Selection of *AraB* and *AraC* Mutants of *Escherichia coli* B/r by Resistance to Ribitol

LEONARD KATZ

Department of Biological Sciences, University of California, Santa Barbara, California 94106

Received for publication 22 January 1970

The growth of strain *araC*67, which produces the enzymes of the *ara* operon constitutively, is inhibited by the addition of ribitol. Isolation of strains resistant to ribitol yields mutants of either the *araB* or *araC* genes. A model to account for the inhibition by ribitol is discussed.

During the course of a study in which compounds related to L-arabinose were examined for their possible effect upon the expression of the *ara* operon in *Escherichia coli* B/r (see Fig. 1), it was noted that ribitol produced a marked effect upon the growth of an *ara* constitutive mutant, *araC*67. Addition of ribitol to a growing culture of strain *araC*67 resulted in a drastic reduction in the growth rate, from a doubling time of 45 min in the absence of ribitol to a doubling time of 186 min in its presence (Fig. 2A). The growth rate of the wild-type strain, however, was unaffected by the presence of ribitol.

When the strain *araC*67 is plated on 2,3,5-triphenyltetrazolium chloride (TTC)-ribitol tryptone medium, light-pink colonies, less than 1 mm in diameter, are observed after 24 hr. After an additional 24 hr, however, large red colonies begin to arise. A ribitol-resistant mutant, *RR500*, was isolated and purified, and its growth in casein hydrolysate medium, with and without ribitol, was examined. Its growth rate was only slightly affected by the presence of ribitol (Fig. 2B).

One-hundred independent spontaneous ribitol-resistant mutants were isolated from strain *araC*67 on TTC-ribitol tryptone plates. These were purified once on homologous media and screened for the presence of *ara* mutations in a complementation test on mineral arabinose plates employing various homogenotes which possessed *araA*, *araB*, *araC*, or *araD* mutations (5). The presence of the *araC*67 allele in each of the resistant strains was also examined in a complementation test with the homogenote F*araCS/araC5* on mineral arabinose d-fucose plates. [The presence of the dominant *C*67 allele results in fucose resistance (2).] It was found that 59 of the 100 resistant mutants screened contained mutations in the *araB* gene by failing to complement only the homogenotes which carried *araB* muta-

---

1 Present address: Department of Biology, Revelle College, University of California, San Diego, La Jolla, Calif. 92037.
The basis for the resistance to ribitol has not yet been determined.

The ribitol inhibition of the araC\(^{-}\) strain closely resembles the inhibition by arabinose of araD gene mutants described by Englesberg et al. (1). They demonstrated that a deficient L-ribulose-5-phosphate-4-epimerase results in the accumulation of the phosphorylated intermediate, L-ribose-5-phosphate, which is inhibitory to growth. Mutation to resistance involves a mutation which results in the prevention of the accumulation of the phosphorylated compound; either araD\(^{-}\)A\(^{-}\), araD\(^{-}\)B\(^{-}\), or araD\(^{-}\)C\(^{-}\).

In attempting to explain the ribitol effect, we note the fact that ribitol is a substrate of the L-ribulokinase (N. Lee, personal communication). It is proposed that ribitol is converted by the enzyme L-ribulokinase to the phosphorylated, non-metabolizable compound, ribitol phosphate, which accumulates in the cell, thereby inhibiting growth. Mutation to resistance involves prevention of accumulation of the phosphorylated compound by either loss of kinase activity (araB mutation) or cessation of its synthesis (araC mutation). The observation that a double mutant, araA39C\(^{-}\)67, is sensitive to ribitol and the failure to find any araA or araD mutants among the resistant strains is consistent with this interpretation. Furthermore, because the wild-type strain and the araC\(^{+}\)-resistant revertant are refractory to the ribitol effect, it is further argued that the kinase must be produced constitutively for the inhibition by ribitol to occur. This model is also in agreement with the earlier finding that ribitol is not an inducer of the L-arabinose operon in the wild-type strain.

Two types of mutations, not previously described in the ara system, can account for the resistance to ribitol in the strains which appear to be similar to the araC\(^{-}\)67 parent strain. Mutation in the araB gene may result in the alteration of the structure of the L-ribulokinase such that it is no longer capable of binding or converting ribitol to the phosphorylated compound. A loss of ability to bind ribitol need not be accompanied by the loss of ability to bind the natural substrate, L-ribulose. Alternatively, mutation in the araC gene may result in the loss of constitutivity without loss of fucose resistance. Experiments are in progress to understand the nature of resistance to ribitol in these strains.

This investigation was supported by National Science Foundation grant GB5342 and was conducted during my tenure as a Quebec Scholar.

I thank Ella Englesberg for assistance and encouragement during the course of this work.
LITERATURE CITED


