THE CONTROL OF ENZYME SYNTHESIS BY GLUCOSE AND THE REPRESSOR HYPOTHESIS[1]

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It has been proposed that induced enzymes are those whose synthesis is repressible (Pardee et al., 1959). Repression of induced enzyme synthesis is produced in response to endogenously synthesized repressors and is considered to be relieved by exogenously added inducers. The classical "glucose effects" (the inhibition of enzyme synthesis by glucose) have lately been interpreted in terms of this repression theory. Thus glucose, or derivatives of glucose, are precursors of an internal repressor which specifically inhibits the formation of various inducible enzymes (Neidhardt and Magasanik, 1956; Englesberg, 1959) by competing with the inducer for the enzyme-forming system (Cohn and Horibata, 1959). An analogous phenomenon has recently been described by Gorini (1960) for the control of the synthesis of a biosynthetic enzyme in E. coli.

The repressor hypothesis for glucose effects predicts that a) constitutive enzyme synthesis should be less sensitive to glucose inhibition than induced enzyme synthesis and b) inducer should reverse the inhibition. These predictions were verified for β-galactosidase synthesis

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in Escherichia coli by Cohn and Horibata (1959). However, in yeast the first prediction has been violated (MacQuillan et al., 1960). The synthesis of constitutive and inducible β-glucosidase are equally sensitive to glucose. These findings have led us to test in yeast the second prediction of the hypothesis.

The organism used in these studies was a yeast hybrid Saccharomyces fragilis x Saccharomyces dobzhanskii. This yeast is semi-constitutive with respect to β-glucosidase production, i.e. high amounts of the enzyme are produced constitutively but the addition of inducers, methyl-β-D-glucoside (βMG) or ethyl-β-thio-D-glucoside (TEG), to the medium will elicit a doubling of the differential rate of enzyme synthesis. A study of factors controlling β-glucosidase synthesis in this yeast has revealed, among other things, that many carbon sources, including glucose, acetate, lactate, isopropanol and oxalacetate, will inhibit production of this enzyme.

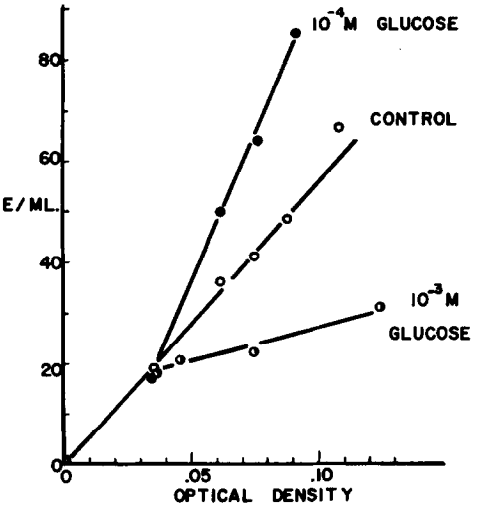

Fig. 1. Glucose effects on constitutive β-glucosidase synthesis. The differential rate of β-glucosidase (E) synthesis in exponentially growing cells was measured as previously described (Duerksen and Halvorson, 1959). Glucose was added at an optical density of 0.035.

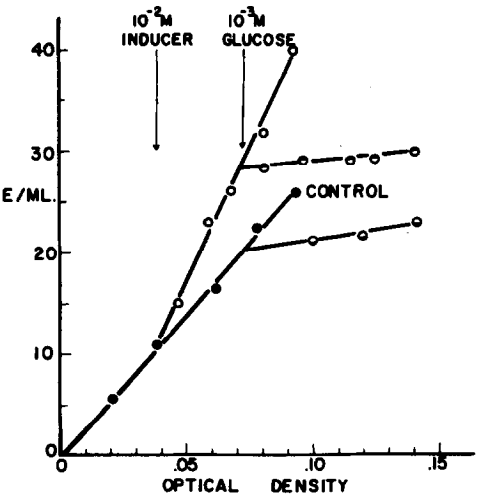

Fig. 2. Effect of preinduction on glucose inhibition of β-glucosidase synthesis. Glucose and 10^{-2} M inducer (βMG) were added as indicated.

Examination of the glucose concentrations necessary to effect inhibition of constitutive synthesis revealed that 10^{-3} M glucose will inhibit β-glucosidase production by more than 70 per cent (Fig. 1). Higher concentrations of glucose will inhibit synthesis completely. At lower concentrations of glucose, 10^{-4} M, increased β-glucosidase synthesis was observed. This stimulated rate of synthesis parallels that observed on addition of an inducer, and may be due either to the synthesis of an internal inducer or to the inhibition of the synthesis of an internal repressor. This problem is under current investigation.

The results in Fig. 1 provide a system for testing the second prediction of the glucose-repressor hypothesis. At limiting, inhibitory concentrations of glucose, the inhibition of β-glucosidase synthesis was not reversed by 10^{-2} M βMG (Fig. 2) or TEG. No inhibitory levels of glucose have been found which were reversed by inducer. Further, preinduction did not render the system resistant to glucose inhibition. Other carbon compounds tested also exhibited this non-competitive inhibition of β-glucosidase synthesis.

The present studies on the glucose inhibition of β-glucosidase synthesis in yeast contradict both predictions of the simple glucose-repressor hypothesis. At least two distinct sites must be involved in the regulation of β-glucosidase synthesis: an induction site and a repressor site. The repressor compound therefore exerts its effect at some site other than the stereospecific one involved in induction. Observations consistent with these results have been reported in other systems. Cohn and Horibata (1959) found a second non-competitive glucose inhibition of β-galactosidase synthesis in E. coli exhibited at higher concentrations of glucose, a finding more in accord with a two site hypothesis. Also, Vogel (1957) observed that the repression by arginine of N-acetyl ornithinase synthesis in E. coli was not reversed by enzyme substrate which therefore constitutes a repressible, but non-inducible, constitutive enzyme system.

We are continuing studies on the specificity of the glucose-repression phenomenon in yeast.

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