THE CONTROL OF ORNITHINETRANSCARBAMYLASE ACTIVITY BY ARGINASE IN SACCHAROMYCES CEREVISIAE

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1. Introduction

The synthesis of ornithinetranscarbamylase (OTC) in *Saccharomyces cerevisiae* is repressed by arginine and its activity is regulated by a mechanism which involves a regulatory binding protein induced by arginine [1]. The regulatory mechanism was reconstituted *in vitro* with partially purified OTC and regulatory protein. This enabled us to show that ornithine in addition to being a substrate is also an effector of this system and that arginine which is an inducer of the regulatory protein becomes an inhibitor of OTC when the regulatory protein is present [2]. In the present paper, we wish to report conclusive experiments which show that the regulatory protein is an arginase which, when its substrate, arginine, and the product of its action, ornithine, are simultaneously present, binds OTC and inhibits its activity. This mechanism prevents the formation of a wasteful urea cycle.

2. Material and methods

Culture and purification methods and enzymatic assay are the classical ones [1,3,4]. The OTC activity (μmoles citrulline formed per hour) is measured at 15° for 5 min. Urea which interferes with citrulline determination is eliminated by urease (1 mg of Sigma type III, in 1 ml of incubation mixture). Arginase-less mutants are described elsewhere [6]. Arginase activity is given in μmoles of urea formed in 1 hr at 30°. An arbitrary value of 100 units is given to the amount of regulatory activity found in 1 mg of total protein from wild-type strain 1705d grown on arginine as only nitrogen source. This preparation is used as a standard and inhibition of OTC is measured routinely in the presence of 10^{-3} M arginine and an excess of ornithine (10^{-2} M). The inhibition is a saturation-type function of the amount of regulatory protein and accurate determinations are done at less than 50% inhibition [3].

3. Results

a. The regulation of OTC by a limited amount of regulatory protein and the actions of effectors are summarized in fig. 1. OTC is an enzyme which is inhibited by an excess of ornithine. Arginine does not affect its activity. Addition of regulatory protein increases the inhibition by excess of substrate and this inhibition is strongly reinforced by arginine.

b. A striking property of this regulatory process is that regulatory mutants (argR) simultaneously have lost most of the repressibility by arginine and the capacity to produce the regulatory protein [1] suggesting a link between this regulation and the anabolism. However, the role that ornithine appears to play in this regulation, focused attention on this intermediate which is known to be common to the anabolism and catabolism of arginine in a number of microbes, including yeast [7,4]. Moreover, an unexpected connection between the two metabolisms was disclosed by the discovery that the argR mutants were unable to grow on arginine or ornithine as nitro-

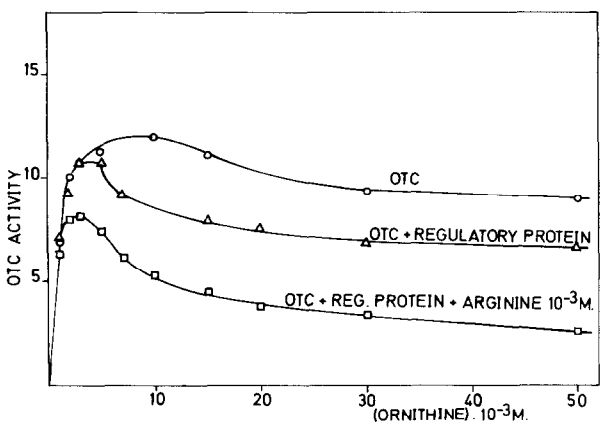

Fig. 1. *Inhibition of OTC by the regulatory protein as a function of ornithine and arginine concentrations.* The regulatory protein is a preparation from the mutant AG_1 deprived of arginase activity grown on glutamate + arginine (1 mg ml^{-1} each): 62 units (0.6 mg per protein) per ml of a dialized precipitate at 50% $(NH_4)_2SO_4$ saturation. Preparation from wild-type strains give similar results except that a small correction for ornithine concentration ($< 0.5 \times 10^{-3}$ M) has to be made due to the formation of ornithine by arginase.

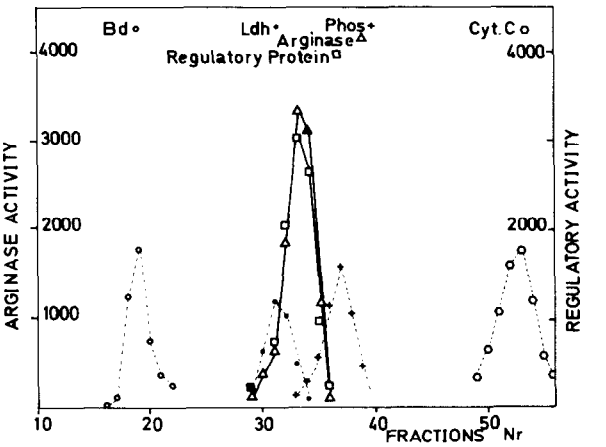

Fig. 2a. *Arginase and the regulatory protein have the same molecular weight.* Arginase and regulatory activities in fractions from an elution on Sephadex G200 superfine (Pharmacia) [5] of 28,000 units of regulatory protein (purified 150 fold) by Tris 0.05 M pH 8, KCl 0.2 M, arginine 10^{-3} M. Bd (–○–): blue dextran (Pharmacia). Phos (–+–): *E. coli* alcaline phosphatase (Worthington Biochem. Corp.) M.W. 75,000. Ldh (–●–): rabbit muscle lactic dehydrogenase (Boehringer) M.W. 135,000. Cyt-C (–◯–): cytochrome C (Boehringer) M.W. 12,400.

