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"Self-Catabolite Repression" of Pectate Lyase in *Erwinia carotovora*

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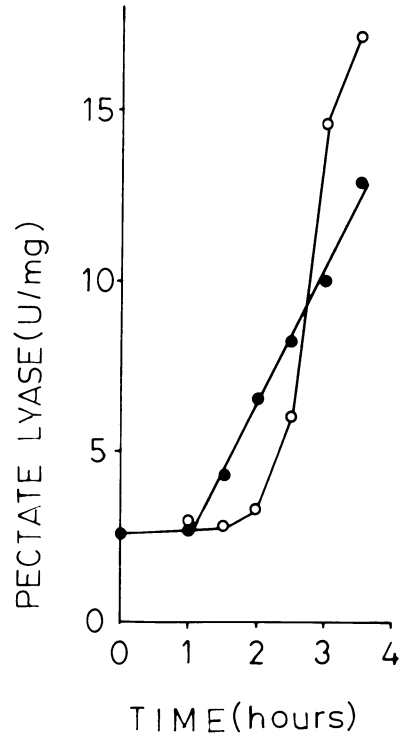
The induction of pectate lyase of *Erwinia carotovora* was repressed by a high concentration of its inducer. The concomitant addition of cyclic adenosine 3',5'-monophosphate reversed this repression.

Extracellular pectate (polygalacturonate) lyase formed mainly by bacteria catalyzes the transeliminative cleavage of pectate (1). I have reported that the true inducer of pectate lyase in *Erwinia carotovora* strain EC-1 was not pectate but its breakdown product and have termed this mechanism of induction "product induction" (11). The main breakdown product of pectate by this enzyme, *O*-(4-deoxy-β-L-threo-hexopyranos-4-enyluronate)-(1-4)-D-galacturonate (unsaturated digalacturonate), was suspected to be the true inducer.

Ca^{2+} has been shown to be the activator of pectate lyase (10). Thus, when the optimum concentration (10^{-4} M) of Ca^{2+}, together with pectate (supplier of the inducer) was added to an EC-1 culture in minimal medium (8), the induction started earlier than the control (i.e., no addition of Ca^{2+}) (Fig. 1). This early start of induction may be due to the effective supply of inducer caused by activation of the basal level of extracellular pectate lyase by Ca^{2+}. However, as induction proceeded and the induced pectate lyase accumulated in the medium, the synthesis rate in the culture with Ca^{2+} became lower than the control. These results suggest the existence of repression caused by the high concentration of inducer.

When 2×10^{-3} M unsaturated digalacturonate (inducer) was added directly into EC-1 culture (Fig. 2), the induction rate of pectate lyase during the first 60 min after its addition was almost the same as the rate repressed by the concomitant addition of 0.5% glucose, which is known to be a good mediator of catabolite repression (7). However, after this period only repression by the high concentration of inducer was relieved and a new high induction rate was observed. Since EC-1 grew well in the medium containing



2×10^{-3} M $CaCl_2$ (●) or without it (○) to the same volume of EC-1 cultures grown in minimal (8) + 2% glycerol medium to an absorbance at 655 nm of 0.280. At intervals, 3-ml samples were withdrawn, washed twice, and suspended in 1.5 ml of tris(hydroxymethyl)aminomethane-hydrochloride buffer (0.05 M, pH 8). Suspensions were sonically treated, and the cell debris was removed by centrifugation for 15 min at 10,000 × g. The activity of pectate lyase was measured by a modified thiobarbituric acid method (12). One unit of pectate lyase is defined as the amount of enzyme which produces 1 μmol of unsaturated products per h. Specific activity is expressed as units of enzyme activity per milligram of protein. Protein concentrations were determined by the method of Lowry et al. (6).

