THE EFFECT OF GLUCOSE REPRESSION ON THE LEVEL OF RIBOSOMAL-BOUND β-GLUCOSIDASE*

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Received June 6, 1961

Accumulating evidence suggests that proteins are synthesized on ribosomes and that the information for coding a specific enzyme is derived from DNA by an unstable messenger RNA (Nomura et al, 1960; Brenner et al, 1961). It has further been suggested that induction (derepression) and repression function at the gene level (Riley et al, 1960), presumably by controlling messenger RNA synthesis. This view is supported by the observation that the level of ribosomal-bound enzyme increases during induction (Cowie et al, 1961) and decreases during repression (Warren and Goldthwait, 1961).

Ribosomes contain nascent precursors of soluble protein and enzymes in both a latent and active form (Kihara et al, 1961). From these findings as well as studies on the metabolic release of ribosomal bound protein (Morris and Schweet, 1961), discrete stages must exist in the release of finished enzymes from ribosomes. The question is thus raised whether or not the release mechanism(s) is subject to cytoplasmic control. A control of enzyme synthesis at the release stage should in principle differ in one important respect from controls at the gene level. That is, under conditions of repression one would expect that the ribosomal-bound level of enzyme would not decrease but would remain unchanged or actu-

*Supported in part by research grants from the National Institute of Health (E-1459), National Science Foundation (B-7150) and the U.S. Air Force Office of Scientific Research of the Air Research and Development Command (AF 49(638)-314.

ally increase because of the accumulation of bound, fully finished enzyme.

Glucose has frequently been observed to inhibit induced enzyme synthesis, presumably by the production of metabolic repressors. It is not clear, however, at which level this repression is exerted. In order to further examine this phenomenon the effect of glucose inhibition on β-glucosidase synthesis was examined in the yeast diploid Saccharomyces fragilis X Saccharomyces dobzanskii. Yeast cells were grown in a synthetic-succinate or synthetic-glucose (2%) medium. In both cultures the ribosomes were extracted and repeatedly washed on a sucrose density gradient until a residual level of β-glucosidase was observed. The results are shown in Table I.

TABLE I

Effect of Glucose on the Level of Ribosomal-Bound β-Glucosidase

Exp.	Succinate-grown		Glucose-grown		%	Rib.(gluc.)/ Rib.(succ.)
	Soluble	Ribosomal	Soluble	Ribosomal	Repressed	
1	1480	0.009	210	0.025	86	2.8
2		0.010	149	0.013	90	1.4
3		0.008	90	0.022	94	2.5
4		0.010	120	0.009	92	1.0

The organism was grown and the ribosome fraction extracted, washed and assayed as described by Kihara et al (1961), with the exception that 0.006 M cysteine was included in the assay medium. Enzyme activities are given as units/mg protein for the soluble enzyme, and units/ml/E_{260} for the ribosomal enzyme. The soluble enzyme value for the succinate-grown cells is an average from 25 experiments.

Glucose reduces the level of soluble enzyme from 1480 to an average of 142 units/mg protein. In spite of a ten fold decrease in the soluble enzyme concentration by growth on glucose, the ribosomal level rather than decreasing was somewhat increased, from an

average of 0.009 to 0.019 enzyme units/ml/E_{260}. These findings are consistent with the hypothesis that the control of β-glucosidase formation at glucose concentrations of 10^{-3} M or higher operates through blocking the release of finished enzyme from the ribosome.

The above experiments support our previous conclusion that two distinct sites are involved in the glucose control of β-glucosidase formation in yeast. Addition of 10^{-4} M glucose leads to an induced synthesis of β-glucosidase, presumably by the synthesis of an internal inducer (β-glucoside), while at 10^{-3} M glucose an inducer-insensitive inhibition of β-glucosidase formation is observed (MacQuillan et al, 1960).

The picture emerging from these studies is that glucose may exert a dual control of enzyme synthesis. The first, probably operating at the primary template level, is stereospecific, whereas the second operating on the release from the secondary template may be a relatively unspecific control exerted by glucose or some derived metabolite. By this model, it is also possible to account for apparent competitions between glucose and specific inducers: An inducer-stimulated increase in the number of functional enzyme-forming sites could be balanced by a glucose-retarded release from the same sites.

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