SUBCELLULAR MISLOCATION OF CYSTEINE SYNTHASE IN A CYSTEINE AUXOTROPH OF *ASPERGILLUS NIDULANS*

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1. Introduction

The factors governing enzyme locational specificity within the cell are not well understood. The study of mutants with defective enzyme compartmentation may prove valuable as an approach to the problem. In *Escherichia coli* such mutants were found for two systems. Some strains, unable to grow on non-fermentable carbon sources, display the release of their ATPase from the membrane complex [1,2]. Certain chlorate-resistant mutants are unable to form a proper membrane aggregate of nitrate reductase [3,4]. In *Neurospora crassa*, some mutants, unable to use malate as sole carbon source, have been reported to show redistribution of malate dehydrogenase within the cell [5]. The developed system of organellar membranes should make enzyme mislocation in fungi a not uncommon mutational event.

In the cysteine biosynthetic pathway of *Aspergillus nidulans* an auxotrophic mutant *cysE-1* was selected, which appeared to have no cysteine synthase (*O*-acetyl-L-serine acetate-lyase, adding hydrogen-sulphide, EC 4.2.99.8) activity in vivo. It was, however, demonstrable in vitro [6]. The findings, presented in this communication, indicate that at least one-third of cysteine synthase is associated with particulates in *Aspergillus nidulans*. This association of cysteine synthase is lost in *cysE-1* mutant.

2. Materials and methods

2.1. *Strains, media and chemicals*

The strains, *biA-1 mecA-1* and *biA-1 mecA-1 cysE-1* were isolated as described previously [6,7]. The mycelial cultures were initiated from heavy conidial suspensions in flasks with 800 ml of minimal medium [8], containing (per litre) 2.5 g sodium nitrate and 5 mg Tween 80, and supplemented with 35 μg biotin and 100 mg L-cysteine. The cultures were grown for 14–16 h in an orbital incubator at 30°C and harvested when the fresh weight reached 4–5 g. Cytochrome *c* was obtained from Biomed, Kraków, Poland, sorbitol and Triton X-100 from Koch-Light, Colnbrook, Bucks, England. The reagents used in cysteine synthase assay were as in [7]. Snail digestive juice was prepared after Jarige and Henry [9] and lyophilized.

2.2. *Preparation of cell lysate*

Modified *Neurospora crassa* procedure [10] was used. The mycelium, collected on nylon fabric, was washed with water and twice in softening buffer (0.1 M citrate – K_2HPO_4 at pH 5.5, 1 mM EDTA, 0.6 M sorbitol and 0.14 M 2-mercaptoethanol). It was then suspended in the same buffer with 500 mg of snail juice preparation in a total vol of 100 ml. The digestion was carried for 45 min at 37°C with slow agitation. The material was centrifuged at 500 *g* for 5 min and the pellet washed twice with fractionation buffer (10 mM potassium phosphate pH 7.5, 1 mM EDTA and 1 M sorbitol) by gentle resuspension and centrifugation. The cells were suspended in 30 ml of fractionation buffer and lysed by six strokes at 1500 rev/min in a glass–Teflon homogeniser. Unbroken cells and cell debris were removed by centrifugation at 600 *g* for 10 min and the supernatant (=cell lysate) taken for examination.

2.3. *Gradient centrifugation*

The particulate fractions were obtained by centri-

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fugation of cell lysate at 15 000 *g* for 30 min. The pellet was brought to 0.4 ml with 30% (w/w) sorbitol and suspended gently with a Pasteur pipette before layering on the gradient. Linear 30 to 60% (w/w) sorbitol gradients (12 ml) were prepared in 10 mM potassium phosphate pH 7.5, 1 mM EDTA. Centrifugation was done in a Beckman-Spinco SW 41 Ti rotor at 28 500 rev/min (g_{av} = 98 500) at 4°C for 60 min. Gradients were withdrawn with capillary and divided into approximately 20 fractions of 25 drops each. For density measurment a linear gradient of carbon tetrachloride and petroleum ether was prepared in a graduated cylinder and calibrated with a series of sucrose solutions. The drops of fractions were allowed to reach their equilibrium position and their densities were read from the graph. Each fraction was mixed with a drop of 10% Triton X-100 and after 30 min samples were taken directly for enzyme assays. Cysteine synthase and cytochrome oxidase were assayed as described in [7] and [11], respectively.

