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WATER CONCENTRATION AND THE RATE OF HYDROLYSIS OF SUCROSE BY INVERTASE

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Many investigators[1] have studied the relation between the rate of hydrolysis of sucrose by invertase from yeast and the concentration of the sugar. This relationship, as observed by Ingersoll, is shown graphically by the heavy lined Curve 1, in Fig. 1. It will be seen that the velocity

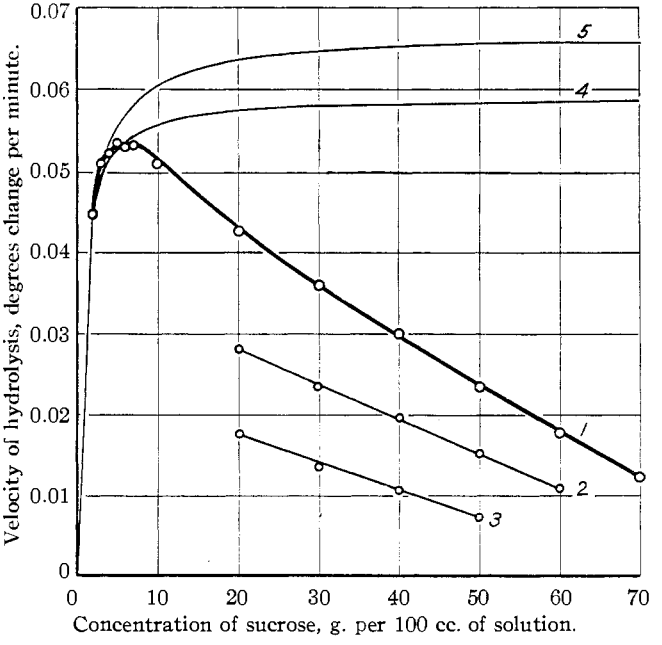

Fig. 1.—Curve 1, sucrose and no alcohol; 2, sucrose and 10% alcohol; 3, sucrose and 20% alcohol; 4, theoretical curve, K = 0.017; 5, theoretical curve, K = 0.030.

rises gradually as the sucrose concentration is increased until the latter reaches about 5%, after which there is a dropping off, and at 70% of sucrose the velocity is only about one-fourth of the maximum value reached at 5%. So far no very satisfactory explanation has been found

[1] (a) O'Sullivan and Tompson, *J. Chem. Soc.*, **57**, 834 (1890); (b) Brown, *ibid.*, **81**, 373 (1902); (c) Henri, *Z. physik. Chem.*, **39**, 215 (1902); (d) Michaelis and Menten, *Biochem. Z.*, **49**, 333 (1913); (e) Euler and Myrbeck, *Z. physiol. Chem.*, **124**, 159 (1922); (f) Achalme and Bresson, *Compt. rend.*, **152**, 1328, 1420, 1621 (1911); (g) Colin and Chaudun, *J. chim. physik.*, **20**, 4719 (1925); (h) Ingersoll, *Dissertation*, Columbia University, **1925**; (i) *Bull. soc. chim. biol.*, **8**, 264, 276 (1926).

for this peculiar relationship. It is apparent from the shape of Curve 1 in Fig. 1 that in the more concentrated region, 10 to 70% sucrose, it approaches very closely to a straight line and at a first glance it might seem that the velocity is some simple function of the sucrose concentration. But, as Ingersoll has pointed out, the water concentration also drops off linearly with the increase in sucrose concentration and if the velocity is plotted against the concentration of water instead of the concentration of sucrose, then a curve, heavy lined in Fig. 2, is obtained which it will be noticed is very similar in shape to the velocity–sucrose concentration

