Genet. Res., Camb. (1972), **19**, *pp.* 27–32
With 1 *text-figure*
Printed in Great Britain

Mutants in *Saccharomyces lactis* controlling both β-glucosidase and β-galactosidase activities*

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(*Received* 15 *June* 1971)

SUMMARY

In *Saccharomyces lactis*, a class of mutants isolated for low β-glucosidase activity are reduced in activity for β-galactosidase as well. Genetic studies indicate that their properties are the result of a single mutation in a nuclear gene. In diploids containing a wild-type and mutant β-galactosidase allele, the mutant phenotype is partially dominant. The two enzymes can be separated physically and under appropriate conditions are induced independently in wild-type strains.

1. INTRODUCTION

β-Glucosidase in *Saccharomyces lactis* (*Kluyveromyces lactis*, Van der Walt, 1965) is under the control of the B locus. A number of mutations in the B locus have been found which lead to altered forms of β-glucosidase (Tingle, 1967). In addition, about 30 % of these mutant stocks were simultaneously reduced in β-galactosidase activity. The gene responsible for β-galactosidase activity, LAC_1, is tightly linked to the B locus (Herman, 1963). Several mechanisms could explain the simultaneous reduction in β-galactosidase and β-glucosidase activities. For example, the two genes could be co-ordinately controlled by a common linked regulator gene or the gene products could be physically associated *in vivo* as a bifunctional protein or as a multi-enzyme aggregate. However, the results from the experiments reported here indicate that neither explanation is satisfactory and that another mechanism may be involved in the control of the two enzyme systems.

2. MATERIALS AND METHODS

(i) *Organisms, media and growth*

The following strains of *Saccharomyces lactis* were used: Y-123 and 1009D (α, B^H, LAC_1, Lac_2) and Y-14 (a, B^m, Lac_1, LAC_2). LAC_1 and LAC_2 are the gene designations for the two polymeric genes which control lactose utilization in this yeast. Lac_1 and Lac_2 are the naturally occurring recessive alleles. The gene designa-

* This investigation was supported by Public Health Grant AI-01459 and a National Science Foundation Grant GB 6993.
† Supported by a National Institutes of Health training grant (GM-265).
‡ National Institutes of Health Research Career Professorship.

https://doi.org/10.1017/S0016672300014233 Published online by Cambridge University Press

tion, lac_1, refers to the altered LAC_1 locus. LAC_1 and LAC_2 are unlinked (Herman, 1963). B^h, B^m and B^l are the gene designations for strains carrying high, medium or low levels of β-glucosidase which is closely linked to LAC_1 (Herman & Halvorson, 1963*a*). Mutants were obtained by ultraviolet irradiation of wild-type strains carrying the α, B^H, LAC_1, Lac_2 markers as described previously (Tingle, 1967). The conditions for mating, the techniques for the isolation of spores and the procedure for plate assays for β-glucosidase and β-galactosidase activities have been described (Herman & Halvorson, 1963*a*; Tingle, Herman & Halvorson, 1968). Cell-free extracts were prepared from logarithmically growing cultures in synthetic succinate medium (MacQuillan & Halvorson, 1962) and prepared for enzymes assays as described previously (Tingle *et al.* 1968).

(ii) *Enzyme assays*

β-Galactosidase activity was determined by measuring the release of *o*-nitrophenol (ONP) from *o*-nitrophenyl-β-D-galactoside (ONPG). Enzyme, 0·05 M sodium phosphate buffer pH 7·1 ($MnCl_2$ $1{\cdot}6 \times 10^{-5}$ M) and ONPG (final concentration 10^{-2} M) were incubated at 30 °C and the optical density of the ONP released was recorded continuously at 420 nm ($E^{M}_{1\,cm} = 1{\cdot}83 \times 10^3$). β-Glucosidase activity was determined by measuring the release of *p*-nitrophenol (PNP) from *p*-nitrophenyl-β-D-glucoside (PNPG) as previously described (Duerksen & Halvorson, 1958). One unit of β-galactosidase or β-glucosidase is the amount of enzyme required to produce 1·0 nmole nitrophenol/min. ATP-PRPP pyrophosphorylase was assayed as described by Ames, Martin & Garry (1961). One unit is defined as the amount of enzyme necessary to bring about an absorbance change at 490 nm of 0·001/min.

(iii) *Inductions*

Methods for the growth and sampling of cells and for measurement of the differential rate of enzyme synthesis have been described (Herman & Halvorson, 1963*b*).

(iv) *Chemicals*

Chemically pure galactose, lactose, thiomethyl-β-D-glucoside (TMG), methyl-β-D-glucoside (BMG), *o*-nitrophenyl-β-D-galactopyranoside (ONPG) and *p*-nitrophenyl-β-D-glucoside (PNPG) were purchased from Calbiochem.

