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Dextransucrase, an Induced Enzyme from *Leuconostoc mesenteroides*

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Sucrose has been shown to induce the formation of dextransucrase in the organism *Leuconostoc mesenteroides*. Furthermore, the results imply that the fructofuranose portion of the sucrose molecule is the trigger which causes induction to take place. No other inducer for dextransucrase has been found.

The study of the ability of microorganisms to adapt to a new environment is an important and fascinating area of research. One example of this ability is the ease with which microorganisms evolve new enzyme system(s) to act on foreign substrates. This phenomenon is commonly known as induction, and many excellent review articles have been written; for example see Pollock (1959) or Dixon and Webb (1958). This facet of adaption provides a convenient method for following the formation of a catalytically active protein. Unfortunately, in studying this phenomenon it is difficult to separate *in vivo* synthesis of enzyme from simple conversion of inactive protein to active protein, and these two possibilities should always be considered.

The action of the enzyme dextransucrase from *Leuconostoc mesenteroides* on the substrate sucrose has been studied by a number of investigators (for a review see Neely, 1960). The reaction catalyzed by this enzyme is shown in equation (1). One of the characteristics of dextransucrase is specificity for sucrose. No other sugar has been

$$n \text{ sucrose} + \underset{\text{Acceptor}}{\text{HOR}} \longrightarrow \underset{\text{Dextran}}{(\text{glucose})_n\text{-OR}} + n \text{ fructose} \quad (1)$$

shown to act as a donor of glucosyl groups for the formation of the polymer dextran. Since the enzyme was specific for sucrose, we began to wonder if its biosynthesis was also dependent on the sucrose molecule. The only work of this nature was done by Karstrom (1938). He stated that the enzyme system from *L. mesenteroides* for dealing with sucrose was constitutive and not adaptive. However, he grew the organism at 37° and carried out his experiments with the washed cells at 45°. Since Neely (1959) and Tsuchiya *et al.* (1955) have shown that dextransucrase is quite unstable at this high temperature, it appears unlikely that Karstrom was dealing with this particular enzyme. We decided to reinvestigate the nature of the enzyme elaborated by *L. mesenteroides* in an effort to learn more about its biosynthesis.

Experimental

Enzyme Production and Assay.—The medium for the *Leuconostoc mesenteroides* NRRL-B512F was similar to the one described by Tsuchiya *et al.* (1952). The culture was maintained on a 2% sucrose medium and transferred every other day to fresh medium. Since the enzyme dextransucrase is exocellular, centrifugation at 8500 rpm (5500 × *g*) of the whole culture after adjustment of the *p*H to 5.2 gave a crude cell-free extract. The enzyme assay was based on the measurement of free reducing sugar when 1 ml of appropriately diluted extract was incubated with 1 ml of 100 mM sucrose at 25° for ½ hour. The dextransucrase unit (D.S.U.) as used in this paper was the amount of enzyme which would convert 1 mg of sucrose to dextran in one hour (releasing 0.52 mg of fructose) under the above-described conditions. Reducing sugars were measured by the Somogyi method (1945), with the colorimetric technique of Nelson (1944), incorporated.

Preparation of Washed Cells.—A 24-hour culture containing 2% sucrose was centrifuged at 8500 rpm (5500 × *g*) for ½ hour. The supernatant was discarded. The cells were dispersed in half their original volume with 0.05 M phosphate buffer, *p*H 6.5. They were recentrifuged. The washing and centrifugation were repeated three times and the cells were finally stored in the phosphate buffer. This preparation was fairly unstable and had to be used within 24 hours.

