Formation of Hydrogen Peroxide by a Polyvinyl Alcohol Degrading Enzyme

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There have been much interest in microorganisms which are capable of degrading and utilizing synthetic polymers and in enzymes which catalyze the reactions involved. Microbial deteriorations of synthetic polymers have so far been reported occasionally, but mechanisms of degradation are obscure in most cases and enzymes responsible for are scarcely studied. In 1973, Suzuki *et al.*[1] have reported that a bacterium, *Pseudomonas boreopolis*, isolated from soil degrades and assimilates PVA,*1 a water soluble synthetic polymer, and that the bacterium produces an inducible enzyme which catalyzes an oxidative and endowise cleavage of PVA molecules. The enzyme, however, has not yet been purified, and neither the natures of the enzyme nor the mechanism of the reaction are elucidated.

Another species of pseudomonad bacterium has been isolated from soil in a medium in which PVA serves as the sole carbon source.*2 The bacterium is found to be not identical with *P. boreopolis* in that it turns the milk medium to alkaline and did not produce acids from some sugars.

A PVA degrading enzyme was purified from the culture broth of this organism by the following procedures: ammonium sulfate precipitation (0.4 saturation), SP-Sephadex column chromatography (pH 6.5, NaCl-gradient elution) and Sephadex G-75 gel filtration.*3 The enzyme reaction was performed in 20 ml of 0.05 M phosphate buffer (pH 7.3) containing 1.0% PVA at 27°C with reciprocal shaking. The viscosity of reaction mixture was measured by an Ostwald-type viscometer at 27°C. The activity was expressed as a relative decrease in viscosity, assuming a decrease down to the viscosity of water as 100%, and was calculated according to the following equation,

$$\text{Relative decrease in viscosity } (\%) = (V_0 - V_t / V_0 - V_w) \times 100$$

where V_0 was the time in second measured for the reaction mixture at zero min, V_t for the reaction mixture at t min and V_w for water. A unit of the enzyme was defined as the amount which brought about a relative decrease in viscosity of 10% per hr under the assay conditions.

A typical purification is shown in Table I. The purified enzyme sedimented as a single protein in ultracentrifugation as shown in Fig. 1, and migrated in a single band in disc electrophoresis as seen from Fig. 2. Concerning the reaction of the enzyme, it was found that the enzyme brought about a decrease in viscosity of a reaction mixture in which PVA was used as substrate, a drop of

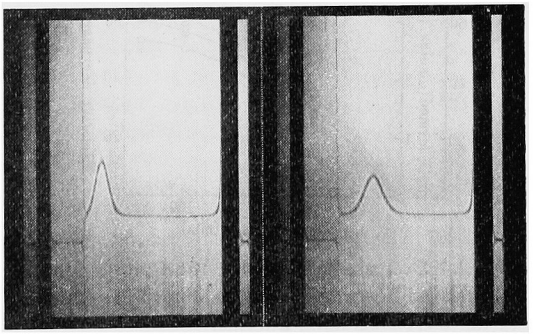


FIG. 1. Sedimentation Analysis of the Purified PVA Degrading Enzyme.

The enzyme protein was dissolved in 0.01 M acetate buffer (pH 6.5) at a cencentration of 10 mg per ml and subjected to a sedimentation analysis by a Hitachi ultracentrifuge UCA-1 at 20°C at 55,000 rpm. The photographs were taken at (left) 30 and (right) 60 min of centrifugation.

*1 Abbreviation used; PVA, polyvinyl alcohol.

*2 N. Hamada, Y. Watanabe and Y. Tsujisaka, Abstr. Ann. Meeting Agr. Chem. Soc. Japan, p. 136 (1973).

*3 N. Hamada, M. Morita, Y. Watanabe and Y. Tsujisaka, Abstr. Ann. Meeting Agr. Chem. Soc. Japan, p. 209 (1974).