RESULTS

(i) *Properties of mutants*

Five mutants from our collection of *Saccharomyces lactis* strains simultaneously reduced in β-galactosidase and β-glucosidase activities were examined. Cell-free extracts of these mutant strains, previously shown to be mutants of B locus and which produce altered β-glucosidase proteins, display either little or no β-galactosidase activity compared to the wild-type strain (see Table 1). The enzymic lesions are apparently specific since no drastic alterations in the levels of ATP-PRPP

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Table 1. *Enzyme levels in wild-type and mutant strains*

Strain	Genotype	β-Galactosidase (units/mg)	β-Glucosidase (units/mg)	ATP-PRPP pyrophosphorylase (units × 10^3/mg)
Wild-type				
Y-123	α, B^H, LAC_1, Lac_2	449·7	66·8	14
Mutant				
Y-123-2	α, B^l, lac_1, Lac_2	< 1	3·6	—
Y-123-9		< 1	6·3	10
Y-123-40		54	28·0	12
PE-25		21	10·1	8
X-33		25	6·6	12

Enzyme activities were measured as described in Materials and Methods.

pyrophosphorylase, β-glucanase (not shown) or α-glucosidase (not shown) could be detected. When crude extracts from wild-type and mutant strains were mixed in various proportions only strictly additive enzyme activities for β-galactosidase and β-glucosidase were observed, ruling out potent inhibitors or activators as possible causes for the altered activities.

Genetic studies were carried out to determine the inheritance pattern of β-galactosidase in these mutants. The mutants were first mated to stocks carrying the LAC_2 gene. Thus, in a cross of mutant Y-123-2 (α, B^l, lac_1, Lac_2) × Y-14 (a, B^m, Lac_1, LAC_2) 2:2 (high:low β-galactosidase activity) segregation ratios should result if the mutation is at the LAC_1 locus, since only one polymeric gene, LAC_2, is segregating. However, more complex segregation ratios (3:1, 4:0 and 2:2) would be observed if a third modifier locus was involved. In 17 tetrads from a cross of Y-123-2 × Y-14; 55 tetrads from a cross of Y-123-9 × Y-14; 38 tetrads from a cross of Y-123-40 × Y-14 and 29 tetrads from a cross of X-33 × Y-14 only 2:2 (high:low β-galactosidase activity) segregation ratios were observed. This monogenic segregation pattern indicates that in each mutant a single factor closely linked to or at the LAC_1 locus is responsible for reduced β-galactosidase activity in each strain.

The mutants were then obtained in 'a' mating type strains by crosses to 'a', B^H, LAC_1, Lac_2 strains and backcrossed to Y-123 (α, B^H, LAC_1, Lac_2). In 61 ascospore sets derived from such crosses with the mutants Y-123-2 and Y-123-9 (a, B^l, lac_1, Lac_2) and Y-123 (α, B^H, LAC_1, Lac_2) only 2:2 segregation ratios for β-galactosidase activity were observed supporting the evidence that in each mutant only a single nuclear gene was responsible for reduced β-galactosidase activity. Furthermore, in all tetrads, high and low levels of β-glucosidase and β-galactosidase segregated together, indicating that the factor responsible for the mutant phenotype is either the same factor as the mutation in the B locus or closely linked to it in each strain. Identical ratios showing not only the monogenic segregation of β-galactosidase activity but also the coincident segregation of the LAC_1 and B markers were observed on analysis of over 200 random spores from the same

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Table 2. *Epistasis of β-galactosidase (lac_1) allele*

(Specific activity of β-galactosidase.)

	Haploid		Diploid		
Diploid	Mutant	Wild-type	Theoretical	Observed	Arithmetic mean (%)
Y-123-2 × Y-14 (α, B^l, lac_1, Lac_2) × (a, B^m, Lac_1, LAC_2)	< 1	1270	639	273	43
Y-123-9 × Y-14 (α, B^l, lac_1, Lac_2) × (a, B^m, Lac_1, LAC_2)	< 1	1270	639	336	52

crosses. Similar results were obtained when mutants Y-123-40, PE-25 and X-33 all (α, B^l, lac_1, Lac_2) were backcrossed to Y-123 (α, B^H, LAC_1, Lac_2).

The possibility that the simultaneous reduction in both activities is due to a double mutation seems remote. The frequencies of a mutation in the B locus and LAC_1 locus were approximately 3×10^{-5} and 10^{-5} respectively. The existence of such a large class of double defective mutants observed here (*c.* 10^{-5}) is much higher than the frequency expected for a double mutation produced by ultraviolet light. The extremely low β-galactosidase activity in these mutants precluded a biochemical study to determine if the reduced levels of β-galactosidase activity reflected inherent differences in the protein molecules produced. Since we have previously determined that the reduced activity for β-glucosidase is due to a mutation in the B locus (all of these mutants produced altered forms of β-glucosidase) we conclude that the mutation in the B locus is responsible for reduction of both activities.

The mutant phenotype is more fully expressed in diploids than the unaltered LAC gene. For example, in diploids such as ($LAC_1Lac_2 \times Lac_1LAC_2$), simple additive inheritance of the LAC genes is observed and the specific activity of β-galactosidase is the level expected for classic gene dosage. However, as shown in Table 2, the altered lac_1 gene is partially epistatic to the LAC_2 locus, since the specific activity of β-galactosidase in these diploids is less than the 50% activity level predicted. Thus, the possibility exists that the two enzymes may be controlled by a common regulatory system.

