Some properties of a soluble and particle-bound hydrogenase in *Aerobacter aerogenes*

It is well established that, with the exception of the *Clostridium* type, hydrogenase in homogenates of bacteria is localized in the particles. Many attempts have been made to bring the particle-bound hydrogenase into solution but only in one case has this been successful[1]. In this paper, experiments are reported in which hydrogenase from *Aerobacter aerogenes* was obtained either in soluble or particle-bound form by varying the procedure of cell breakage.

Cells of *A. aerogenes* were grown in mass culture as already described by PENGRA AND WILSON[2]. Preparations in which hydrogenase was associated with the particles were obtained by disrupting the cells with a Hughes press and separating the homogenate by differential centrifugation into the following fractions which were designated according to the nomenclature of ALEXANDER AND WILSON[3]: 0.1 s 20 (crude extract), 9.7 p 30, 9.7 s 30, 144 p 60 and 144 s 60. Soluble hydrogenase was prepared by breaking the cells for 10 min in the Raytheon magnetostrictive sonic oscillator. Whole cells and large cell debris were removed by centrifugation at 25,000 × *g* for 10 min. The supernatant (25 s 10) was centrifuged into the fractions 144 s 60 and 144 p 60.

For the particle-bound hydrogenase, measurement of H_2 uptake in the presence of benzylviologen and H_2 evolution from reduced methylviologen were the most suitable assays. Although when assayed with methylene blue as acceptor the enzyme activity of the soluble preparations was about 3 times greater than the activity measured by the evolution assay, the results with methylene blue were variable. Soluble hydrogenase preparations assayed by H_2 uptake in the presence of methyl- or benzylviologen showed about the same activity as with the evolution assay. For comparison, therefore, the evolution assay was used throughout for both preparations.

Data in Table I demonstrate that the hydrogenase activity of the Hughes press preparation is mainly localized in the particle fraction (9.7 p 30) while the activity of the sonic-treated preparation is still entirely in the supernatant fraction (144 s 60).

The stability of these two preparations was also quite different. The particle-bound hydrogenase (fraction 9.7 p 30) lost activity in 2 h when stored in air at 4°

TABLE I

HYDROGENASE ACTIVITY OF THE PARTICLE-BOUND AND SOLUBLE HYDROGENASE FROM *Aerobacter aerogenes*

Activity measured at 30° with 0.05 *M* phosphate, pH 7, and 40 μmoles reduced methylviologen in a reaction vol. of 3 ml. Gas phase, helium.

Method of cell disruption	*Fraction*	*Activity (μl H_2/h/mg N)*
Hughes press	0.1 s 20	2,300
	9.7 p 30	7,200
	9.7 s 30	530
	144 p 60	610
	144 s 60	0
Sonic treatment	25 s 10	13,300
	144 p 60	0
	144 s 60	11,200

and had a half life of about 2 days when stored under H_2 at 4°. Although fraction 25 s 10 of the sonic extract also lost activity when stored under air at 4° for 24 h, it was stable for 5 days when kept under H_2 at 4°.

The action of some inhibitors is shown in Table II. Although we failed to demonstrate light reversibility of CO inhibition of hydrogenase activity the experiments with cyanide, *o*-phenanthroline and ethylenediaminetetraacetate suggest that a metal, probably iron, is involved in the hydrogenase system.

TABLE II

EFFECT OF SOME INHIBITORS ON AEROBACTER HYDROGENASE ACTIVITY

Conditions as in Table I.

Inhibitor	*Concentration*	*Inhibition (%)*	
		Sonic extract (fraction 25 s 10)	*Hughes-press preparation (fraction 9.7 p 30)*
CO	10%	43.0	30.0
	20%	67.0	85.0
	30%	82.0	95.0
KCN	$3.3 \cdot 10^{-4}$ M*	30.0	—
	$3.3 \cdot 10^{-3}$ M*	95.0	80.0
	$33.0 \cdot 10^{-3}$ M*	100.0	100.0
	$3.3 \cdot 10^{-3}$ M* + 100 μmoles Fe^{++}	34.0	—
	$3.3 \cdot 10^{-3}$ M**	10–40	—
o-phenanthroline	$16.6 \cdot 10^{-3}$ M	51.0	—
	$33.3 \cdot 10^{-3}$ M	69.0	50.0
Ethylenediamine tetraacetate	$66.6 \cdot 10^{-3}$ M	73.5	75.0
	$> 66.6 \cdot 10^{-3}$ M	100.0	—

* Preincubated with cyanide.
** No preincubation.

No H_2 was liberated from pyruvate by any preparation, but CO_2 was evolved and acetoin was formed at an appreciable rate by the sonic extract (25 s 10). With the Hughes-press preparation only fraction 144 s 60 carried out these reactions.

All attempts to bring the particle-bound hydrogenase (fraction 9.7 p 30) into solution by sonic treatment caused a total loss of activity.

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