Hydrogenase Activity in the Dry State

Sir:

Enzymes act on substrates in aqueous solution, where the only molecular species present are hydrated ones. Thus the effect of water must not be overlooked in discussing the mechanism of enzyme action. For this reason, it is desirable to compare the enzyme activity in aqueous solution to that in the dry state or in nonaqueous media. It is, however, almost impossible to study the activity in dry state for most enzymes.

Hydrogenase, on the other hand, is a unique enzyme whose substrate is gaseous hydrogen. It catalyzes reactions 1–3. In reaction 3, substrates of the both

$$H_2 + \text{acceptor (in solution)} \rightleftharpoons \text{reduced acceptor (in solution)} \quad (1)$$

$$H_2 + D_2O \text{ (liquid)} \rightleftharpoons HD + HDO \text{ (liquid) or } D_2 + H_2O \text{ (liquid)} \quad (2)$$

$$o\text{-}H_2 \rightleftharpoons p\text{-}H_2 \quad (3)$$

reactions, forward and backward, are gaseous. Thus it could be possible to demonstrate whether the enzyme retains activity in the dry state. This paper describes the catalytic properties of a dry hydrogenase preparation.

Hydrogenase[1] was purified carefully from *Desulfovibrio desulfuricans* as described by Yagi, Honya, and Tamiya.[2] In this study, hydrogenase fraction A obtained from a Sephadex G-200 column was used throughout.

Enriched parahydrogen was prepared by charcoal-catalyzed conversion of normal hydrogen at about 20°K as reported in a previous paper.[3] The contents of the hydrogen mixture, o-H_2 + p-H_2 and H_2 + HD + D_2, were assayed by the gas chromatographic technique developed in our laboratory.[3]

A sample of purified hydrogenase and 0.3 ml of 0.2 *M* phosphate buffer (pH 7.0) containing 2.4 mg of $Na_2S_2O_4$ were placed in a reaction vessel described in the previous paper.[3] The contents were frozen at −78°, evacuated, filled with hydrogen, thawed, frozen again at −78°, and lyophilized by evacuation at 10^{-6} mm. After the contents became dry powder by visual observation, further evacuation at 10^{-6} mm was continued successively for at least 6 hr at room temperature. The hydrogen gas containing 70% para modification (at 150 mm) was admitted into the reaction vessel, which was placed in a thermostat. At intervals the gas was withdrawn and analyzed for parahydrogen. Figure 1 shows the results.

Figure 1 shows that the dried hydrogenase preparation which had been reduced with $Na_2S_2O_4$ catalyzed the parahydrogen–orthohydrogen conversion reaction whereas the dried hydrogenase preparation which had not been reduced did not catalyze the conversion reaction. $Na_2S_2O_4$ alone did not catalyze the same reaction. Bovine serum albumin, a typical protein of no enzymatic activity, when reduced with $Na_2S_2O_4$ in the presence or the absence of iron(II) sulfate did not catalyze the conversion reaction.

The apparent activation energy for the enzymatic conversion reaction in the dry state was found to be 7.5 kcal/mol, and that in an aqueous solution at pH 7.0, 11.8 kcal/mol.

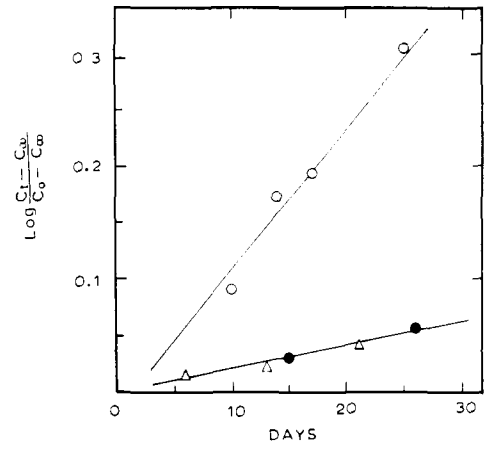

Figure 1. The parahydrogen–orthohydrogen conversion on hydrogenase. The reaction conditions are described in the text; (—○—○—) with ten units of fraction A hydrogenase containing $Na_2S_2O_4$; (—△—△—) same as the above sample except that $Na_2S_2O_4$ had been omitted; (—●—●—) $Na_2S_2O_4$ only.

In 1955, Couper, Eley, and Hayward[4] tried to carry out parahydrogen–orthohydrogen conversions in dry *E. coli* and *P. vulgaris*[5] cells and found no conversion to occur. In our present study, dry hydrogenase preparation was found to retain catalytic activity if it had been activated by $Na_2S_2O_4$ prior to being dried. In a reaction vessel devoid of $Na_2S_2O_4$ little conversion was observed. Activation by adding $Na_2S_2O_4$ to hydrogenase[6] has frequently been observed, and this case is not exceptional. In another experiment, dry hydrogenase (activated by $Na_2S_2O_4$) was kept in contact with a mixture of D_2 and H_2 at room temperature, and a small amount of HD was produced in a few weeks. Further, when the enzyme had been inactivated by heating the vessel at 200° for 2 hr, no hydrogen conversion occurred.

The above-mentioned observations clearly demonstrate that hydrogenase in the dry state actually binds hydrogen molecule and renders it activated, resulting in eventual parahydrogen–orthohydrogen conversion, where aqueous protons do not participate in the reaction mechanism. Probably one hydrogen atom of a hydrogen molecule bound on the enzyme surface (at the active center) might possess protonic character indistinguishable from that of bound protons of the protein molecule (such as NH_2). Far less activity of the D_2–H_2 exchange reaction compared to orthohydrogen–parahydrogen conversion suggests the limited availability of such bound protons on the enzyme surface.

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