# PURIFICATION OF HYDROGENASE FROM *DESULFOVIBRIO DESULFURICANS*

by

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Hydrogenase, which catalyses the reduction of various organic and inorganic substances by hydrogen, has been shown to be present in several bacteria[1] and algae[2]. The preparation of cell-free extracts of hydrogenase and its partial purification has been reported by HYNDMAN *et al.*[3] from *Azotobacter vinelandii* and by GEST[4] from *Escherichia coli* and *Rhodospirillum rubrum*. But the preparation obtained by the former workers was mostly insoluble and the final activity of the purified enzyme obtained hitherto has been low ($-Q_{H_2}^{\text{methylene blue}}$ of 30,000 $\mu$l per hour per mg nitrogen).

The present report deals with the isolation of hydrogenase of $-Q_{H_2}^{\text{methylene blue}}$ of 2,500,000 $\mu$l from *Desulphovibrio desulphuricans*, an anaerobe in which the presence of hydrogenase was shown by STEPHENSON AND STICKLAND[5] and POSTGATE[6].

Hydrogenase was extracted from the acetone-dried bacteria with 0.2 *M* phosphate buffer of pH 6.4 and centrifuged at 18,000 *g* for two hours. The supernatant was heated at 60° for 10 minutes and centrifuged to remove denatured proteins. The supernatant, which contains hydrogenase, was then adjusted to pH 4.5 and the small precipitate which formed was removed by centrifugation. Hydrogenase was then precipitated from the supernatant by adjustment of the pH to 4.0 and redissolved in tris(hydroxymethyl)aminomethane buffer, pH 5.0. The solution was heated at 50° for two minutes and centrifuged to give a water-clear, pale pink solution containing hydrogenase. The results are given in Table I and show that a recovery of about 90% of the initial activity was obtained.

The activity of the enzyme was determined by measuring hydrogen uptake in Warburg manometers with methylene blue as the hydrogen acceptor under the conditions described in Table I. It was necessary to remove traces of oxygen from the Warburg flasks since the enzyme showed very little or no activity in the absence of alkaline pyrogallol, especially at low enzyme concentrations. The presence of both cysteine and serum albumin was essential for obtaining proportionality between enzyme concentration and activity and for preventing rapid inactivation of the enzyme during the test. Cysteine could be replaced by glutathione or sodium sulfide.

## TABLE I

### PURIFICATION OF HYDROGENASE

The test system for the determination of enzyme activity contained 20 $\mu$moles cysteine; 0.2 mg crystalline bovine albumin and enzyme in 0.1 *M* tris (hydroxymethyl) aminomethane buffer, pH 7.2; 15 $\mu$moles methylene blue (side arm of Warburg flask); final volume 1.5 ml; temperature, 34°; gas phase, hydrogen; alkaline pyrogallol in center-well. The results given below were obtained with 1 g (dry wt) of acetone-dried bacterial cells.

| *Enzyme preparation* | *Total activity ($\mu$l $H_2$ absorbed per hour)* | *Specific activity $-Q_{H_2}^{\text{methylene blue}}$ ($\mu$l $H_2$ absorbed per hour per mg nitrogen)* |
|---|---|---|
| 1. Non-proliferating-washed bacterial cells | 4.0 to 6.0·10⁶ | 5 to 7·10⁴ |
| 2. Acetone dried cells | 4.0·10⁶ | 5·10⁴ |
| 3. Phosphate extract, centrifuged at 18,000 *g* for 2 hours | 3.9·10⁶ | 21·10⁴ |
| 4. Extract (3) heated at 60° for 10 minutes | 3.8·10⁶ | 34·10⁴ |
| 5. pH 4.0 precipitate | 3.6·10⁶ | 97·10⁴ |
| 6. No. 5 reheated at 50° for 2 minutes at pH 5.0 | 3.5·10⁶ | 250·10⁴ |

The purified enzyme, which was pink in colour, was stable and could be stored in the frozen state for a week with no loss of activity. It was precipitated by ammonium sulfate between 70 to 100% saturation at pH 5.5 and was not sedimented by centrifugation for one hour at 80,000 *g*. There was no hydrogen uptake in the absence of enzyme or methylene blue. Riboflavin-5'-phosphate was also active as a hydrogen acceptor, but the rate of hydrogen uptake was less than 5% of that obtained with methylene blue. Ferricyanide, sulfite and sulfate were active as hydrogen acceptors with the crude enzyme preparations, but not with the purified enzyme.

Electrophoretic and ultracentrifugal studies on the enzyme are in progress.

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# THE VISCOSITY AND STREAMING BIREFRINGENCE OF CONCENTRATED SOLUTIONS OF SODIUM DESOXYRIBONUCLEATE

by

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PETERLIN[1] has recently proposed a semi-empirical theory of the viscosity and streaming birefringence of concentrated solutions of macromolecules, which led to the relationships

$$\lim \frac{(\pi/4 - \chi)c}{(\eta - \eta_0)G} = \frac{\beta M}{2RT} \qquad (1)$$

($\beta = 1$ for flexible, 3 for rigid molecules)

$$\text{and } \frac{\Delta n}{n} = \frac{4\pi}{5}\left(\frac{n^2+2}{3n}\right)^2 \frac{(\alpha_1 - \alpha_2)}{kT}(\eta - \eta_0)G \qquad (2)$$

(where $\chi$ is the orientation angle, $G$ the gradient, $M$ the molecular weight, $\eta$ and $\eta_0$ solution and solvent viscosities, $(\alpha_1 - \alpha_2)$ the optical factor, $\Delta n$ the birefringence, and $c$ the concentration in grams per ml).

These equations agree well with existing data for several uncharged polymers.

The solution properties of sodium desoxyribonucleate (DNA) are extremely dependent on concentration and gradient, and extrapolation to zero concentration and gradient, previously necessary to obtain molecular parameters from viscosity and birefringence measurements, is often difficult. This, together with its polyelectrolyte properties, makes DNA a very interesting substance with which to test PETERLIN's theory.

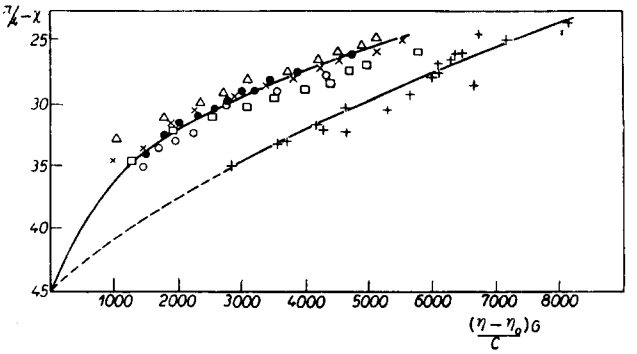


Fig. 1. Extinction angle $\chi$ vs. $(\eta - \eta_0)G/c$ for DNA in 0.2 *M* salt (upper curve) and in aqueous solution (lower curve). DNA in 0.2 *M* salt:
○, 0.0190% DNA;
●, 0.0384%;
□, 0.0582%;
×, 0.0768%;
△, 0.0960%. DNA in aqueous solution:
+, 0.00900–0.0177% DNA.