MUTANTS OF ESCHERICHIA COLI HAVING TEMPERATURE SENSITIVE REGULATORY MECHANISM IN THE FORMATION OF ARGININE BIOSYNTHETIC ENZYMES*

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Since the first discovery by Horiuchi et al.(1961), there have been several reports on the thermo-labile regulatory system of bacterial enzyme synthesis. The reports are limited so far to the catabolic enzymes such as β-galactosidase and alkaline phosphatase. Because of the importance of temperature sensitive system in elucidating the mechanism of regulation of enzyme synthesis, we looked for such a system concerning biosynthetic enzymes.

We found a mutant which has characteristics to form thermo-labile repressor, and another mutant whose permeability for corepressor appeared to be temperature dependent.

EXPERIMENTAL

Mutants were derived from E. coli K12. All cultures except those used for mating were grown aerobically in minimal salts-glucose media with appropriate supplement. The arginine rich medium contained 200 μg/ml of L-arginine. Mating was carried out in Penassay broth (Difco) with gentle shaking.

Ornithine transcarbamylase (OTC) activity was measured on toluenized cells according to the method of Jones et al.(1955) and

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argininosuccinase was according to the method of Ratner (1955). One unit of activity is the amount of enzyme which produce 1 μmole of reaction product in 1 hour at 37°. Specific activities are expressed in units/mg protein of toluenized cells.

RESULTS AND DISCUSSION

Isolation of mutants: It was attempted to isolate temperature sensitive regulatory mutants among canavanine resistant mutants by taking advantage of the fact that mutants resistant to canavanine were often non-repressible strains. Ultraviolet light irradiated cells were spread on the AF agar medium (an enriched but arginine free medium) of Novick and Maas (1961) which contained 200 μg/ml of canavanine. The plates were incubated at 25° for 2 to 3 days and then at 42° for 2 days. The incubation at 42° first and at 25° second was also employed. Among the colonies appeared, those which exhibited fast growth at 42° and slow growth at 25° and *vice versa* were selected. The mutants were purified by plating on the canavanine medium. Thus, two mutants, KR18 and KR22, which had entirely different levels of OTC activity in the presence of arginine at 25° and 42° were obtained.

Temperature effect on enzyme formation: Fig. 1 shows enzyme levels of the mutants at various temperatures as well as those of the parent strain and a non-repressible strain, KR11. The figure indicates that KR18 has fully derepressed level of OTC and argininosuccinase when grown at 42° in contrast to the nearly repressed level at 20°. On the other hand, OTC activity of KR22 increases 30 times as high as that of the parent at 20° and decreases gradually to the repressed level at 40°. In both mutants, the levels of two enzymes vary in a parallel fashion with the change of the incubation temperature.

Analysis of temperature sensitive locus: Since it is known

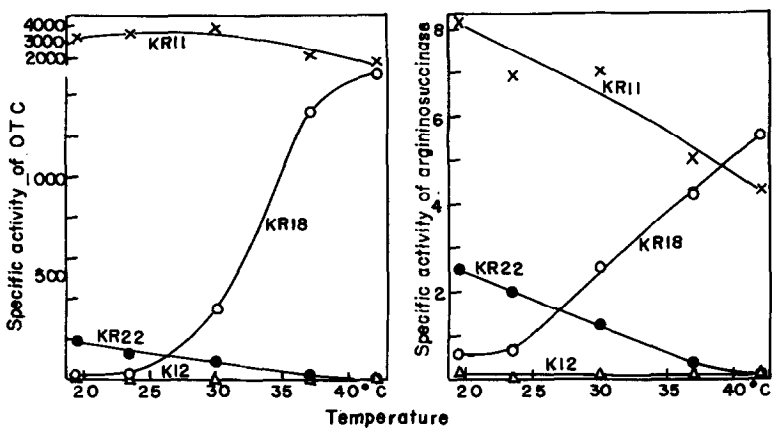

Fig. 1 Enzyme levels at various cultural temperatures. The bacteria were cultivated in the arginine rich medium. The cells at the logarithmic phase of growth were collected by centrifugation, resuspended in small volume of 0.01M tris buffer, pH 8, and toluenized at 37° for 10 minutes. Enzyme activities were determined as described in the text. Upper part of the scale in OTC activity applies only for KR11.

