LXIII. THE SELECTIVE FERMENTATION OF GLUCOSE AND FRUCTOSE BY YEAST.

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Interest in the phenomena of selective fermentation of mixtures of sugars by yeast has been revived in the last decade, following the more precise measurements of Willstätter and Sobotka [1922]. The facts are briefly as follows.

(1) That separate solutions containing more than 1 % of glucose or fructose are fermented at equal rates by living yeast.

(2) That glucose and fructose in mixtures are fermented at unequal rates, glucose being fermented faster than fructose by most yeasts, including *S. cerevisiae*, while fructose is fermented faster than glucose by some yeasts, among which are Sauterne yeast, *S. pombe* and *S. exiguus*.

(3) That zymin generally exhibits selective properties similar to those of the yeast from which it is prepared, although the zymins obtained from *S. pombe* and *S. exiguus* show a selectivity opposite to that of the yeasts from which they are obtained [Fernbach, Schoen and Mori, 1928].

(4) That zymin prepared from brewer's yeast when acting on separate sugar solutions esterifies fructose more rapidly than glucose if a suitable concentration of inorganic phosphate is added.

Willstätter and Sobotka expressed their results in terms of a selectivity constant

$$K = \frac{\ln y_0 - \ln y}{\ln z_0 - \ln z},$$

where y_0 and z_0 represent the initial concentrations of glucose and fructose, and y and z represent sugar concentrations after a fermentation period t. K, therefore, is the ratio of the separate velocity constants calculated for unimolecular reactions, and its use appears to imply the assumption that the rate of fermentation of each separate sugar is proportional to its concentration at any time. The values of K obtained by Willstätter and Sobotka from different experiments showed good agreement.

Hopkins [1928], using the same formula as Willstätter and Sobotka, found $K = 1{\cdot}8$ for a top brewery yeast and $K = 2{\cdot}1$–$2{\cdot}8$ for a bottom yeast. In a later paper Hopkins [1931] determined the selectivity constants of brewer's yeast and of Sauterne yeast, and he also estimated the rates at which these two

yeasts ferment separate solutions containing glucose and fructose. These separate rates of fermentation are of particular interest since Hopkins found that, at concentrations of sugar below 1 %, brewer's yeast fermented glucose faster than fructose. The Sauterne yeast, on the other hand, fermented fructose faster than glucose at all concentrations of sugar tested, the effect being more marked in dilute sugar solutions. According to the ideas to be expressed in the present paper, the explanation of the phenomena of selective fermentation is to be found in the relative rates of fermentation of solutions containing low concentrations of the separate hexoses.

On the basis of the theory of Michaelis and Menten [1913] the relative rates of reaction of two substrates competing for the same enzyme can be expressed by the formula

$$\frac{V_1 K_2}{V_2 K_1} = \frac{\ln y_0 - \ln y}{\ln z_0 - \ln z},$$

where K_1 and K_2 are the Michaelis constants (expressed in concentrations) of the two substrates, and V_1 and V_2 the maximum velocities for each substrate alone [Haldane, 1930].

Since, as was shown by Slator [1908], the maximum rates of fermentation of glucose and fructose by brewer's yeast are equal, it is evident that the above equation is identical with that used by Willstätter and Sobotka to express selectivity. The selectivity constant thus represents the ratio of the affinities of the two sugars for the enzyme. From the figures given in Table IV by Hopkins [1931], the concentrations of sugar at which half the maximum velocity of fermentation is observed are 0·2 % and 0·4 % for glucose and fructose respectively. These figures yield a value of 2 for the selectivity constant; Hopkins obtained from mixed fermentations the value 2·3 at 31°.

The extent to which Hopkins's results on the rates of fermentation of separate sugar solutions agree with the Michaelis equation

$$v = \frac{VS}{K+S},$$

where V is the maximum velocity, v is the velocity at substrate concentration S, K is the Michaelis constant, can be tested by plotting the values of $\frac{1}{v}$ against $\frac{1}{S}$. Since the Michaelis equation may be transformed to $\frac{1}{v} = \frac{1}{V} + \frac{K}{V} \cdot \frac{1}{S}$, the figures plotted in this manner should yield a straight line. The agreement found is not sufficient to enable this method to be used to obtain values for the Michaelis constants more accurate than those obtained by direct observation of Hopkins's data. The determination of the rate of fermentation of low concentrations of sugar is, however, complicated by the autofermentation of yeast carbohydrate, and the amount of autofermentation is not independent of the sugar concentration. Fair agreement with Hopkins's experimental data is obtained by calculating the rates of fermentation at different sugar concentrations from the Michaelis equation using 0·2 % and 0·4 % as the values of the Michaelis constants for glucose and fructose respectively.

When using Sauterne yeast Hopkins observed that the rate of fermentation of glucose was less than that of fructose at all concentrations tested. In order, therefore, to calculate the selectivity constant from the Michaelis constants this difference in maximum velocities has to be taken into account. Analysis of Hopkins's data shows, however, that better agreement with the Michaelis equation is obtained if it is assumed that the maximum velocities of fermentation of glucose and fructose by Sauterne yeast are identical. This assumption can, to some extent, be justified since Hopkins's figures show that at 10·5 % glucose the velocity of fermentation was still increasing. Higher concentrations were not tested, but the theoretical maximum velocity is not likely to be achieved in practice on account of inhibition by hypertonic sugar solutions. Calculated on this basis Hopkins's data yield the values 2·5 % and 0·22 % for the Michaelis constants of glucose and fructose respectively, from which the calculated value of the selectivity constant is 0·09. Hopkins [1931, Table I] found the value 0·098 for Sauterne yeast at 31°. It is clear, therefore, that the phenomena of selective fermentation by living yeast can be satisfactorily explained by the Michaelis theory of enzyme affinity.

