[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLUMBIA UNIVERSITY]

Influence of the Extent of Hydrolysis upon the Determination of the Initial Velocity of α-Amylase Action[1]

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In reactions catalyzed by α-amylases, the velocity depends markedly upon the extent of hydrolysis. If this fact is neglected in the treatment of kinetic data, erroneous conclusions will result. A simple method for treating kinetic data, which permits comparable and well defined initial velocities to be obtained with α-amylases, is presented.

Introduction

In random or random-like depolymerization of condensation polymers, the molecular weight and usually the molecular weight distribution of the polymer changes during the reaction. In some cases, particularly in enzymatically catalyzed reactions where steric factors are important, the velocity of the reaction depends upon these molecular characteristics of the polymer.[3] Such a dependence is marked with α-amylases.[4-9] The data reported here for the action of crystalline pancreatic amylase from swine[10] on defatted waxy maize starch[7,8,11] illustrate the erroneous and ambiguous conclusions that are obtained when these molecular characteristics are neglected in kinetic studies. A simple method for obtaining comparable and well defined initial velocities for such complex systems is suggested.

Experimental

Amylase.—Crystalline pancreatic amylase from swine[10] was used in this work.

Substrate.—The substrate was waxy maize starch[12] that had been used in previous studies with this amylase.[7,8] It had been defatted exhaustively[11] and gave no evidence of contamination by linear components.[13-15] It had been washed thoroughly with cold distilled water to remove low molecular weight impurities.

Methods.—The hydrolyses were carried out at 40° as described previously.[10] The extent of hydrolysis of the higher substrate concentrations investigated was measured by an iodometric procedure.[16] A modification of this method[17] was used for the lower substrate concentrations studied (0.03 to 0.01%).

Results

As part of an investigation[18] of the hydrolysis of waxy maize starch by pancreatic amylase from swine, the authors were interested in determining the dependence of the velocity of hydrolysis upon the substrate concentration. Starch concentrations between 0.5 and 0.08% were investigated first, because previous studies with this and other α-amylases[4-6,19,20] had indicated a dependence of velocity on substrate concentration within this range. Since previous work in this Laboratory had indicated that the velocity of hydrolysis by α-amylases is strongly dependent on the extent of reaction or on the molecular weight of the products[7,8,20] the data were plotted in a manner which permitted comparison of the velocities at the same extent of reaction. This comparison was made by plotting the fraction of the bonds hydrolyzed *versus* the time divided by the initial substrate concentration. Since, in this type of plot the ordinate is the extent of reaction and the slope of the curve is the rate, the desired comparison is made easily. The single curve obtained for the four substrate concentrations, Fig. 1, shows that the velocity of hydrolysis is independent of the substrate concentration for the range studied. The curvature of the line shows the previously mentioned dependence of the rate on the extent of reaction.

The conclusion that the velocity of hydrolysis of waxy maize starch by swine pancreatic amylase is independent of the substrate concentration within the range of substrate concentrations studied (0.5 to 0.08%) does not agree with the findings of Bernfeld and Studer-Pécha[5] for the action of swine pancreatic amylase with substrate concentrations within the same range. The reason for this discrepancy is shown in Fig. 2 where the data given in Fig. 1 are plotted as amount of products formed *versus* time of hydrolysis, the form employed by Bernfeld and Studer-Pécha[5] and by other previous investigators.[4,6,19,20] In this case, Fig. 2, four different curves are obtained, and from these one can calculate four apparently different "initial

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[2] The data reported here are taken, in part, from a dissertation submitted by John W. Van Dyk in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Chemistry under the Faculty of Pure Science of Columbia University.

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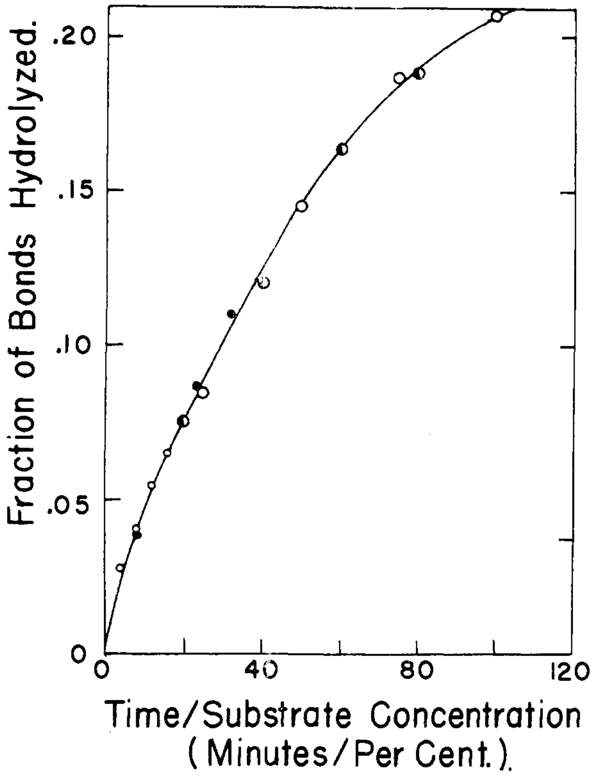

