carbamyl phosphate synthetase by ornithine [7, 8].

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