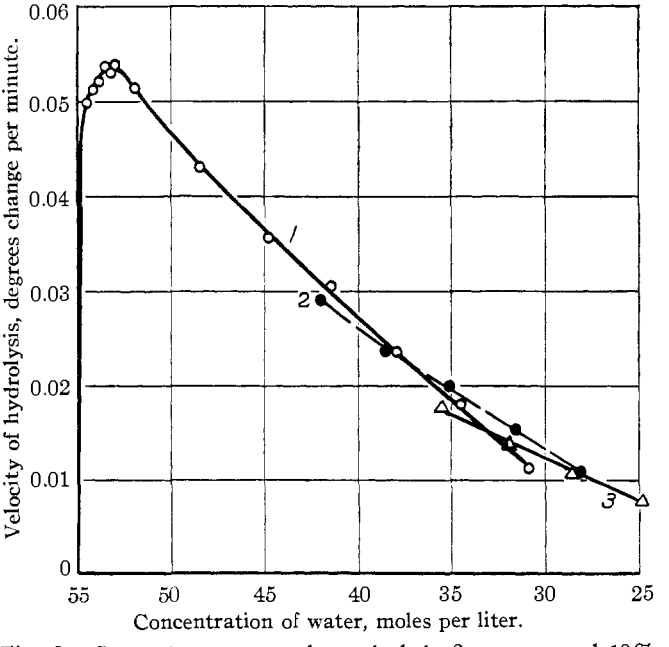

Fig. 2.—Curve 1, sucrose and no alcohol; 2, sucrose and 10% alcohol; 3, sucrose and 20% alcohol.

Curve 1, in Fig. 1. In other words, the velocity in the case of sucrose solutions ranging in concentration from 10 to 70%, drops off as the sucrose concentration increases and as the water concentration decreases, and for this reason Ingersoll was unable to decide whether it is the concentration of sucrose or the concentration of water or both together which determines the magnitude of the velocity of hydrolysis when the concentration of sucrose is greater than 5%.

In the present study, the authors have tried to avoid the difficulty encountered by Ingersoll, that is, not being able to vary the sucrose concentration without simultaneously varying that of the water, by adding to the sucrose solutions alcohol, which has only a small retarding

effect, thereby making it possible to vary the sucrose and water concentrations independently of each other. For this purpose several series of solutions, varying in sucrose content from 20 to 70%, were made up. Each solution of these series contained a constant quantity of alcohol, and the composition of the solutions and their respective rates of hydrolysis are given in Table I. These values have been plotted in two different ways; that is, in Fig. 1 the velocities have been plotted against the corresponding sucrose concentrations, while in Fig. 2 the velocities are plotted against the corresponding concentrations of water. Upon comparing the curves in the two figures, it will be seen that when the velocities are plotted against water concentration, then Curves 2 and 3, Fig. 2, lie very close to the curve (1) for sucrose alone. On the other hand, when the velocities are plotted against sucrose concentration, Curves 2 and 3, Fig. 1, then they are spread much farther apart.

TABLE I

HYDROLYSES AT VARIOUS CONCENTRATIONS OF SUCROSE AND ALCOHOL

Constant amount of a yeast invertase preparation, "Convertit," diluted 200 times used in all hydrolyses. Buffer, 0.01 molar sodium citrate, $P_H = 4.5 \pm 0.1$. Velocity given in degrees change in rotation per minute. Hydrolyses run at 25.00°. All rotations determined at 25.0°; light source, 5461 Å. Water concentrations given in moles per liter of solution

Sucrose, g./100 cc. of soln.	No alcohol		10% alcohol		20% alcohol	
	Vel.	Water concn.	Vel.	Water concn.	Vel.	Water concn.
2	0.0448	54.6	...	..	...	..
3	.0511	54.2	...	..	...	..
4	.0522	53.9	...	..	...	..
5	.0534	53.5	...	..	...	..
6	.0530	53.2	...	..	...	..
7	.0532	52.8	...	..	...	..
10	.0512	51.8	...	..	...	..
20	.0428	48.3	0.0280	41.9	0.0177	35.5
30	.0360	44.9	.0236	38.4	.0137	32.0
40	.0301	41.4	.0197	35.0	.0106	28.5
50	.0235	38.0	.0153	31.5	.00716	24.9
60	.0178	34.4	.0109	28.0	...	..
70	.0124	30.9	...	..	...	..

