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Studies on the Kinetics of Amyloglucosidase as Affected by Native and Derivatized Corn Starch

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ABSTRACT

The influence of the degree of starch etherification on the kinetic parameters of amyloglucosidase was studied. The K_m *and* V_{max} *constants were affected by the degree of substitution of corn starch. The hydroxyethyl derivatives of corn starch were prepared using three molar ratios of starch: ethylene oxide (7:1, 7:2 and 7:3). The degrees of etherification of starch were calculated as 0·05, 0·09 and 0·2 for the three derivatives.*

The kinetic parameters clearly show that the treatment of corn starch with ethylene oxide leads to a one-and-a-half times increase in the K_m *value which is 43·5 mM with a low degree of corn starch etherification (0·05). At higher degrees of etherification* K_m *values were not affected.*

The active site studies prove that amyloglucosidase has one substrate binding site and the equilibrium constant (K′) *was calculated as 22·9 mM.*

INTRODUCTION

Starch ether derivatives (hydroxyethyl starch) are prepared by the reaction of alkaline starch and ethylene oxide. These derivatives are

considered to be suitable for use in food; they have desirable properties for pie fillings, salad dressings and as food thickeners. In recent years they have been used in numerous industrial processes (Whistler & Paschall, 1967). The textile and paper industries constitute the largest and most diversified industries in which hydroxyethyl starch is used (Hjermstad & Whistler, 1959). The use of hydroxyethyl starch as a blood volume expander and as a cryophytic agent for erythrocytes has recently been suggested (Knorpp *et al.*, 1967).

In this paper the effect of the etherification of starch (degrees of etherification; 0·05, 0·09 and 0·20) on the kinetic parameters of amyloglucosidase [α-D-(1 → 4)-glucan glucohydrolase] is studied.

MATERIALS AND METHODS

Source of samples

Corn starch was provided by the Egyptian Starch and Glucose Manufacturing Co., Mostorod, Egypt, and amyloglucosidase by the NOVO Company, Denmark.

Preparation of starch derivatives

The method described by Kesler & Hjermstad (1964) was adopted to prepare hydroxyethyl derivatives of corn starch. The molar ratios of starch:ethylene oxide were 7:1, 7:2 and 7:3 for the preparation of three derivatives. The degree of substitution was determined using the technique adopted by Fritz & Schenkin (1959).

Enzyme assay

Amyloglucosidase activity was determined by the method of Attia & Ali (1974). It is summarized as follows.

To 1 ml of 1 % starch solution (in acetate buffer at pH 4·3), 1 ml of the enzyme solution (0·02 units AG/ml) was added. The mixture was then incubated at 60 °C for 5 min. After the incubation period, 2 ml of chromogenic reagent (3,5-dinitrosalicylic acid) were added. The mixture was heated in a water bath for 15 min, then cooled and diluted with 20 ml of distilled water; absorbance at 530 nm was measured.