Fructose Measurement.—Samples from the cul-

TABLE I

DEXTRANSUCRASE ACTIVITY PRODUCED BY *Leuconostoc mesenteroides* GROWN ON THE STOCK MEDIA WITH VARIOUS SUGARS AT THE 2% LEVEL USED AS CARBON SOURCES

Sugar	Growth[a] Optical Density	pH	Days[b]	Activity (Units/ml)
Sucrose	1.47	5.05	1 (5.05)	50.6
Invert sugar	1.43	5.05	1 (5.05)	0
Glucose	1.38	5.15	1 (5.15)	0
Maltose	1.15	5.55	2 (4.6)	1.18
Trehalose	1.14	5.15	2 (4.5)	0.65
Melibiose	1.14	6.45	2 (5.2)	0.52
Fructose	0.890	6.85	2 (4.8)	0
α-Methyl glucoside	0.121	7.05	3 (5.2)	0
Mannose	0.107	6.90	2 (5.4)	0
Galactose	0.090	6.90	6 (6.1)	0
Melezitose	0.057	7.05	3 (6.2)	0
Xylose	0.020	7.05	6 (6.8)	0
Raffinose	0.012	6.90	3 (5.3)	0
Turanose	0.004	7.05	3 (4.8)	0
Cellobiose	0.004	7.05	6 (6.2)	0
β-Methyl xyloside	0.003	7.05	3 (6.5)	0
Lactose	0.003	7.05	6 (7.1)	0

[a] Relative rate of growth was measured by determining both the optical density of the culture at 650 mμ and the pH at the end of 24 hours' incubation. [b] The organism was allowed to grow for the indicated time before the assay for activity. The figure in brackets represents the pH of the culture at the time of assay.

ture were taken at regular intervals and filtered through Millipore filters with a pore size of 0.45 μ to remove cells. A portion (2 ml) was subjected to the alkaline hypoiodate oxidation of Hirst *et al.* (1949) to destroy aldoses and the final volume made up to 25 ml. The keto hexoses remaining were then analyzed in the manner described above for reducing sugars.

Total Carbohydrate.—Total carbohydrate was measured with the anthrone technique of Morris (1948). After correction for reducing sugars, the results were expressed in terms of polysaccharide.

Warburg Measurements.—The Warburg measurements were conducted at 25°. The description of the vessel contents is given in the legends to the appropriate figures.

RESULTS

The enzyme activity produced by the organism growing in the presence of various sugars is indicated in Table I. These results were obtained by inoculating the stock medium plus the indicated carbohydrate at the 2% level. Several of the sugars were poor carbon sources, and growth was consequently much slower. As a general rule the cultures were assayed for activity when the pH of media had fallen from the initial value of 7.0 to approximately 5.0. That pH is a fairly good criterion of growth is illustrated in Table II, where sucrose, maltose, mannose, fructose, glucose, and raffinose are examined in greater detail. A differential rate of synthesis of dextransucrase was obtained by adding sucrose to an exponentially growing culture of *Leuconostoc mesenteroides*. This is shown in Figure 1.

TABLE II

DEXTRANSUCRASE ACTIVITY PRODUCED BY *Leuconostoc mesenteroides* WITH VARIOUS SUGARS AT THE 2% LEVEL USED AS CARBON SOURCES

The cultures were assayed for activity at the end of 30 hours' incubation at room temperature (24°).

Sugar	pH	Cell Dry Wt. (mg/10 ml culture)	Activity (units/mg cell dry wt.)
Sucrose	4.8	55.9	8.2
Glucose	4.7	54.5	0
Fructose	5.2	56.6	0
Maltose	5.0	56.5	0
Mannose	7.2	19.9	0
Raffinose	7.0	19.2	0

Figure 2 shows the effect of increasing sucrose concentration on enzyme production in a 24-hour culture. These solutions were also assayed for total polysaccharide. To show what effect the combination of glucose and sucrose would have on the resulting activity, sucrose was added in varying amounts to media containing 1% glucose. These results are shown in Figure 3.

Figure 4 gives a more detailed analysis of the various parameters. A 2% sucrose medium was inoculated and the following analyses were conducted: (1) pH, (2) appearance of fructose, (3) disappearance of total carbohydrate, (4) increase in enzyme activity, and (5) uptake of O_2 as measured by the Warburg technique.