The fact that the alcohol curves in Fig. 2 fall so close to the sucrose only curve indicates that the velocities of hydrolysis are nearly the same, irrespective of the amounts of alcohol and sucrose which the solutions contain (provided the water concentration is the same). Thus it does not make so very much difference in the magnitude of the velocity whether, for example, the following three solutions contain: 20% of alcohol and 20% of sucrose; 10% of alcohol and 45% of sucrose; or no alcohol and 60% of sucrose, the velocities for these three solutions being 0.0177, 0.0175 and 0.0178, respectively. In other words, the above results indicate that the concentration of water is the primary factor, while the

concentrations of alcohol and sucrose and possibly some other influences are of minor significance in determining the magnitude of the velocity of hydrolysis when the concentration of sucrose is beyond 20%.

Significance of Results for Current Theories.—The theory of the mechanism of the invertase inversion of sucrose most widely accepted is that of Michaelis. The two fundamental assumptions of this theory are (1) that sucrose combines with invertase according to the mass law and (2) that the velocity of inversion is proportional to the amount of invertase combined with sucrose. With these assumptions, the mass law is used to calculate from measurements of velocities of hydrolysis the affinity constants of various invertases for sucrose. The relation between velocity of inversion and sucrose concentration derived on these assumptions is represented in Curve 4, Fig. 1. According to this theory, as the sucrose concentration is increased, more and more of the invertase combines with sucrose to form a complex and the velocity of hydrolysis increases until a sufficiently high sucrose concentration is reached so that practically all of the invertase exists in the combined form. Then the velocity has reached a maximum value and further increase of sucrose should have no effect on the velocity.

One of the methods for determining the affinity constant of invertase for sucrose requires the determination of the maximum velocity of hydrolysis with a given amount of invertase, the assumption being that then all the invertase is in combination with sucrose. But the experimentally determined relation (Curve 1, Fig. 1) shows that the velocity maximum is attained at 5% or 0.176 *M* sucrose. Since the velocity drops off beyond this sucrose concentration, it has been customary with workers determining affinity constants to neglect the portion of the curve corresponding to sucrose concentrations above 5 to 6% and to assume that, at the experimental maximum, all of the enzyme is combined with sucrose. Yet examination of the curves in Fig. 1 shows that at 5% of sucrose the theoretically derived curve (4) is still 10% short of the true maximum velocity attainable. Curve 4 is plotted,[2] using Michaelis and Menten's original value of 0.017 as the dissociation constant of the sucrose–invertase complex. More recent work, like that of Kuhn and Münch[3] and also the results obtained by Nelson and Larson, give an average value for the above dissociation constant of about 0.030. Using this value in the Michaelis and Menten equation, Curve 5, in Fig. 1 is obtained, where at 5% of sucrose the velocity is still 17% short of its maximum. This means that the affinity constant

[2] The data for Curves 4 and 5 in Fig. 1 were obtained by substituting for S, in the equation $V = S/(S + K)$, the sucrose concentrations above, and multiplying the values obtained by the factors 0.590 and 0.6688, respectively, to make the curves coincide roughly with the first portion of Curve 1.

[3] Kuhn and Münch, *Z. physiol. Chem.*, **163**, 1 (1927); Nelson and Larson, *J. Biol. Chem.*, **73**, 223 (1927).

of invertase for sucrose determined by the method of Michaelis and Menten, if it exists at all, is probably wide of its true value.

The results obtained in the present work with alcohol and more concentrated sucrose solutions make it look as if the decrease in water concentration might be the disturbing factor. As the sucrose concentration increases, the influence of the decreasing water concentration becomes relatively more significant and therefore will tend to mask the increase in velocity brought about by the increasing sucrose concentration forming a higher concentration of the enzyme–sucrose complex. From this point of view, the peculiar shape of the curve relating velocity of hydrolysis to sucrose concentration (Fig. 1, Curve 1) must be considered to be the resultant of at least two effects, both due to the increase in sucrose: (1) the increasing formation of sucrose–invertase complex and (2) the decrease in water concentration. Furthermore, the influence of the decreasing water concentration, which predominates at sucrose concentrations above 7% has very probably begun to operate at lower sucrose concentrations. Thus it seems that even in the first segment of the velocity–sucrose concentration curve (Curve 1, Fig. 1) up to 6% of sucrose, the water concentration factor of which the Michaelis theory takes no account will have to be considered. In view of the extensive application of the affinity constant of an enzyme as an essential characteristic in its description, and also in accounting for its specificity,[4] this influence of the water concentration becomes a matter of considerable importance.