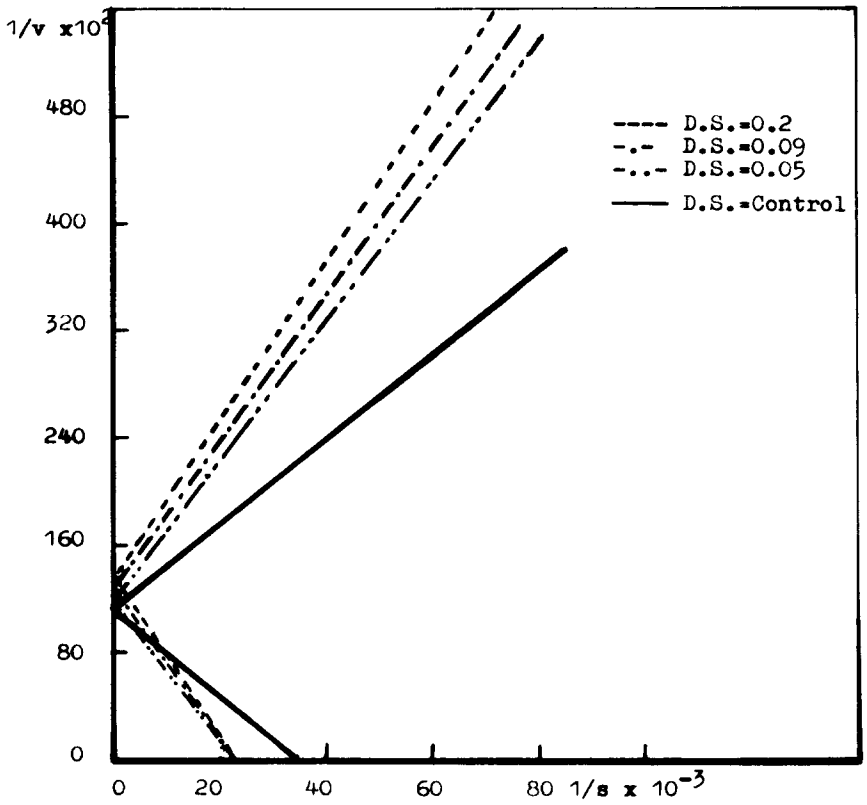

Fig. 1. $1/v$ versus $1/s$ (corn starch).

TABLE 1
Kinetic Parameters of Amyloglucosidase on Corn Starch

Treatment	*Molar ratio starch: ethylene oxide*	*Degree of substitution (D.S.)*	*Kinetic parameter* V_{max}	K_m (mM/litre)	K_m/V_{max}
Corn starch	—	—	0·892 8	28·5	31·93
Corn starch DS_1	7:1	0·05	0·833 3	43·5	52·20
Corn starch DS_2	7:2	0·09	0·781 3	43·5	55·68
Corn starch DS_3	7:3	0·20	0·735 3	43·5	59·16

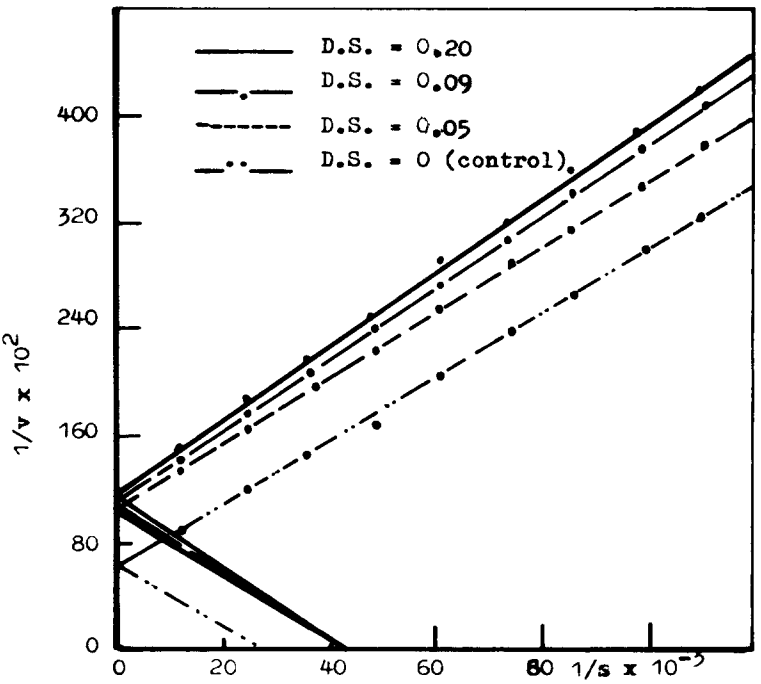

Fig. 2. $1/v$ versus $1/s$ (corn starch).

RESULTS AND DISCUSSION

Determination of Michaelis constant

The Michaelis constant (K_m) was determined experimentally using two techniques adopted by Lineweaver & Burk (1934) and Harper *et al.* (1979). The results represent the average of three replicates. Results in Table 1, illustrated by Figs 1 and 2, show that the K_m values of all etherified starches were the same—43·5 mM. The K_m value of native corn starch was 28·5 mM. Maximum enzyme velocities (V_m) were 0·892 8, 0·833 3, 0·781 3 and 0·735 3 for native corn starch (0·05, 0·09 and 0·2 degrees of substitution, respectively).