The appearance of fructose during growth prompted an examination of the rates of O_2 utilization by the organism with three different

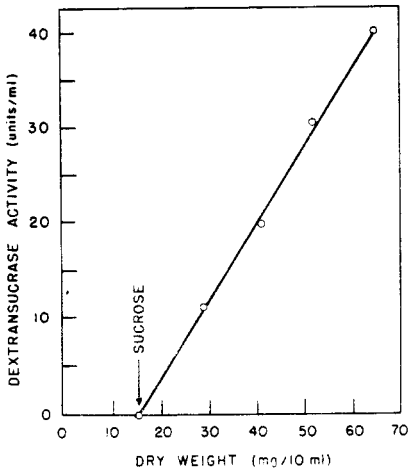

FIG. 1.—Differential rate of dextransucrase synthesis. Sucrose was added as indicated to a culture of *Leuconostoc mesenteroides* growing on a glucose medium that had reached the exponential growth phase.

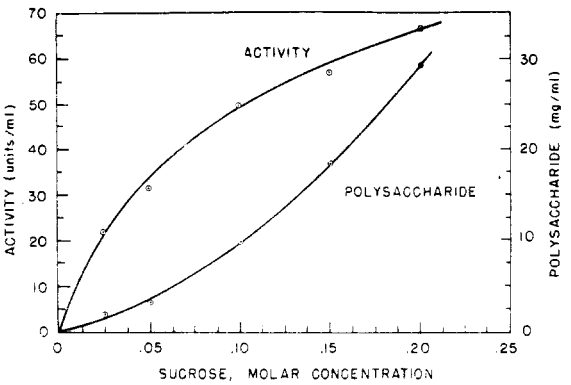

FIG. 2.—Production of enzyme and polysaccharide in the presence of increasing amounts of sucrose. Sucrose concentration of 0.058 M is equivalent to 2%.

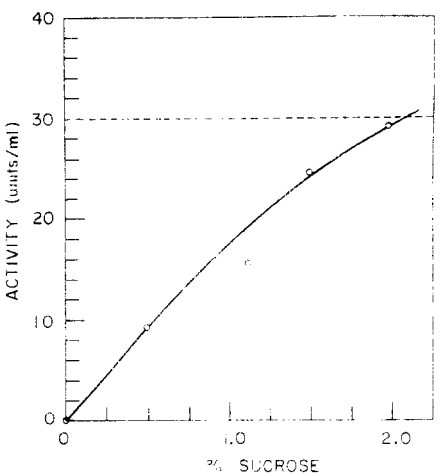

FIG. 3.—Production of dextransucrase as a function of sucrose concentration. All the samples possessed 1% glucose as well as the indicated amount of sucrose. The dotted line represents the enzyme activity when the organism is grown on 2% sucrose as the sole source of sugar.

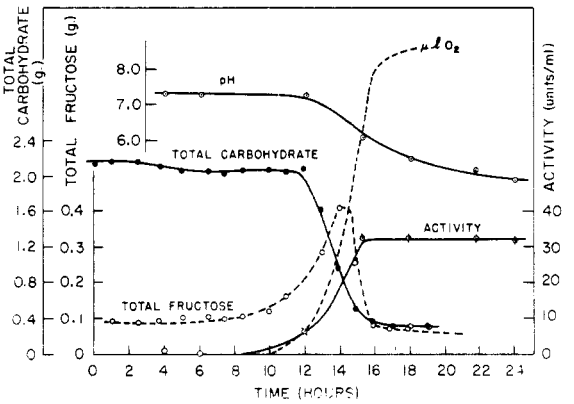

FIG. 4.—The analyses of various parameters against time of incubation in a 2% sucrose medium. The fructose analysis has not been corrected for a residual blank. The oxygen uptake is on an arbitrary scale.