TABLE I. PURIFICATION OF THE PVA DEGRADING ENZYME

Step	Total activity (units)	Total protein (mg)	Specific activity (units/mg)	Recovery (%)
Culture broth	1640	5355	0.306	100
$(NH_4)_2SO_4$ precipitation	1230	1080	1.14	75.0
SP-Sephadex chromatography	600	60.0	10.0	36.6
Sephadex G-75 gel filtration	40.0	3.30	12.1	2.44

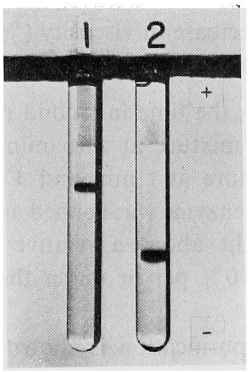


FIG. 2. Disc Electrophoresis of the Purified PVA Degrading Enzyme.

The enzyme protein (0.1 mg) was applied to a column (0.5×5.0 cm) of 7.5% polyacrylamide gel. Electrophoresis was conducted in the pH 4.3 buffer system[2] at 5 mA per column for 2 hr at room temperature, and the gel was stained with amido schwarz. The photographs were taken at (1) 1 and (2) 2 hr of electrophoresis.

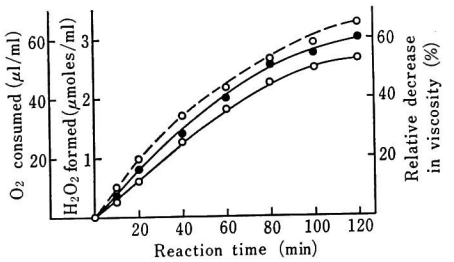


FIG. 3. Decrease in Viscosity, the Consumption of O_2 and the Formation of H_2O_2 during the Reaction of the PVA Degrading Enzyme.

○—○, viscosity decrease; ○---○, O_2 consumption; ●—●, H_2O_2 formation.

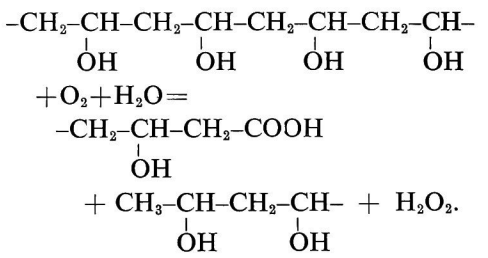
pH of the mixture and a consumption of O_2 in the reaction vessel.

It has been found recently that H_2O_2 is formed during the reaction of the enzyme on PVA. The reaction mixture contained 1.0% PVA, 0.05 M phosphate buffer (pH 7.3) and 0.03 mg of the purified enzyme in 2.0 ml,

TABLE II. CONSUMPTION OF O_2 AND THE FORMATION OF H_2O_2 BY THE PVA DEGRADING ENZYME

Reaction time (min)	O_2 consumed (μmoles)	H_2O_2 formed (μmoles)	H_2O_2/O_2
30	2.11	2.10	0.995
60	3.09	3.65	1.18
90	4.04	4.75	1.18

and the reaction was carried out at 30°C with shaking in a vessel of a Warburg's manometer. H_2O_2 was detected by the color development in peroxidase-*o*-tolidine system, and determined using the titanium reagent reported by Teranishi *et al.*[3] As shown in Fig. 3, the decrease in viscosity, the consumption of O_2 and the formation of H_2O_2 all occurred in parallel, and 1 mole of H_2O_2 was formed for 1 mole of O_2 consumed as seen from Table II. From these findings and other natures of the reaction, the enzyme appeares to be one of oxidases in nature, and to catalyze the cleavage of PVA molecules as follows:



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1) T. Suzuki, Y. Ichihara, M. Yamada and K. Tonomura, *Agr. Biol. Chem.*, **37**, 747 (1973).
2) R. A. Reisfeld, U. J. Lewis and D. E. Williams, *Nature*, **195**, 281 (1962).
3) Y. Teranishi, A. Tanaka, M. Osumi and S. Fukui, *Agr. Biol. Chem.*, **38**, 1213 (1974).