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that the gene locus of OTC is distinct from the locus of argininosuccinase on the chromosome of *E. coli* K12, parallelism observed in the activities of two enzymes with varied temperatures cannot be attributed to mutation in the structural gene of each of the enzymes, but may be due to mutation in the regulator gene of arginine pathway, Rarg, or mutation which causes modification of the regulatory system. Preliminary genetic analysis was carried out to see whether the mutated locus was identical with the known Rarg locus.

Hfr AB312 of Adelberg (origin-$Rarg^+$-Sm^r-Thr^--Leu^-) and Hfr #15 of Kada (origin-Thr^--Sm^r-$Rarg^+$) were crossed with KR18 $F^-$and F^+, KR22 F^+, and KR11 F^+. All of the recipients were prototrophic and streptomycin (Sm) sensitive. From each cross, 50 prototrophic and Sm resistant recombinants were selected and tested for their enzyme levels after cultivation in the arginine rich medium at 20° and 42°. The temperature sensitive regulatory character of KR18 and non-

repressible character of KR11 both were found to be closely linked with the Sm locus (about 90% linkage), while that of KR22 was not (about 30% linkage). Thus, it may be concluded that KR18 is a mutant at the Rarg locus and KR22 is a mutant at a different locus.

Some characters of mutants: Fig. 2 illustrates an experiment with KR18 in which the enzyme is rapidly formed with a little growth after heat treatment of the cells. There is no effect of heat on the parent strain. In addition, arginine when present during heating has an action to decrease the formation of enzyme as shown in curve 2 of Fig. 2. The effectiveness of arginine to prevent the derepression by heat varied considerably depending on the experimental conditions. However, similar effect has not been observed so far by the addition of canavanine or ornithine.

It is tempting to speculate from these findings that the cytoplasmic repressor elaborated by Rarg of KR18 is thermo-labile one similar to the repressor of the strain E103 in the case of β-galactosidase (Horiuchi et al., 1961), and the repression system of KR18 is different from that of mutants in which repression was released at higher temperature only with concomitant growth (Gallant, 1962; Novick et al., 1963). Furthermore, the effect of arginine is of special interest in regard to the possible study of the interaction between aporepressor and corepressor.

As regard to KR22, it was suspected that the disturbance in the permeation of arginine added externally might be the cause of the derepression observed at lower cultivation temperature, since the enzyme level of KR22 grown in the presence of arginine is about the same as the level of the parent grown in the absence of arginine. It was in fact found that arginine is almost impermeable to the cells of KR22 at various temperatures. The varied extent of derepression with temperature could be the reflection of the degree of impermea-

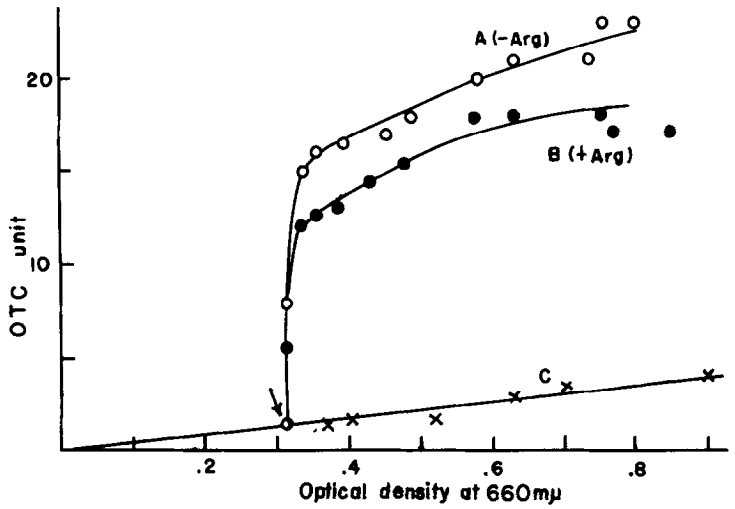

Fig. 2 Thermal derepression of OTC synthesis. The strain KR18 was grown in the arginine rich medium at 24°. At the time indicated by arrow the culture was centrifuged, washed once with the minimal salts medium without added glucose and arginine, and resuspended in the one twentieth volume of the same medium. The suspension was divided into three portions. One portion was transferred to 44° bath and heated for 30 minutes, then was diluted with the arginine rich medium to the original volume and cultivated at 24° (curve A). Another portion was treated as above except that L-arginine (200 μg/ml in final concentration) was added during the heating procedure (curve B). Another portion received no heat treatment (curve C).

bility of arginine to the cells; that is, arginine is slightly permeable at elevated temperatures.

Further study of these mutants is in progress.

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