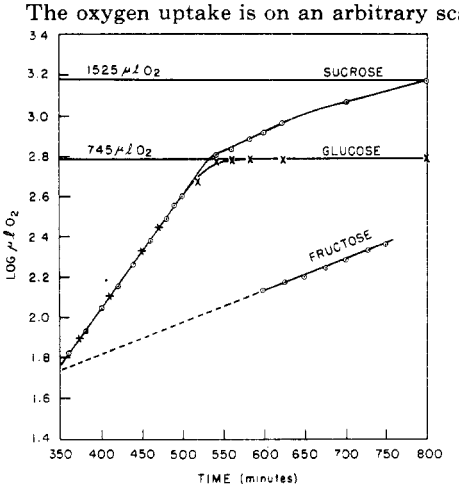

FIG. 5.—Oxygen utilization curves for equimolar glucose, fructose, and sucrose solutions. Each Warburg vessel contained 2 ml of inoculated stock medium with 50 μmoles of the indicated sugar, and 0.3 ml of 10% KOH in the center well. The flasks were shaken at 25°.

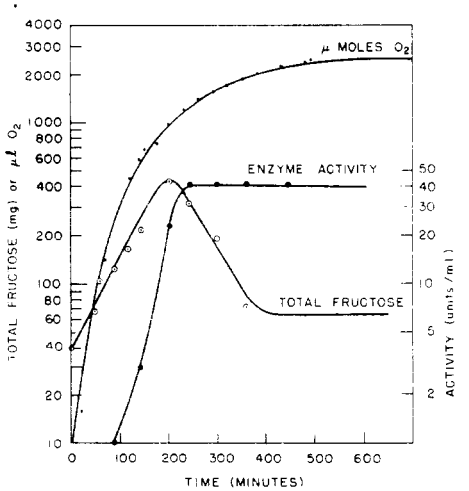

FIG. 6.—Production of enzyme and fructose superimposed on the oxygen utilization curve from a standard 2% sucrose medium inoculated with washed cells. The reaction was carried out at 25°.

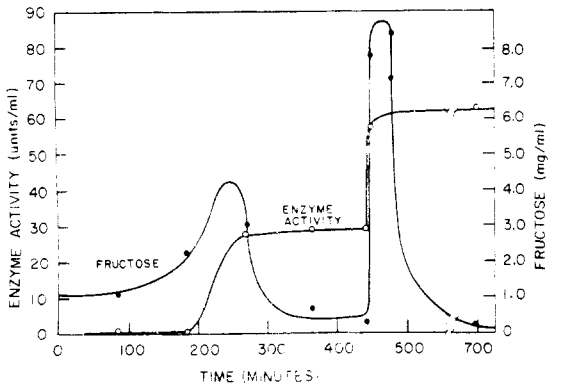

FIG. 7.—Results of enzyme and fructose production from a 2% sucrose medium inoculated with washed cells. At 375 minutes the *p*H, which had fallen to 5.2, was readjusted to 7.0. An additional portion of sucrose equivalent to the initial amount was then added at 425 minutes.

carbon sources: glucose, sucrose, and fructose. Figure 5 shows the result.

The use of washed cells shortened the duration of the experiment by removing the long lag phase in the growth cycle. Consequently, washed cells were used as the inoculum in the following experiments, which were conducted at 25°. The first experiment, illustrated in Figure 6, shows the liberation of fructose and the rise in enzyme level from washed cells on a 2% sucrose medium. Superimposed on this curve is the rate of O_2 uptake taken from Warburg measurements. The next experiment was designed to show what influence a second addition of sucrose would have after the initial sucrose had been utilized. The first time this was attempted a marked increase in free fructose occurred with no increase in the enzyme. It was observed that the *p*H of the medium had fallen to 5.0 after the first cycle. On the second attempt the *p*H was readjusted to 7.0 before the second portion of sucrose was added. The results of this experiment are given in Figure 7. It will be noticed that there was a marked increase in enzyme along with the appearance of free fructose.