(ii) *β-Galactosidase and β-glucosidase control in wild-type strains*

If β-galactosidase and β-glucosidase share a common regulatory mechanism, one might expect that the two enzymes would be synthesized co-ordinately. The basal levels of β-galactosidase and β-glucosidase were examined during growth in a liquid succinate medium. The specific activity of β-glucosidase remained constant during logarithmic and stationary phase of growth while β-galactosidase activity increased almost threefold from late logarithmic to stationary phase. Apparently, the basal levels of the two enzymes are not strongly correlated.

Induction of the two enzymes is also under independent control. As shown in Table 3, lactose, galactose and TMG specifically increase the differential rate of

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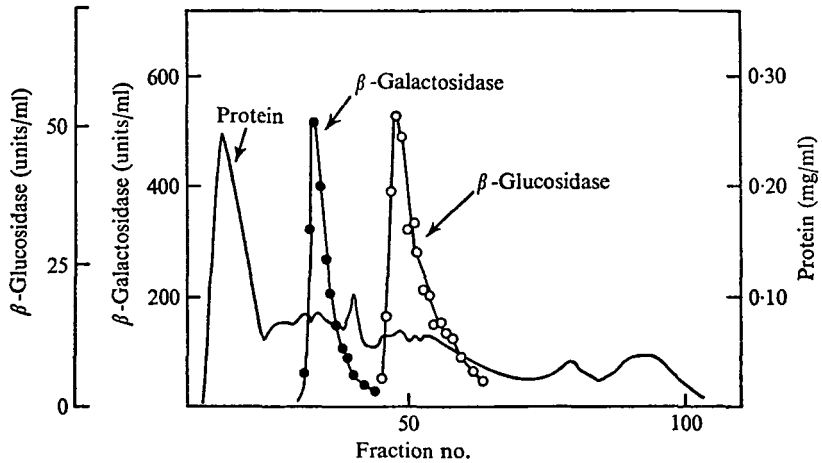

Fig. 1. DEAE-cellulose chromatography. A column (31 × 2·2 cm) was prepared containing washed DEAE-cellulose. Two ml (17·7 mg protein/ml) of a crude extract of strain Y-123 (α, B^H, LAC_1, Lac_2) containing 2880 units of β-glucosidase and 9980 units of β-galactosidase was placed on the column and eluted with 500 ml of phosphate buffer, pH 6·8, containing a linear (0–0·5 M) NaCl gradient. Fractions of 4·3 ml were collected and assayed for enzyme activities as described in Materials and Methods.

Table 3. *Effect of various inducers on β-glucosidase and β-galactosidase activity in wild-type strains*

Strain	Inducer	Inducibility factor	
		β-Glucosidase	β-Galactosidase
Y-123	10^{-2} M BMG	**3**	1
	10^{-3} M glucose	1	1
1009D	10^{-2} M BMG	**3**	1
	10^{-3} M glucose	**3**	1
Y-123	6×10^{-3} M galactose	1	6·4
	3×10^{-3} M lactose	1	4·6
	10^{-2} M TMG	1	5·0

The inducibility factor is the ratio of the specific enzyme activity in induced cultures to that found in control or uninduced cultures.

β-galactosidase synthesis without affecting the basal level of β-glucosidase. Similarly, β-methyl-glucoside (BMG) and glucose induce the synthesis of β-glucosidase without changing the rate of β-galactosidase synthesis.

Thus, no co-ordinate control of β-glucosidase and β-galactosidase synthesis was observed for either basal or induced enzyme synthesis. Since the action of a common regulation mechanism seemed unlikely, the possibility that the two enzymes were physically associated *in vivo* was investigated.

In wild-type strains β-galactosidase and β-glucosidase were easily resolvable by ion exchange chromatography, eluting from a DEAE-cellulose column

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at 0·025 M and 0·15 M-NaCl respectively (Fig. 1). Both enzymes could also be separated by starch-gel electrophoresis and exhibited different sedimentation rates and patterns on sucrose density gradient centrifugation, indicating that they did not exist as a multi-enzyme aggregate *in vitro* (Tingle, 1967).

DISCUSSION

The hypothesis that an altered promotor or operator type gene is responsible for the simultaneous loss of both enzyme activities seems unlikely from the results of diploid tests and the absence of any detectable common regulatory system. β-Glucosidase and β-galactosidase are clearly not physically associated *in vitro* and appear to be distinct protein species. β-Glucosidase activity can be altered independently of β-galactosidase activity and full β-glucosidase activity is observed in strains in which β-galactosidase activity cannot be detected.

β-Glucosidase has been isolated and characterized from a number of yeast species (Fleming & Duerksen, 1967). The enzyme is a multimeric enzyme of molecular weight approximately 300000 and is composed of several subunits. One possibility is that this mutation leads to a defective subunit of β-glucosidase which is common to the β-galactosidase molecule. Such a mutation would explain the simultaneous reduction of both activities and could account for partial epistatis of the mutation in diploids. Unequal mixing of altered subunits could reduce β-galactosidase activity below the level anticipated for independent gene expression.

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