ENZYMIC DEGRADATION OF CRYSTALLINE HYDROCELLULOSE

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In all instances where study of the enzymic degradation of cellulose has included consideration of the effect of the size of the substrate particles on the velocity of the reaction it has been reported that smaller particles are more rapidly attacked than larger ones. The reviews of Tracey (1953), Cowling (1963), and King (1964) discuss a large number of instances where this relationship of particle size to reaction velocity has been described qualitatively.

Other than the statement that the velocity appears to depend on the exposed surface area, however, no quantitative relationship has been developed. With only limited experimental support the assumption that enzymic attack on cellulose is an exclusively surface process has become generally accepted.

The data presented here demonstrate a direct proportionality between substrate surface area and rate of enzymic solubilization of cellulose. In addition, they establish that something more than surface erosion is involved, a process appearing to represent fragmentation of the original substrate particles into sub-units which are still particulate.

Experimental system: The enzyme was a partially purified cellulase derived from Trichoderma viride as described by Li, Flora and King (1965). The substrate was a predominantly crystalline hydrocellulose in the Type I crystal lattice prepared as described by Flora (1964). Substrate particles of radius less than 3 μ were selected by differential flotation in filtered distilled water.

Particle size distributions were determined using a Fisher Autocytometer calibrated over the size range radii of 0.1 to 12 μ using polystrene latex, polyvinyltoluene latex, and styrenedivinylbenzene latex spheres. All solutions were filtered through a 100 mμ Millipore filter to remove dust.

Reaction systems included the following in a volume of 70 ml incubated at 40°C: 15 mg of substrate, 20 mg of enzyme protein, and 50 μmoles of potassium phosphate at pH 5.0. Control tubes contained no enzyme. At 10-minute intervals turbidity was measured in a Klett-Summerson colorimeter with a 660 mμ filter, and 0.10 ml portions were added to 40 ml of 90% saturated $NaHCO_3$ to stop the action of enzyme until particle size distribution measurements could be made.

The course of the reaction in terms of total solubilization of substrate in a typical experiment follows "Schutz' Rule" (1885) as seen in Fig. 1 as do all enzymic hydrolyses of insoluble forms of cellulose. This unusual kinetic behavior has been demonstrated by Flora (1964) to be independent of product inhibition and of the presence of inhibitors in the crude enzyme as suggested by Dixon and Webb (1958). Rather it reflects the insolubility of the substrate.

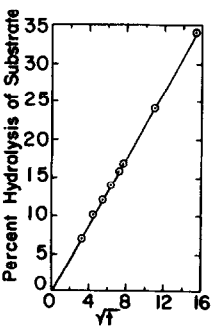

Fig. 1. Kinetics of enzymic hydrolysis of crystalline hydrocellulose

From the size-distribution data after each time increment, the corresponding amount of cellulose solubilized was determined by multiplying the volume of each particle by the change in number of such particles per unit volume of solution and this product by the density of the substrate, 1.1 g/ml. The

surface area of particles in each size class was then calculated assuming that the particles were spherical, the assumption being recognized as an approximation. Plotting the resulting values yielded Fig. 2.

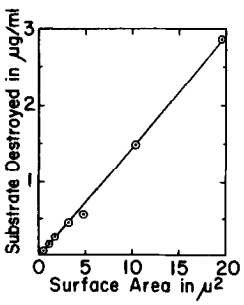

Fig. 2. Relationship of the rate of degradation of crystalline hydrocellulose particles to their surface area.

If the action of the enzyme were exclusively a surface erosion, the total number of particles per unit volume of reaction mixture should remain constant until particles too small to be detected were produced. From that point on the number of particles per unit volume should decrease. The experimental data, however, show a marked increase in the total number of particles during the initial stages of the reaction. This increase was attributable to a fragmentation of the larger initial particles into 800 to 1500 smaller ones

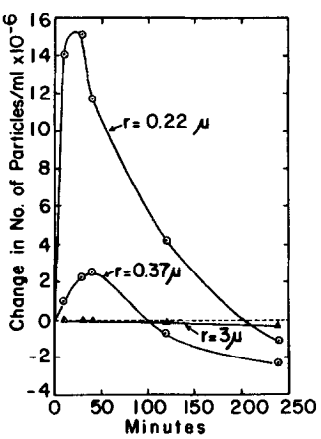

Fig. 3. Changes in the number of three size classes of hydrocellulose particles per ml during enzymic degradation.

as seen in Fig. 3. The precise number of sub-particles produced cannot be calculated until data of greater accuracy in the lower size classes can be obtained.

This fragmentation phenomenon appears to occur simultaneously with the surface erosion. It indicates action of a cellulase component not previously recognized as being involved in enzymatic cellulose degradation. In terms of both the nature of the substrate and the size of both the substrate and the sub-units it is distinct from the fragmentation of cotton fibers recently described by Halliwell (1965).

ACKNOWLEDGMENT

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