3. Results and discussion

Centrifugal fractionation at 15 000 *g* of cell lysate from *biA-1 mecA-1* strain has shown that 30.3 ± 6.6

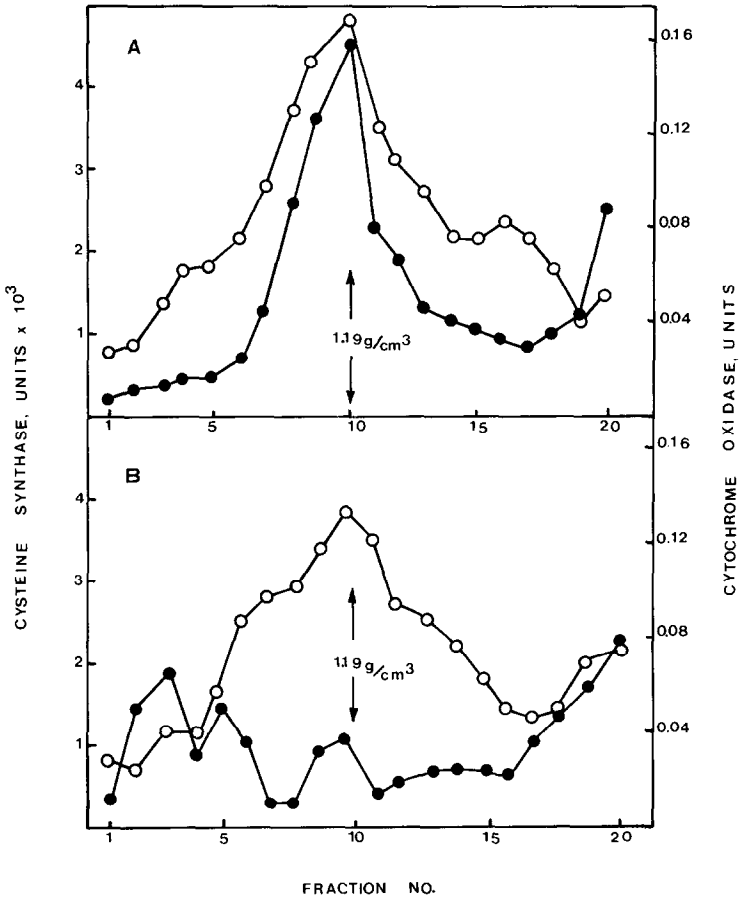

Fig.1. Sorbitol density gradient profiles of cytochrome oxidase and cysteine synthase. The particulate fractions of *biA-1 mecA-1* strain (A) and *biA-1 mecA-1 cysE-1* strain (B) were prepared and centrifuged, and enzyme activities were measured in the gradient, as described in Materials and methods. (○) Cytochrome oxidase (●) Cysteine synthase. One enzyme unit corresponds to 1 μmol of product formed per min per ml. Recovery of the activities (activities layered on the gradient = 100%) was 70% for cysteine synthase and 100% for cytochrome oxidase.

percent of cysteine synthase activity is sedimentable and consequently the particulate protein was about 3-fold enriched in the enzyme. Cytochrome oxidase, a mitochondrial marker enzyme, was sedimentable to a similar extent (38.1 ± 1.9 percent) in the same experiments. These results would even be compatible with cysteine synthase being wholly particulate, if we assume that non-sedimentable activity was released from particulate fractions during cell lysis.

The sedimentation of particulate fractions through a density gradient produces a peak of cysteine synthase which bands at the same equilibrium position as the peak of cytochrome oxidase (fig.1A). The fractions with highest activity of cysteine synthase have density of 1.19 g per cm^3, typical of fungal mitochondria. This evidence, although not complete, favours the mitochondrial location of cysteine synthase.

In the strain *biA-1 mecA-1 cysE-1* the particulate fraction is poorer in cysteine synthase activity than is the cell lysate and the distribution of the enzyme between supernatant and pellet is different from that of cytochrome oxidase. Only 4.4 ± 1.8 percent of cysteine synthase was sedimentable in the experiments where 40.3 ± 4.0 percent of cytochrome oxidase was found in pellet. The density gradient obtained with this strain does not exhibit a typical distribution of cysteine synthase (fig.1B). The main peak of enzyme coincident with the peak of cytochrome oxidase is missing, and the residual activity present on the gradient remains mostly on the top.

The change in density gradient profile of cysteine synthase introduced by the *cysE-1* mutation points to changed subcellular location of the enzyme. The nature of this change is not yet clear. Previous work on *Aspergillus nidulans* failed to show the presence of more than one cysteine synthase species. The activity remained as one component during gel filtration and ion-exchange chromatography [6,7]. This does not entirely exclude, however, the possibility of cysteine synthase being in the form of heterotopic isozymes. Therefore it cannot be decided yet whether the *cysE-1* mutation results in selective loss or change of location of organellar enzyme form, or affects the location specificity properties of the entire population of cysteine synthase molecules. Further study of *cysE*-type mutants and mutants deficient in cysteine synthase activity is expected to clarify this problem. It may also make it possible to determine if *cysE*-type mutations lie in the structural gene(s) of the enzyme.

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