Comparison of Activities for Hydrogen Evolution from Water of Hydrogenase and Colloidal Platinum

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Summary The activities for hydrogen evolution from water were compared for both hydrogenase and colloidal platinum and the turnover number per active site of hydrogenase was 500 times larger than that for colloidal platinum.

HYDROGENASE is an enzyme which can catalyse the decomposition of water in the presence of an electron-donating agent. Since enzymes are not ideally suited for long-term use, the replacement of hydrogenase by heterogeneous or homogeneous catalysts has been studied.[1-4] Hall and his co-workers[1,2] have compared the efficiencies of platinum and hydrogenase and have found that they both lead to the evolution of hydrogen at similar rates (7—9 μmol H_2/h/mg chlorophyll) on illumination of isolated chloroplasts with water as the electron source and methyl viologen as the electron carrier. To compare their efficiencies in detail, we describe an estimation of the number of active sites on colloidal platinum by an inhibition technique, and a comparison of the efficiencies of hydrogenase and colloidal platinum under the same reaction conditions.

All reagents were obtained from commercial sources and were of the highest purity available. Hydrogenase from *Desulfovibrio vulgaris* was purified according to Yagi's method.[5] Colloidal platinum was prepared by the reduction of chloroplatinic acid with sodium citrate.[6] The platinum sol thus prepared has been found by electron microscopy to have particles with an average diameter of 34 Å with deviation from the mean of 25%.[6] A typical hydrogen evolution experiment was performed as follows under anaerobic conditions at 30 °C. To methyl viologen ($6{\cdot}32 \times 10^{-7}$ mol) and $Na_2S_2O_4$ ($2{\cdot}30 \times 10^{-5}$ mol), 0·5 ml of colloidal platinum ($1{\cdot}46 \times 10^{-7}$ mol) or hydrogenase ($5{\cdot}71 \times 10^{-10}$ mol) was added. The mixture was adjusted to 3·5 ml with $1{\cdot}14 \times 10^{-2}$ mol l^{-1} of phosphate buffer (pH 7·0; this value is suitable for hydrogenase). In the inhibition experiment, colloidal platinum pre-treated with $HgCl_2$ was used. The evolved hydrogen was collected *via* a sampling valve and analysed by g.l.c.

As shown in the Figure, the initial rate of hydrogen evolution decreases with $HgCl_2$ concentration. Although

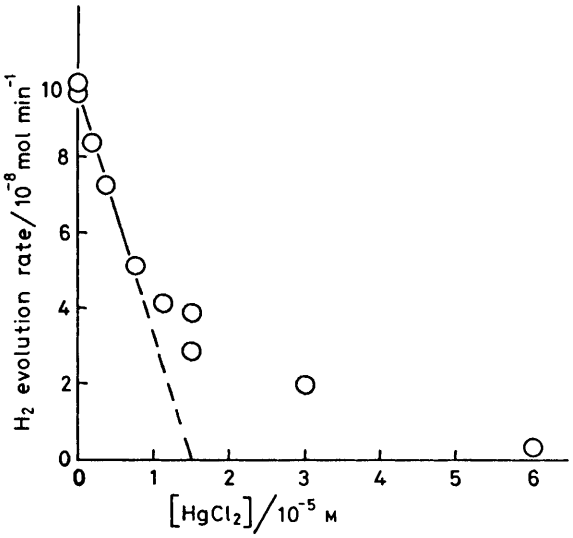

FIGURE. Effect of mercury(II) chloride on hydrogen evolution rate with colloidal platinum. (See text for reaction conditions).

the rate decreases linearly at low $HgCl_2$ concentrations ($< 1 \times 10^{-5}$ mol l^{-1}), there are some deviations from the straight line at higher concentrations. Since at low concentrations the interaction of adsorbed $HgCl_2$ on the colloidal platinum surface may be neglected, the $HgCl_2$ concentration required to inactivate the colloidal platinum completely is calculated as $1{\cdot}5 \times 10^{-5}$M by extrapolation. If $HgCl_2$ adsorbs irreversibly, and one molecule of $HgCl_2$ inhibits one active site, the number of active sites on the platinum surface is calculated as $2{\cdot}7 \times 10^{15}/cm^2$. This value corresponds to the number of platinum atoms exposed on the surface. Therefore, the hydrogen evolution per active site (turnover number) is determined as $3{\cdot}16 \times 10^{-24}$ mol/min. On the other hand, the turnover number per hydrogenase molecule under the same reaction conditions was calculated to be $2{\cdot}83 \times 10^{-21}$ mol/min. As two active sites are involved per hydrogenase molecule,[7,8] the turnover number per active site is $1{\cdot}41 \times 10^{-21}$ mol/min. From these results it is apparent that hydrogenase is about 500 times more active than colloidal platinum.

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