Effect of the Chain Length in Water Soluble Viologen Linked Zinc Porphyrins on Hydrogen Evolved Activity with Hydrogenase

Yutaka Amao, Keisuke Yamane, and Ichiro Okura*
Department of Bioengineering, Tokyo Institute of Technology, Nagatsuta, Midori-ku, Yokohama 226

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Kinetic properties of hydrogen evolution with hydrogenase using water soluble viologen linked zinc porphyrins ($ZnP(C_nV)_4$; n=3-5) as substrate were studied. Among $ZnP(C_nV)_4$, $ZnP(C_5V)_4$ has high affinity for hydrogenase compared with the other compounds.

As we reported previously, efficient photoinduced hydrogen evolution has been observed by using water soluble viologen linked zinc porphyrins ($ZnP(C_nV)_4$).[1] In photoinduced hydrogen evolution using $ZnP(C_nV)_4$, the amount of hydrogen evolved strongly depended on the length of methylene chain of $ZnP(C_nV)_4$.[1] Though these $ZnP(C_nV)_4$ can be substrate of hydrogenase, affinity of $ZnP(C_nV)_4$ for hydrogenase has not been clarified yet. To obtain more detailed information on hydrogen evolution system using $ZnP(C_nV)_4$ and hydrogenase, kinetic studies were carried out.

Water soluble viologen-linked zinc porphyrins ($ZnP(C_nV)_4$; the structures are shown in Figure 1) were synthesized according to the literature.[2] Hydrogenase was obtained from *Desulfovibrio vulgaris* (Miyazaki) and purified according to the literature.[3] Hydrogenase concentration was determined using following molar absorption coefficient, ε=47 $mmol^{-1}dm^3cm^{-1}$ at 400 nm. For hydrogen evolution the sample solutions containing dithionite-reduced $ZnP(C_nV)_4$ and hydrogenase in 50 mmol dm^{-3} Tris-HCl buffer were deaerated. The amount of hydrogen evolved was detected by gas chromatography (detector : TCD, column: active carbon).

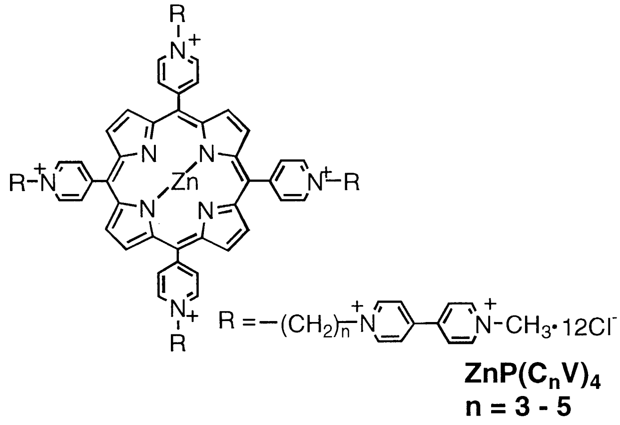

Figure 1. Structure of water soluble viologen linked zinc porphyrin ($ZnP(C_nV)_4$).

In the presence of dithionite-reduced $ZnP(C_nV)_4$ hydrogen evolved with hydrogenase. The amount of hydrogen evolved was almost linearly with reaction time. Dithionite-reduced $ZnP(C_nV)_4$ concentration dependence of initial rate of hydrogen evolution with hydrogenase is shown in Figure 2.

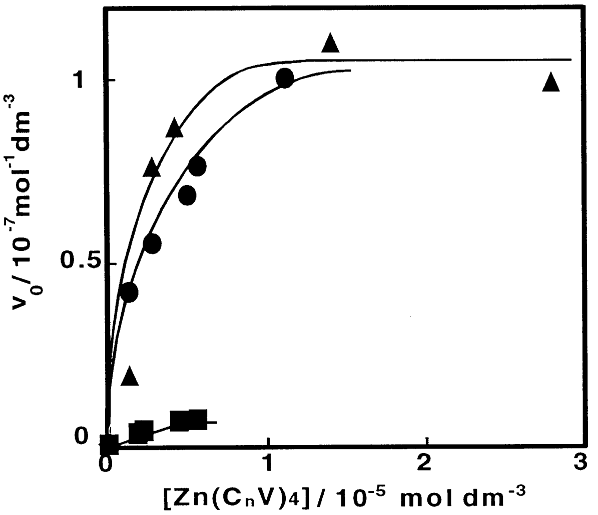

Figure 2. Concentration of dithionite-reduced $ZnP(C_nV)_4$ dependence of hydrogen evolution. The sample consisting of $ZnP(C_nV)_4$ and hydrogenase (5.0×10^{-8} mol dm^{-3}) in 4.0 ml of 50 mmol dm^{-3} Tris-HCl (pH=7.4). ■: n=3; ●: n=4; ▲: n=5.

The initial rate of hydrogen evolution increased with increasing the concentration of $ZnP(C_nV)_4$ and then was constants. The substrate activity of $ZnP(C_5V)_4$ was highest in the other $ZnP(C_nV)_4$. This result coincides with that of photoinduced hydrogen evolution system with $ZnP(C_5V)_4$ and hydrogenase.[1] In this system for hydrogen evolution the affinity constants (K_m), catalytic constant (k_{cat}), catalytic efficiency (k_{cat}/K_m) were determined from the linear double-reciprocal plots as shown in Table 1. The K_m value of $ZnP(C_5V)_4$ was lowest in the other $ZnP(C_nV)_4$, indicating the substrate affinity for hydrogenase of $ZnP(C_5V)_4$ was highest in the other compounds. On the other hand, the V_{max} and k_{cat} of $ZnP(C_nV)_4$ except $ZnP(C_3V)_4$ were almost same value. The catalytic efficiency (k_{cat}/K_m) of $ZnP(C_5V)_4$ was highest in the other compounds. From these results, as $ZnP(C_5V)_4$ has high affinity for hydrogenase compared with the other compounds, effective hydrogen evolution was observed. In case of

Table 1. Kinetic Parameters for hydrogen evolution with dithionite-reduced $ZnP(C_nV)_4$ and hydrogenase

Substrate	K_m / 10^{-6} mol dm^{-3}	V_{max} / 10^{-7} mol dm^{-3} s^{-1}	k_{cat} /s^{-1}	k_{cat} / K_m/ 10^5 mol^{-1} dm^3 s^{-1}
$ZnP(C_3V)_4$	11	0.21	0.46	0.42
$ZnP(C_4V)_4$	2.2	1.1	2.4	11
$ZnP(C_5V)_4$	1.2	1.1	2.4	20

The kinetic parameters K_m, V_{max} and k_{cat} were determined by fitting to the Micheaels-Menten equation.

$ZnP(C_3V)_4$, as the distance between zinc porphyrin moiety and the bonded viologen was short, the affinity for hydrogenase decreases by a steric hindrance among the porphyrin moiety, the bonded viologen and hydrogenase. For $ZnP(C_5V)_4$, on the other hand, as the distance between zinc porphyrin moiety and the bonded viologen was long, streic hindrance was little and the affinity for hydrogenase was highest among other compounds.

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References and Notes

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