Following the Woolf plot technique, the results proved significant. The kinetic studies clearly show that starch, treated with different substitution values of ethylene oxide, reduced the amyloglucosidase affinity (28·5 mM for native corn starch and 43·5 mM for all other degrees of substitution).

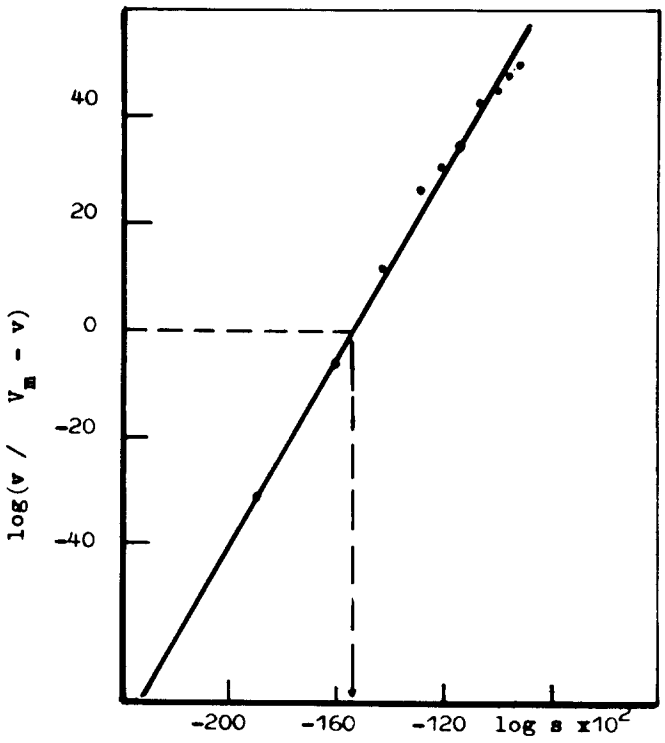

Fig. 3. Hill plot for amyloglucosidase on corn starch.

Enzyme properties using Hill plot

An expression describing the velocity (v) as a function of substrate concentration is obtained from the following equation after Piszkiewicz (1977):

$$\log v/V_m - v = n \log (s) - \log K'$$

where n represents the number of molecules and K', the equilibrium constant, is equal to $K_2 + K_3/K_1$.

Figure 3 is a plot of $\log (v/V_m - v)$ versus $\log (s)$ which gives a straight line. The slope of this line (n) is equal to the number of substrate binding sites, as mentioned by Piszkiewicz (1977).

From the Hill plot (Fig. 3), $\log K'/n$ is calculated as $-1{\cdot}36$ and the slope (n) is equal to 0·883 1. Thus, $\log K'$ is equal to 1·54 mM. It can be concluded that amyloglucosidase has one substrate binding site and its equilibrium constant (K') is 22·9 mM.

CONCLUSIONS

It can be concluded from the action of amyloglucosidase on corn starch and its derivatives that the affinity of the enzyme was altered when the natural substrate was treated with ethylene oxide. We suggest that the decrease in affinity of the enzyme is due to the action of ethylene oxide on (OH^-) groups at C_1, C_3 and C_6, forming a new bond with different binding energy which affects the conformation of substrate and hinders the enzyme–substrate interaction.

Also, at low degrees of etherification, the corn starch reacts with a large amount of ethylene oxide. We suggest that the saturation of binding sites on the corn starch molecule reached a maximum at etherification degree 0·05, any further increase in etherification degree not affecting the enzyme. Substrate interaction may, however, be affected inasmuch as maximum velocity decreases with increasing etherification degree (0·05, 0·09 and 0·20 D.S. being 0·833 3, 0·781 3 and 0·735 3, respectively).

Thus, industries which use the corn starch substitutes should take a molar ratio of starch:ethylene oxide of 7:1. Avoiding an excess of ethylene oxide is very important as it inhibits amyloglucosidase action. Otherwise, the enzyme concentration must be increased.

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