DISCUSSION

Tables I and II demonstrate quite clearly that *Leuconostoc mesenteroides* is capable of good growth, as represented by increase in cellular dry weight, drop in *p*H, and increase in turbidity, on a number of different carbon sources. In fact, the growth patterns on sucrose and glucose are quite similar not only with respect to the above-mentioned parameters but also with respect to the rate of oxygen uptake, as shown in Figure 5. The one outstanding difference, however, is the fact that dextransucrase is formed only on sucrose. Figure 1 indicates that when sucrose is added to a culture in the log phase, enzyme formation proceeds at a maximal rate and represents a constant fraction of new protein synthesis. These data indicate that we are dealing with an induced enzyme.

Figure 3 again indicates the negative effect of glucose as an inducer of dextransucrase and in addition demonstrates that the enzyme activity is directly related to the amount of sucrose present. Figure 2 substantiates the earlier observations of Tsuchiya *et al.* (1952) that increased concentrations of sucrose cause increased levels of enzyme. In addition, the total carbohydrate analysis in this figure indicates a considerable lag in the synthesis of dextran. In fact at the 2% sucrose level there is very little polysaccharide formation, and this suggests that sucrose at this concentration is being used completely as an energy source. As the amount of sucrose is increased, more and more substrate becomes available for the newly created polymerizing enzyme. The dextran that is formed from the excess sucrose cannot be used by the organism as a carbon source, and consequently accumulates in the medium. These points are further demonstrated in Figure 4, which also shows a rise in fructose during the logarithmic period and its subsequent decline. Tsuchiya *et al.* (1952) also showed this appearance of fructose, which they interpreted as being due to the release of fructose by dextransucrase in the production of dextran from sucrose. In view of our observation that at the 2% sucrose level there is very little accumulation of dextran, we prefer to think of the increase of fructose in the medium as resulting from a difference in the rates of metabolism of glucose and fructose. In other words, the glucose portion of sucrose is metabolized much faster than the fructose, so that the keto sugar accumulates. The Warburg measurements shown in Figure 5 substantiate these points. Note that sucrose and glucose have the same initial oxidation rate until the glucose residue of sucrose is metabolized, at which time the rate of oxidation of sucrose follows that of fructose. These observations imply that during sucrose metabolism the fructose residue accumulates and then is slowly utilized. The analyses shown in Figure 6 helped confirm these results. The important feature of this experiment is the observation that the concentration of fructose and the enzyme activity reached a maximum at nearly identical times. The failure of the second addition of sucrose to stimulate enzyme formation when the *p*H of the medium was 5.0 reflects the earlier observations of Tsuchiya *et al.* (1952) that the maximum elaboration of enzyme occurred at *p*H 6.7. At the lower *p*H values there was a marked reduction in enzyme synthesis, which is in good agreement with our present observation. When the *p*H of the medium was readjusted to 7.0 the organism was stimulated in the direction of further enzyme synthesis (Fig. 7).

This study has several implications. First, dextransucrase appears to be an induced enzyme,

and it is induced by sucrose; we have found no other satisfactory inducer. The inability of invert sugar to induce dextransucrase leads to the second implication. The basic difference between sucrose and invert sugar is the fact that in sucrose the fructose residue is in the furanose form. As soon as fructose is liberated from sucrose it rapidly converts to the pyranose form. It is very tempting to state that fructofuranose is the trigger that initiates the formation of dextransucrase. This hypothesis would fit with the observations. Figure 6 substantiates this point. The curves show a parallel rise in free fructose with enzyme activity. From the Warburg experiment in Figure 5 we may conclude that the maximum concentration of free fructose in Figure 6 represents the point where the glucose residue of sucrose has been metabolized. It is at this point that fructose in the furanose conformation has disappeared. This also represents the optimum in enzyme production (Fig. 6). Adding more fructofuranose in the form of sucrose causes more build up of enzyme as illustrated in Figure 7.

Additional work is required in order to establish these conclusions unequivocally; however, the implications are present and point the way to further investigation in this important area of enzyme biosynthesis.

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