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Note

Enzymic hydrolysis of sucrose*

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The inversion of sucrose is a reaction that takes place between a molecule of water and a molecule of the sugar, and proceeds at a noticeable rate only in the presence of a catalyst (H^+ ions or invertase). The questions as to how the rate of reaction is affected by the concentration of sucrose, and what is the real mechanism of the reaction, still remain incompletely answered.

Conventional descriptions of molecules give no clue as to their reactivities with water. The geometrical position, in space, of each group in the molecule of sucrose must be known, together with some idea as to the strength of the association of water to the sucrose molecule. A distinction must also be made between the description of the rate of the reaction of hydrolysis and an understanding of the reaction (which involves a concept of its mechanism).

Generally, it is satisfactory when a rate of hydrolysis is described by an equation, even if the path of the reaction is not well understood. This does not seem to be the case for the inversion of sucrose; the experimental results do not fit a classical equation describing the inhibition of invertase by the concentration of the substrate. In this connection, it is edifying to recall a statement made over 30 years ago by Moelwyn-Hughes[1] in the introduction to his book *The Kinetics of Reactions in Solution*:

> "The inversion of sucrose is a classical reaction in several respects. It was the first reaction which was timed and which was used for studying the relation between temperature and the rate of reaction. This reaction has attracted the attention of hundreds of investigators and over one thousand publications dealing with its kinetics have appeared since Wilhelmy (1850) [ref. 2]. That we are still very far from under-

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*Relation Between the Structure and the Properties of Carbohydrates in Aqueous Solutions. Part I.

standing its mechanism is an illuminating comment on the difficulties attending the problem in general".

The difficulties outlined by Moelwyn-Hughes in achieving an understanding of the mechanism of the reaction of sucrose inversion are still with us, and they have recently been noted by Klostergaard[3] as a source of textbook errors. We now interpret the effect of substrate concentration on the rate of the reaction of inversion of sucrose in the light of our recent[4,5] results on the structure, and the molecular association, in aqueous sucrose solutions of various concentrations.

EXPERIMENTAL

Aqueous sucrose solution (20 mL) was placed in a buffer (acetic acid and 0.1M sodium acetate, pH 4.5) in the presence of 0.2 mL of invertase (Sigma Product, Grade VI) solution at a concentration of 2 $g.L^{-1}$. The concentrations of sucrose ranged between 0.01M and 2.2M. The enzymic activity of the invertase was determined at 25° by monitoring the formation of reducing sugars as a function of time, in order to determine the initial rate of the reaction. The content of reducing sugars was determined by the dinitrosalicylate method[6]. Standardization of the method was made with an equimolecular mixture of D-glucose and D-fructose. Enzymic activities are expressed in micromoles of reducing sugars formed during 1 minute (U) under the conditions of the test. The specific activity of the sample of invertase was 700 U per mg of protein.

TABLE I

VARIATION OF THE INITIAL RATE OF HYDROLYSIS (U) AS A FUNCTION OF THE SUCROSE CONCENTRATION (M)

Sucrose concentration (M)	*Initial rate of hydrolysis* (*U*)
0.01	21.2
0.02	46.5
0.05	73.9
0.10	95.3
0.20	102.6
0.30	103.4
0.50	88.5
0.60	81.2
0.80	69.5
0.90	63.4
1.00	62.6
1.10	60.6
1.20	56.2
1.40	40.8
1.60	35.1
1.80	32.3
2.00	21.4
2.20	19.8

RESULTS AND DISCUSSION

The initial rates of sucrose hydrolysis catalyzed by invertase are shown in Table I. They indicate a very rapid decrease in the enzymic activity at sucrose concentrations >0.3M. The experimental curve shown in Fig. 1 cannot be described by a standard model of an equation fitting the inhibition by an excess of substrate, such as Eq. *1*.

$$v = V_{max}[S]/(K_M + [S] + [S]^2/K_s), \qquad (1)$$

where v = the rate of the reaction of hydrolysis catalyzed by invertase, V_{max} = the value of the maximum of the rate of the enzymic reaction, K_M = the Michaelis constant, K_s = the constant of inhibition by an excess of substrate, and [S] the sucrose concentration. The values of constants determined at sucrose concentrations <0.3M are V_{max} = 140 μmol.min^{-1}, and K_M = 42mM.

The decrease in the enzymic activity as the sucrose concentration is increased does not occur in a regular way. It shows two plateaus, at sucrose concentrations of M and 1.8M (see Fig. 1). It should be noted that such plateaus, even if they were not taken into account, appeared in the experimental curves reported in previous papers[7,8].

