SYNTHESIS OF TWO STRUCTURALLY DISTINCT β-GLUCOSIDASES BY A YEAST HYBRID*

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Introduction: Using a purified β-glucosidase from a yeast hybrid (Saccharomyes dobzhanskii x Saccharomyces fragilis), Hu, et al. (1960) obtained physicochemical evidence indicating the existence of a single molecular species of enzyme. MacQuillan and Halvorson (1962) showed that the addition of various carbon compounds to this particular hybrid yeast led to a repression of constitutive β-glucosidase synthesis. Preliminary serological evidence has been obtained in our laboratory, however, which indicates the presence of two distinct species of β-glucosidase --- each corresponding to the β-glucosidase produced by the parent strains. Simple gene dosage effect has not been demonstrated by our results.

Methods and Results: S. fragilis (Y-18), S. dobzhanskii (Y-19), and the hybrid (Y-42) were grown in an enriched succinate medium (Hu, et al., 1960). The yeasts were cultured in small quantities to the late log. phase and, following centrifugation, the cells were broken by three passages through a French pressure cell. The cell-free preparations were treated as described in the individual experiments.

Antiserum against the S. dobzhanskii (Y-19) β-glucosidase was obtained by inoculating rabbits with a 50-fold purified preparation (Fleming and Duerksen, 1963). β-glucosidase activity was determined by using p-nitrophenyl-β-D-glucoside (PNPG) as a substrate (Duerksen and Halvorson, 1958).

For serology studies, cell-free preparations of Y-18, Y-19 and Y-42 were diluted to approximately 300 units of enzyme/ml. One ml aliquots of the various enzymes were then mixed with an equal volume of different concentrations of the Y-19 antiserum. The mixtures were incubated at 6°C for six to eighteen hours and subsequently centrifuged for 25 minutes at approximately 10,000 x G. The resulting supernatant fluids were assayed for residual enzyme activity.

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Results indicated that Y-18 β-glucosidase activity was not decreased in the presence of 0.2 ml (the highest concentration tested) of Y-19 antiserum (Fig. 1a). In the homologous system, less than 0.1 ml of antiserum completely precipitated Y-19 β-glucosidase activity. With the hybrid Y-42 enzyme, a neutralization curve was obtained which suggested a mixture of Y-18 and Y-19 enzymes. Precipitated enzyme-antibody complexes were shown to be completely active. In all cases, normal serum controls showed no neutralization of β-glucosidase activity.

Since approximately 20% of the Y-42 β-glucosidase activity appeared to be due to the Y-18 enzyme, an artifical mixture was made containing 80% Y-19 and 20% Y-18 enzyme. This mixture, as well as an aliquot of original Y-42 enzyme, was combined with various concentrations of Y-19 antiserum and processed as described earlier. The results again suggested that Y-42 β-glucosidase actually consists of two molecular species which are similar respectively to β-glucosidase from the parents Y-18 and Y-19 (Fig. 1b).