Selective fermentation by zymin.

In the above treatment of selective fermentation by living yeast no attempt has been made to ascribe the selection to any particular enzyme in the zymase complex. The most probable stage for selection seems to be during the reaction which results in the formation of hexosephosphate. A study of the apparently contradictory facts reported on selective fermentation by zymin yields some evidence for this hypothesis.

If the selection is due to the enzyme which catalyses the reaction between hexose and phosphate, it follows that the concentration of phosphates present should be taken into account. In this connection it should be noted that yeast-juice, dried yeast and zymin, when fermenting sugar, differ from living yeast in the manner of their response to added phosphate [Harden, 1932]. The hypothesis is now advanced that the selectivity shown by zymin when fermenting mixtures of glucose and fructose is controlled by the phosphate concentration.

Hopkins [1928] investigated the velocity of esterification of glucose and fructose by zymin (brewer's yeast) in the presence of varying concentrations of phosphates, and, in agreement with the results obtained by Harden and Young using yeast-juice, found that the maximum rate of esterification of fructose was greater than that of glucose, while the concentration of phosphates required to produce this maximum effect was greater for fructose than for glucose. The concentration of hexose in these experiments was 13 %. It is probable, therefore, that the affinity of the enzyme system for phosphate is smaller for the reaction catalysed between fructose and phosphate than for the reaction between glucose and phosphate. By applying Hopkins's figures for the maximum rates of esterification of separate sugar solutions to the Michaelis

equation it is found that the Michaelis constants are 0·05 *M* phosphate for the reaction with glucose and 0·12 *M* for the reaction with fructose, while the ratio

$$\frac{\text{Maximum rate of esterification of fructose}}{\text{Maximum rate of esterification of glucose}} = 1{\cdot}6.$$

These values are approximate ones, and the calculated maximum velocities are greater than those observed by Hopkins. Excluding the lowest phosphate concentration in the case of fructose and the higher concentrations in the case of glucose, the velocity figures given by Hopkins agree with the values calculated by use of the above constants.

A consideration of the values obtained for the Michaelis constants and for the ratio of the maximum velocities showed that, at low concentrations of phosphate (0·02 *M*), the rate of esterification of glucose should be greater than that of fructose, since at low concentrations of phosphate the greater affinity of the enzyme for glucose should outbalance the effect of the greater maximum velocity of the reaction with fructose. The effects on zymin of concentrations of phosphate of this order are not easy to measure, but they are of importance in the consideration of the phenomena of selective fermentation. Thus Hopkins [1928, Exp. 4] observed that zymin preferentially fermented glucose from a mixture of glucose and fructose. The solution fermented contained 10 g. zymin in 75 cc., and no phosphate was added. An estimate of the effective concentration of phosphate present in these experiments can be obtained from the data of Euler and Myrbäck [1923] who investigated the influence of phosphate concentration on the rate of fermentation of glucose by dried yeast. From their results these workers calculated that 1 g. of their dried yeast preparation contained 0·015 g. PO_4. Assuming the effective phosphate concentration of Hopkins's preparation to be of the same order, the phosphate concentration in his experiments would be about 0·02 *M*, under which conditions, according to the hypothesis developed above, selective fermentation of glucose is to be anticipated.

It is probable that a similar consideration of the various Michaelis constants concerned will explain the results of Fernbach, Schoen and Mori [1928] who found that zymins prepared from two yeasts exhibited selective fermentation opposite to that shown by the fresh yeasts, but in this case the interpretation is complicated owing to the use of a mixture of racemised sugars.

Discussion.

The degree of selectivity exhibited by any particular yeast is not constant, but depends on the cultural conditions to which the yeast has been subjected during growth. Thus Willstätter and Sobotka showed that the value of the selectivity constant of a yeast which preferred glucose to fructose could be lowered by cultivating the yeast in a medium containing fructose. This result was confirmed by Sobotka and Reiner [1930] who found that the preference of a Sauterne yeast for fructose was lowered by cultivating the yeast in

maltose. It thus appears that living yeast possesses the power of adapting itself to the medium in which it is propagated. Explained by the Michaelis theory, this type of adaptation must be considered as being due to an alteration in the affinity of the enzyme for the proffered substrate.

Evidence for the variation in the affinity of an enzyme for its substrate already exists in the results of Kuhn [1923] who showed that the affinity of yeast invertase for sucrose varied in different samples of *S. cerevisiae* examined, while Willstätter, Kuhn and Sobotka [1924] observed a similar lack of constancy in the affinity of yeast maltase for maltose and for α-glucosides. Since a variation of this type is observed with what are regarded as different races of *S. cerevisiae*, it is not surprising that the same race of yeast should, when grown under different conditions, exhibit differences in the affinity constants of its enzymes for particular substrates.

It is not suggested that the above treatment is more than an outline of the methods by which we may arrive at an understanding of the factors governing selective fermentation. A strictly mathematical treatment should include an analysis of the effect of the variation in concentration of one substrate on the affinity constant of the other substrate. The problem is, however, a biological one; selection by living yeast takes place within the cell, and our knowledge of the localisation and co-ordination of the enzyme systems in the cell, and of the methods by which the local concentrations of the substrates near the enzyme may be altered, is negligible. It is well known that the destruction of the organic whole, which occurs when the yeast cell is dried or macerated, injures the mechanism which controls the reaction between hexose and phosphate. It is, therefore, not surprising that with such dead preparations the degree of selectivity, which, in the living cell, is apparently controlled by the affinity and concentration relationships which exist between the enzyme and its competing substrates, should also be profoundly modified.

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