FIG. 1. *Effect of Ca^{2+} on the induction of pectate lyase by pectate. Induction was initiated by adding the solution containing 1% pectate (pH 7) either with*

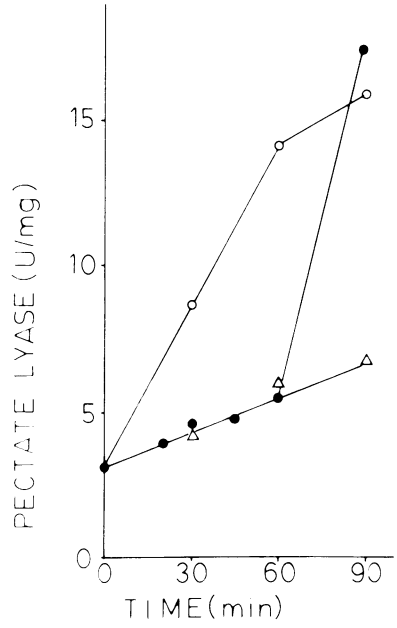


FIG. 2. *Effects of cyclic adenosine 3',5'-monophosphate and glucose on the induction of pectate lyase by unsaturated digalacturonate. The procedures were same as in Fig. 1 except that the induction was initiated by the addition of* 2×10^{-3} *M (final) unsaturated digalacturonate. Symbols:* (●) *inducer alone;* (△) *inducer + 0.5% glucose;* (○) *inducer +* 2×10^{-3} *M cyclic adenosine 3',5'-monophosphate.*

inducer as sole source of carbon (data not shown), a considerable amount of inducer should have been consumed during the first 60 min. This consumption of inducer might have relieved the repression. Furthermore, the repression of the induction by the high concentration of inducer was also relieved by the addition of 2×10^{-3} M cyclic adenosine 3',5'-monophosphate, which has been known to relieve the glucose-mediated catabolite repression of many inducible enzymes (9) including pectate lyase of *E. carotovora* (3). Thus, these results suggest that the accumulation of the breakdown product(s) (catabolite) of the inducer repress the induction of pectate lyase possibly by lowering the intracellular level of cyclic adenosine 3',5'-monophosphate (7, 9). This type of catabolite repression is analogous to what Katz and Englesberg (5) have found in the L-arabinose operon and have termed "self-catabolite repression."

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LITERATURE CITED

1. **Bateman, D. F., and R. Y. Miller.** 1966. Pectic enzymes in tissue degradation. Annu. Rev. Phytopathol. **4**:119–146.
2. **Cooper, R. M., and R. K. S. Wood.** 1975. Regulation of synthesis of cell wall degrading enzymes by *Verticillium albo-atrum* and *Fusarium oxysporum* f. sp. *lycopersici*. Physiol. Plant Pathol. **5**:135–136.
3. **Hubbard, J. P., J. D. Williams, R. M. Niles, and M. S. Mount.** 1978. The relation between glucose repression of endo-polygalacturonate transeliminase and adenosine 3',5'-cyclic monophosphate levels in *Erwinia carotovora*. Phytopathology **68**:95–99.
4. **Kato, K., T. Sugimoto, A. Amemura, and T. Harada.** 1975. A *Pseudomonas* intracellular amylase with high activity of maltodextrins and cyclodextrins. Biochim. Biophys. Acta **391**:96–108.
5. **Katz, L., and E. Englesberg.** 1971. Hyperinducibility as a result of mutation in structural genes and self-catabolite repression in the *ara* operon. J. Bacteriol. **107**:34–52.
6. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
7. **Magasanik, B.** 1970. Glucose effects: inducer exclusion and repression, p. 189–219. *In* J. R. Beckwith and D. Zipser (ed.), The lactose operon. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
8. **Mikula, J. M., B. I. Stieglitz, and J. M. Calvo.** 1972. Leucyl-transfer ribonucleic acid synthetase from a wild type and temperature-sensitive mutant of *Salmonella typhimurium*. J. Bacteriol. **109**:584–593.
9. **Perlman, R., and I. Pastan.** 1968. Regulation of β-galactosidase synthesis in *E. coli* by cyclic adenosine 3',5'-monophosphate. J. Biol. Chem. **243**:5420–5427.
10. **Starr, M. P., and F. Moran.** 1962. Eliminative split of pectic substances by phytopathogenic soft-rot bacteria. Science **135**:920–921.
11. **Tsuyumu, S.** 1977. Inducer of pectic acid lyase in *Erwinia carotovora*. Nature (London) **269**:237–238.
12. **Weissbach, A., and J. Hurwitz.** 1959. The formation of 2-keto-3-deoxy-heptonic acid in extracts of *Escherichia coli* B. J. Biol. Chem. **234**:705–709.