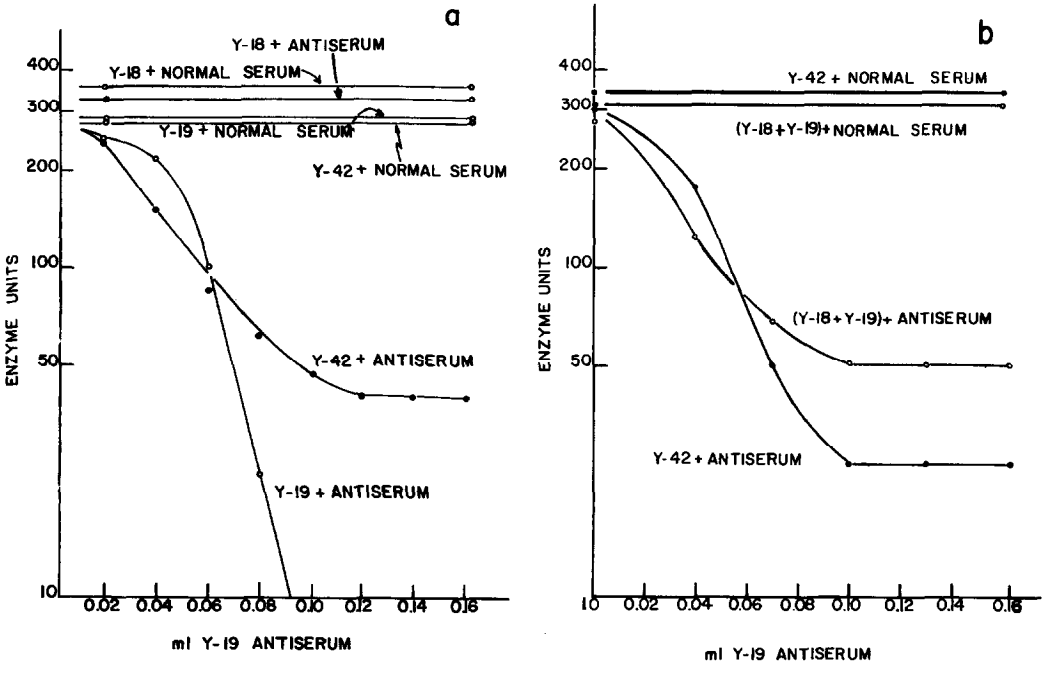

Fig. 1. Neutralization curves of β-glucosidase activity.

Although serologically distinct, the two enzymes demonstrated considerable similarity of physico-chemical characteristics. The two activities could not be separated by DEAE cellulose or by ammonium sulfate precipitation. Continuous

flow electrophoresis (Beckman) in veronal buffer at pH 8.5 produced fractions which described a slightly skewed activity peak. Assays for total activity and non-neutralizable activity of the electrophoretic fractions produced the results shown in Fig. 2a. The non-neutralizable (Y-18) activity showed a slightly greater electromobility than did the neutralizable (Y-19) activity. Neutralization slopes for various key electrophoretic fractions, along with a control Y-42 preparation, are presented in Fig. 2b. It can be seen that fractions 22 and 23 were neutralized in a manner similar to the homologous Y-19 system. Fractions 24 and 25 have slopes similar to the control Y-42 system, whereas fractions 26 mimics the non-neutralizable Y-18 system.

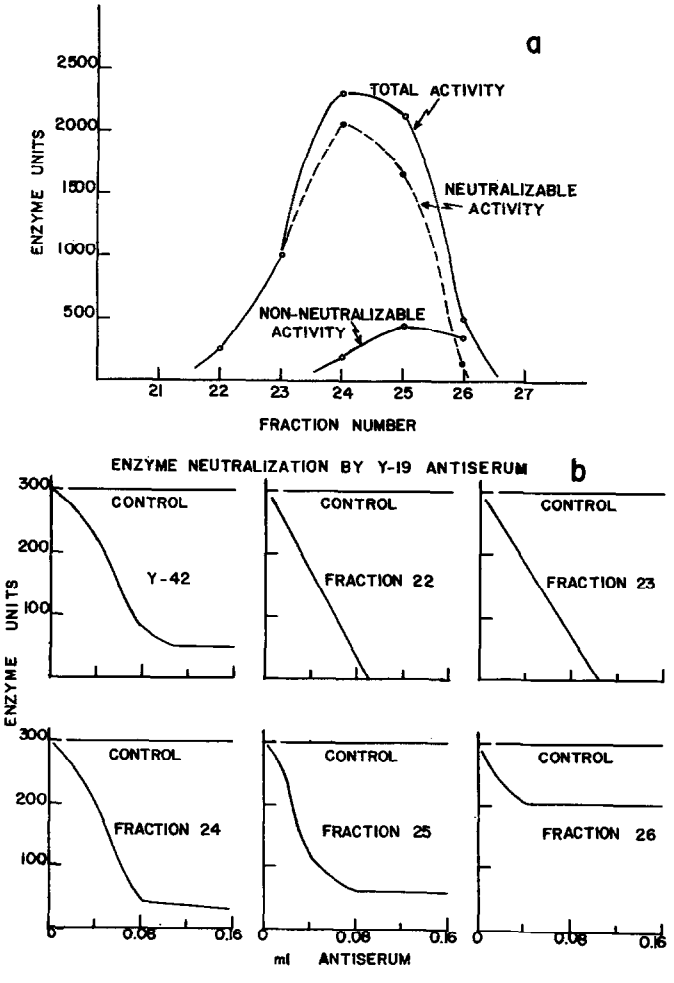

Fig. 2. Electromobility of the neutralizable and non-neutralizable β-glucosidase activity.