Fig. 1.—Hydrolysis of waxy maize starch by swine pancreatic amylase in the presence of excess substrate; substrate concentration: o, 0.5%; ●, 0.25%; ◐, 0.1%; O, 0.08%.

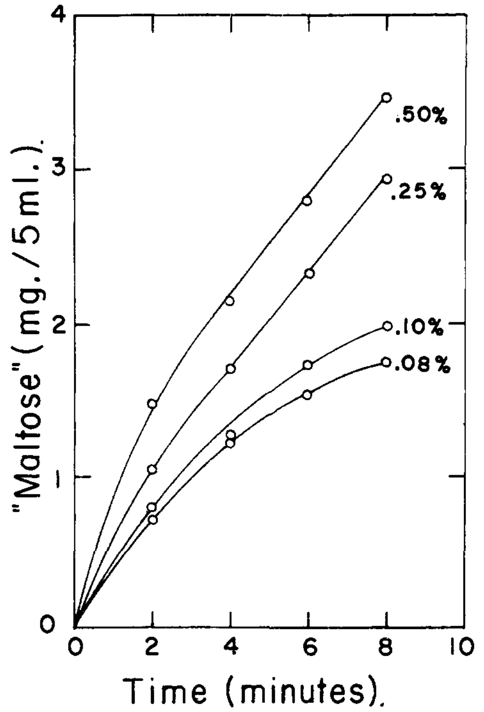

Fig. 2.—Apparent influence of substrate concentration on the rate of the hydrolysis of waxy maize starch by swine pancreatic amylase.

velocities" by an estimation of the slopes of the curves at zero time. That the "initial velocities" calculated in this manner are incorrect is evident from the single curve shown in Fig. 1. The dependence of "initial velocity" on substrate concentration, obtained above, in Fig. 2, is an artifact which arises from neglecting the dependence of the velocity on the extent of the reaction. A true dependence of velocity on substrate concentration is observed with this amylase and substrate only when more dilute substrate solutions (0.03 and 0.01%) are used, as shown in Fig. 3.

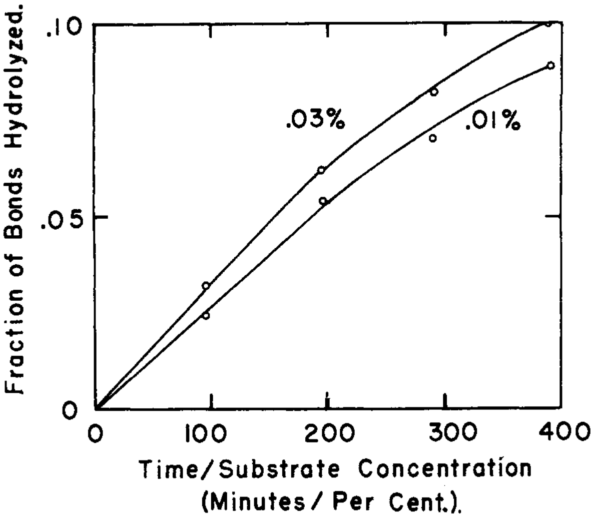

Fig. 3.—Influence of substrate concentration on the rate of the hydrolysis of waxy maize starch by swine pancreatic amylase.

Discussion

Accurate and comparable initial velocities as a function of substrate concentration are essential to a kinetic study. They can be obtained conveniently by defining the "initial" velocity (V_0) in terms of the reaction time (t) necessary for the hydrolysis of a given small fraction of the bonds (F)

$$V_0 = \frac{Fa_0}{t}$$

where a_0 is the initial substrate concentration.

In addition to being comparable, the velocities obtained by the above equation can be reproduced or used by investigators in different laboratories since the vagaries associated with the estimation of the slope at zero time are not involved. It should be noted, however, that V_0 equals the velocity at zero time only if F is infinitesimally small, therefore F should be chosen as small as possible. Since choice of F will be limited by the precision of the measurements, a sensitive method should be used. In the case of the hydrolysis of starch fractions, spectrophotometric measurements are useful for this purpose.[21,22]

The procedure outlined here has been employed in an extensive investigation of the kinetics of the action of swine pancreatic amylase. This work is reported in a subsequent communication.[18]

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