The difficulty in describing, by model equations, invertase action in the presence of concentrated sucrose solutions has been reported by various authors[7-9]. Taking into account the influence of viscosity and of water concentration, they did not

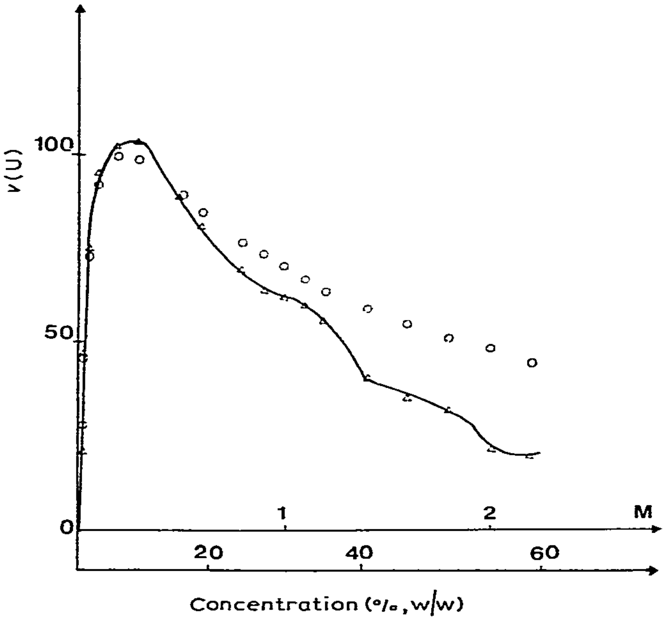

Fig. 1. Initial rate of hydrolysis catalyzed by invertase, v(U), *versus* molar concentration of sucrose solutions. [Key: —△—, experimental values; —○—, values calculated on the basis of model equation of inhibition by an excess of substrate.]

succeed in finding a definite interpretation of the experimental results. The same holds true in respect of acid hydrolysis, for which the different equations proposed[10] to take into account pH values and concentrations in sucrose do not fit the experimental results when the sucrose concentration is high.

Our recent[5] results concerning the structure of the sucrose molecule in aqueous solutions of various concentrations allow us to propose a qualitative explanation of the difference between calculated and experimental values of the rate of hydrolysis (see Fig. 1). The structures of the sucrose molecule that we proposed[5] involve 0, 1, or 2 intramolecular hydrogen-bond(s) in increasingly concentrated aqueous solutions. As splitting of the sucrose molecule occurs at the oxygen atom of the glycosidic linkage[10], the intramolecular bonds must be taken into account when the rate of hydrolysis of sucrose in concentrated solutions is interpreted. Thus, the fact that the experimental values are lower than those estimated by use of the equation for inhibition by an excess of substrate (see Fig. 1) might be assigned to the possibility that the "folded" form of sucrose may not be hydrolyzed by invertase. The effective concentration of substrate should be lower than the apparent concentration of sucrose in the solution. The sole, reactive form is probably that lacking intramolecular bonds.

Moreover, it may be noted that the discontinuities observed in the experimental curve (see Fig. 1) correspond to the thresholds of concentrations found in previous laser–Raman[4] and X-ray diffraction[5] studies of solute–solvent interactions in aqueous solutions of sucrose. The model of organization of these solutions that was proposed[5] supposes a preponderance of the "free-water" structure in dilute solutions (<0.8M), and the coexistence of a regular arrangement of hydrated sucrose molecules and of hydration water in the range of concentrations lying between 0.8 and 2.4M; above this concentration is detected the only organization of associated sucrose molecules.

As the hydrolysis reaction is a consumer of water molecules, its rate will depend on the availability of water molecules. Our previous results[4,5] located a discontinuity in sucrose–water interactions at ~M and ~2.4M concentrations, and these values coincide with those at which the plateaus are observed in the experimental curve (see Fig. 1).

It may be concluded that the hydrolysis of sucrose catalyzed by invertase has a mechanism comparable to that of acid hydrolysis, in that it occurs at the glycosidic oxygen atom. Hence, it might be sensitive to the "folding" of the molecule as the concentration is increased. The intramolecular bonds so formed protect the glycosidic bridge, and prevent sucrose inversion. In addition, the state of association of water with sucrose seems to be important for understanding of the mechanism of the reaction. It seems that hydration water should not be available for hydrolysis, and that the deviation of experimental results from the kinetic model described could probably be due to the fact that water becomes a limiting reactant, as in hydrolysis of "dry" systems[11].

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