An Abnormal Effect of FMN on Hydrogenase Activity

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The effect of a number of additives on the hydrogen evolution activity of hydrogenase [H_2: ferricytochrome C_3 oxidoreductase, EC 1.12.2.1] from *Desulfovibrio vulgaris* (Miyazaki type, IAM 12064) have been studied extensively [1–5]. All the additives studied up to the present were known to serve as inhibitors, though the extent of inhibition depended on the type of additive. When flavin mononucleotide (FMN) or methylene blue is used as an additive, abnormal kinetic behavior is observed, and the effect of the additive is discussed in this letter.

Experimental

All reagents were obtained from commercial sources and were of the highest purity available. *Desulfovibrio vulgaris* cells (which were kindly provided by Professor T. Yagi of Shizuoka University) were cultured according to the literature [6]. Hydrogenase was purified according to Yagi's method [7]. Hydrogenase activity was determined by the initial evolution rate of hydrogen from reduced methyl viologen. Colloidal platinum was prepared by the reduction of chloroplatinic acid with sodium citrate [8]. The reaction mixture contained hydrogenase (or colloidal platinum), methyl viologen, an additive, and 5.0 mg of $Na_2S_2O_4$ in 5.0 ml of phosphate buffer (0.02 *M*, pH 7.0). All the hydrogen evolution experiment was performed under anaerobic conditions at 30 °C.

Results and Discussion

When FMN is used as an additive, the activity increases and then decreases through a maximum point with FMN concentration as shown in Fig. 1, curve a. A similar effect is observed with methylene blue instead of FMN (curve b). When acryflavin is used no activation is observed (curve c). Though colloidal platinum can be used as hydrogen evolution catalyst from reduced methyl viologen in place of hydrogenase, the addition of FMN causes monotonous

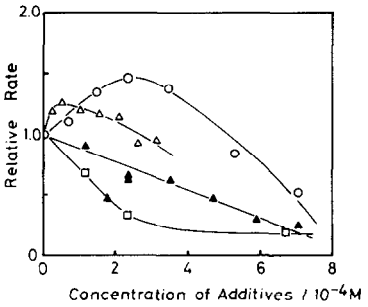

Fig. 1. Relation between relative rate and concentration of additives. Reaction mixture contains hydrogenase (or colloidal platinum (▲)), methyl viologen, $Na_2S_2O_4$ in 3.0 ml of phosphate buffer (0.02 mol dm^{-3}, pH 7.0). Additives; FMN (○, ▲); methylene blue (△); acryflavin (□).

decrease of catalytic activity (curve d). Thus, the abnormal phenomenon of the rate increase may be specific for the combination of hydrogenase and FMN or methylene blue.

This phenomenon may be explained by the following mechanism reported previously on the basis of the activation of hydrogen evolution from reduced methyl viologen by adding cytochrome C_3 [2, 4].

$$E + S \underset{}{\overset{K}{\rightleftharpoons}} ES \qquad ES + S' \overset{K'}{\rightleftharpoons} ESS'$$

$$E + S' \overset{K'}{\rightleftharpoons} ES' \qquad ES' + S \overset{K}{\rightleftharpoons} ESS'$$

$$ES + S \overset{K}{\rightleftharpoons} ES_2 \qquad ES' + S' \overset{K'}{\rightleftharpoons} ES_2'$$

$$ES_2 + 2H^+ \xrightarrow{k} E + 2P + H_2$$

$$ES_2' + 2H^+ \xrightarrow{k'} E + 2P' + H_2$$

$$ESS' + 2H^+ \xrightarrow{k''} E + P + P' + H_2$$

where E is hydrogenase, S and P are reduced and oxidized forms of methyl viologen, and S′ and P′ are reduced and oxidized forms of FMN, respectively. K, K′, k, and k'' are kinetic constants. This mechanism is proposed based on the assumption that two coordination sites per enzyme exist and both electron acceptors can coordinate on the same sites competitively. As FMN can not be an electron acceptor by itself [1], k' should be zero. On the basis of the mechanism, the rate, V, is expressed as follows.

$$V = \frac{kK^2[S]^2 + k'K'^2[S']^2 + 2k''KK'[S][S']}{(1 + K[S] + K'[S'])^2}.$$

According to the equation with $k' = 0$ and [S] = constant, V should increase and then decrease

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through a maximum point with FMN concentration, and the abnormal phenomenon is explained kinetically. The above results show that methyl viologen and FMN can coordinate on the same site on the enzyme, and also show that the rate in the case when methyl viologen and FMN occupy their respective sites in two coordination sites, is larger than that in the case when methyl viologen occupies both sites.

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