Whether or not there is a simple gene dosage effect in the synthesis of β-glucosidase by the hybrid Y-42 can be determined by using the technic described by Duerksen and Halvorson (1959) and used extensively by Rudert and Halvorson (1963) and Herman and Halvorson (1963). This procedure determines the amount of enzyme produced by one optical density (O.D.) unit or 1 mg of protein from logarithmically growing cultures. Samples were taken at increasing optical densities, then washed, lyophilized, and subjected to enzyme determinations. Larger samples were ruptured in the French pressure cell --- enzyme and protein determinations were then performed with the cell-free preparations. The Δ enzyme: Δ O.D. ratio or the Δ enzyme: Δ mg protein ratio were calculated for the three strains of yeast. With the Y-42 preparation, activity due to Y-18 and Y-19 enzyme was determined by measuring the non-neutralizable activity (Y-18 activity).

On the basis of simple gene dosage (additive), the expected Δ E: Δ O.D. ratio for Y-42 is 767, i.e., the rate of synthesis (E/mg protein) in Y-42 would be between the values found in Y-18 and Y-19. The actual value obtained was 1,570 (Table I). The expected ΔE: Δ mg protein ratio for Y-42 in the two experiments is approximately 1,500; those actually obtained were essentially 3,000. The rate of synthesis of β-glucosidase by the Y-42 hybrid cell was approximately equal to the sum of the rates of synthesis of β-glucosidase by Y-18 and Y-19 cells. Therefore, it would seem justified to conclude that a simple gene dosage effect is not demonstrated. When Y-42 enzyme was treated with Y-19 antiserum, a value of 1,162 was obtained. From the actual rates of synthesis obtained for Y-18 and Y-19, values of 1,110 and 800 are expected in the two respective cases for the rate of synthesis of Y-18 β-glucosidase in Y-42.

TABLE I

Gene dosage effect on β-glucosidase synthesis in the yeast hybrid.

Strain	Δ E*/ Δ O.D.		Δ E*/ Δ mg Protein			
	Actual	Expected	Actual	Expected	Actual	Expected
Y-18	524	-	1,110	-	800	-
Y-19	1,186	-	2,093	-	-	-
Y-42	1,570	767	3,050	1,438	2,900	1,500
Y-42 + Y-19 antiserum	-	-	-	-	1,162	-

*β-glucosidase activity determined by the discontinuous assay method (Duerksen and Halvorson, 1958).

Discussion: Evidence presented in this paper indicates that the yeast hybrid (Y-42) synthesizes two serologically distinct β-glucosidases --- each enzyme similar to its respective parental type. The similarity of physico-chemical characteristics of the two molecular species would make their detection difficult (Hu, et al., 1960). It should prove interesting now to determine the response of each enzyme in the hybrid cell to various β-glucosides, as well as high and low concentrations of glucose, and other carbon compounds (MacQuillan and Halvorson, 1962).

Several examples of multiple unlinked structural genes within a single cell have been reported. Nelson (1955) observed in a Saccharomyces strain that galactokinase activity was increased by increasing the G_1 dosage. In another Saccharomyes strain, however, Ogur (1954) found that the dosage effect of a gene controlling isomaltase activity was ploidy-dependent. Halvorson et al. (1962) concluded that each of five genes, M_1 through M_4 and M_6, are structural genes controlling the synthesis of indistinguishable α-glucosidases. Apparently only one dominant form of the unlinked structural genes was necessary for enzyme synthesis (Rudert and Halvorson, 1963). The level of enzyme synthesized was found to be directly proportional to the gene dosage. These authors suggested that the structural genes were under their own individual genetic control. Gene dosage effects have also been observed in bacterial systems (Horowitz and Novick, 1962; Horiuchi et al., 1963). The number of structural and regulatory genes present in each parent and the hybrid is unknown. Therefore the results presented in Table I are not yet open to meaningful interpretation. It is suggested, however, from the results present in this Table and results presented by MacQuillan and Halvorson (1962 and 1962a), that a complex regulatory system exists in the hybrid in the expression of the various structural genes for the two distinct β-glucosidases.

To confirm the results presented here, comparisons should be made between the characteristics of the parental enzymes and the two enzymes produced by the hybrid yeast. Studies of this nature, as well as induction-repression studies, are in progress.

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