Experimental Procedure.—The hydrolyses were run by making up 250 cc. of solution containing sucrose, alcohol for some and buffer of such concentrations that when 200 cc. was placed in a bottle and 25 cc. of invertase solution added, the resulting solution was of a certain desired sucrose and alcohol concentration as well as 0.01 *M* with respect to buffer (sodium citrate was used) and at a P_H of 4.5 ± 0.1. Solutions of 30%, that is, 30 g. per 100 cc. of solution, total (sucrose and alcohol) concentration or less were measured out by pipetting, using calibrated pipets; those of higher total concentration were weighed, using the specific gravities to calculate the weight of 200 cc. Hydrolyses were started by allowing the 200 cc. of sucrose and alcohol solution at least forty minutes to come to the bath temperature (25.00 ± 0.02°), then delivering into it 25 cc. of invertase solution, which had also come to that temperature, and shaking vigorously. The invertase delivery was made by a pipet calibrated to deliver 25.00 ± 0.02 cc. of invertase solution in seven seconds. For solutions below 30% total concentration, 25cc. samples were withdrawn at various intervals of time after the start and added to 5 cc. of 0.2 *M* sodium carbonate solution to stop the reaction and complete mutarotation. In the case of solutions of total concentration above 30%, samples were withdrawn and 25 cc. weighed out as recommended by Ingersoll[1h,i] and then 5 cc. of the carbonate solution added. Hydrolyses were run at 30% total concentration, using both methods to make sure they checked. Individual hydrolyses were run as

[4] Kuhn, *Z. physiol. Chem.*, **125**, 1 (1923); Euler, "Chemie der Enzyme," Bergmann, Munich, **1925**, p. 350; Waldschmidt-Leitz, "Die Enzyme," Vieweg and Son, Braunschweig, **1926**, p. 67; Oppenheimer, "Die Fermente," Georg Thieme, Leipzig, **1925**, p. 193.

far as they could be without showing deviations of more than the experimental error from a linear course.

The method of calculating the initial velocity of hydrolysis consists in dividing the change in rotation for each sample of the hydrolyzing sucrose solution taken by the time in minutes after the start at which that sample was taken. The change in rotation for a given sample is the difference between the initial rotation of the hydrolyzing solution and the rotation of that sample after the addition of 5 cc. of sodium carbonate solution. This ratio was calculated for each sample and the mean taken as the velocity for that particular sucrose solution. This procedure made it quite essential to determine the initial rotation very accurately and this was found to be rather difficult in the more concentrated solutions where slight errors in weighing out samples (weighed to the nearest drop) were liable to make a considerable error in the observed rotation. So in addition to synthetic initials, made by mixing 3.125 cc. of invertase solution with 5.625 cc. of the carbonate solution and then adding 25.00 cc. of the original 250 cc. of sucrose solution made up for the hydrolysis, a sample was taken within three to six minutes after the start of the reaction and its rotation used as a basis for extrapolating back to find the rotation at zero time. The mean of these two, the synthetic and the extrapolated initials, was used as the true initial rotation.

Summary

The above study shows that the concentration of water is a factor in determining the magnitude of the velocity of hydrolysis of sucrose by invertase.

NEW YORK CITY

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF CALIFORNIA]

THE HEAT CAPACITY OF HYDROGEN BROMIDE FROM 15°K. TO ITS BOILING POINT AND ITS HEAT OF VAPORIZATION. THE ENTROPY FROM SPECTROSCOPIC DATA

BY W. F. GIAUQUE AND R. WIEBE

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In a recent paper[1] we have shown that the entropy of hydrogen chloride as calculated with the assistance of spectroscopic data is in agreement with the value as obtained from measurements of heat capacity and the third law of thermodynamics. Here we shall present similar calorimetric data on hydrogen bromide and show, in this case, also, agreement between the two methods of obtaining the entropy.

Preparation of Hydrogen Bromide.—Hydrogen bromide was prepared directly from the elements. The hydrogen was prepared by electrolysis and the oxygen present

[1] Giauque and Wiebe, THIS JOURNAL, 50, 101 (1928).