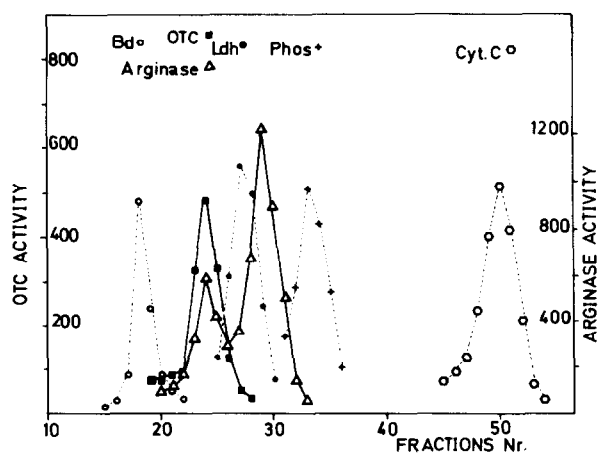

Fig. 2b. *Binding of OTC and arginase.* A mixture of arginase (7000 units, purified 150 fold) and OTC (4800 units) eluted as in fig. 2a except: addition of ornithine 1.5×10^{-2} M.

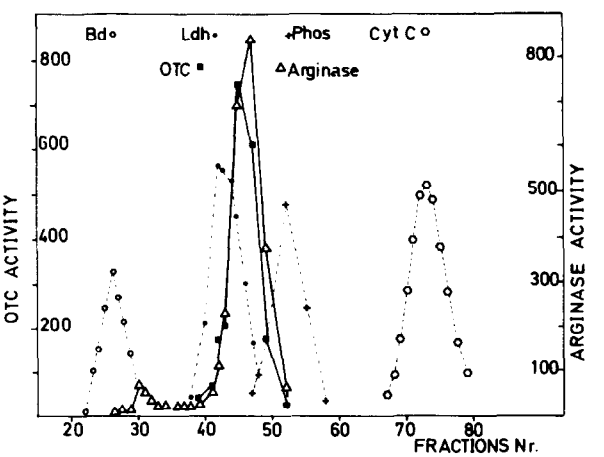

Fig. 2c. *Arginase and OTC do not bind in the absence of effectors.* Same conditions as for fig. 2b except: no ornithine and no arginine.

gen source because of a pleiotropic non inducibility of arginase and δ-L-ornithine transaminase [6]. As appears in fig. 2a, the molecular weights of the materials bearing the regulatory- and the arginase activities are similar (114,000 ± 7,000). The molecular weight of OTC is slightly higher (fig. 2c). It was also observed that the purification of the arginase- and of the regulatory activities are strikingly parallel, one step among others, being a thermodenaturation at 63° for 20 min in the presence of 0.1 M L-arginine [3].

c. A mutant AG_1 deficient in arginase activity [6] (less than 1% of normal activity) retains a full regulatory activity (fig. 1), while two other mutants, AG_2 and AG_3, have lost both catalytic and regulatory activities.

Together these results are much in favour of the identity of arginase and the regulatory protein; the AG_1 strain produces a regulatory protein which has lost its arginase activity.

The experiments reported in figs. 2a–c give a direct demonstration of the binding of arginase with OTC. When an excess of regulatory protein from a wild-type strain is sifted in the presence of OTC, ornithine and arginine, all OTC and a part of arginase activity move together with an apparent molecular weight of 180,000 ± 20,000. The unbound arginase remains at a M.W. of 114,000 (fig. 2b). In the absence of effectors, OTC and arginase move to their usual elution volume (fig. 2c). These data confirm the binding and the stoichiometric character of this interaction [1]. They also exclude the possibility that in the AG_2 and AG_3 strains, a mutation in the gene coding for arginase would, through a polar effect, affect the production of a distinct regulatory protein coded by an adjacent gene.

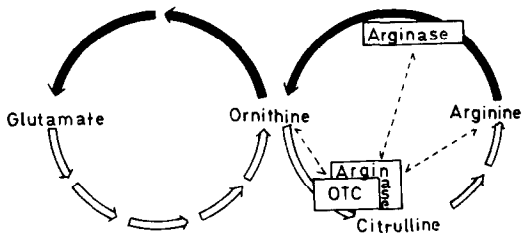

Fig. 3. Simplified scheme of arginine biosynthesis (⇒⇒), catabolism (➡➡) and action of effectors (⇠ – – ⇢) which contribute to OTC inhibition.

The comparison of OTC and arginase of other microbes including many yeasts [2,3], shows that this regulation is very general in the genus *Saccharomyces* and not common in the other ones. Cross reactions show that the specificity is brought by both arginase [3] and OTC [2]. The property of OTC to be inhibited by excess of ornithine seems to be required for this regulation.

d. The physiological result of this regulation is the prevention of the occurrence of a wasteful urea cycle (4 ATP) which could operate when arginine or ornithine (which is also an inducer of arginase [4,6] are catabolized (fig. 3). It should be emphasized that this control is active only when the catabolic pathway is at work and combines the economy of protein synthesis by a genotropic control with the rapidity of enzymotropic control.

The term "epiprotein" has been used to designate the regulatory protein [2]. It is useful to distinguish a regulatory arginase forming an allosteric system by reversible binding with OTC [8] from usual arginase by the name "epiarginase".

Acknowledgements

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