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Photochemical Reduction of NADP to NADPH and Hydrogenation of 2-Butanone using 2,2′-Bipyridinium Salts as Electron Carriers

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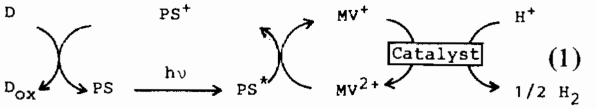
Abstract

The photochemical reduction of NADP was investigated in the four components system containing an electron donor, a photosensitizer, an electron carrier and a catalyst. Methylviologen and 2,2′-bipyridinium salts were effective as electron carriers. The hydrogenation of 2-butanone proceeded by adding alcohol dehydrogenase in the system where the reduction of NADP was achieved.

Introduction

Many studies about photoinduced hydrogen evolution from water have been reported for the utilization of solar energy [1–4]. The systems mostly used for photoinduced hydrogen evolution can be summarized as follows,

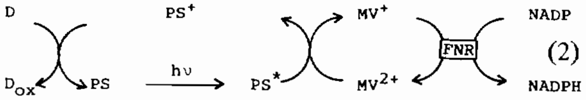


(1)

where D, PS, MV^{2+} are an electron donor, a photosensitizer, and methylviologen as an electron carrier, respectively. However, there are few works capable of the utilization of solar energy except for photoinduced hydrogen evolution.

NAD(P)H is a valuable compound functioning as a coenzyme in organisms, and serves as a hydrogen source for asymmetric reduction with NAD(P)H-dependent dehydrogenases. In vitro system, however, NAD(P)H is consumed stoichiometrically to form NAD(P). To regenerate NAD(P)H from NAD(P), a number of procedures have been proposed [5–15].

In the systems shown in eqn. (1), the reduction of NAD(P) in place of H^+ can proceed by using a suitable catalyst. The new catalyst should accept electron from MV^+ and reduce NAD(P). It is known that ferredoxin–NADP reductase (FNR) catalyzes the reduction of NADP with MV^+ which acts as an artificial electron donor. So, the photochemical reduction systems of NADP as shown in eqn. (2) can be accomplished by using FNR as a catalyst.



This type of the systems for the reduction of NAD(P) has been reported previously [7, 8], in which $Ru(bpy)_3^{2+}$ and Zn–TMPyP^{4+} are used as a photosensitizer. In this work, the kinetic analysis for the photoreduction of NADP was performed to get informations for the improvement of the reaction system. To construct a more efficient system, zinc(II) 5-phenyl-10,15,20-tri(4-sulfonatephenyl) porphyrin (Zn–TPPS$_3^{3-}$) and $Ru(bpy)_3^{2+}$ and 2,2′-bipyridinium salts were used as photosensitizers and electron carriers, respectively.

As NAD(P)H-dependent reductases catalyze the asymmetric reduction of substrates in the presence of NAD(P)H, the asymmetric reduction can be accomplished in the system shown in eqn. (2) by adding the reductases to get the valuable chiral products. In this work the photochemical hydrogenation of ketone was also carried out by adding alcohol dehydrogenase to the photoreduction system of NADP.

Experimental

$Ru(bipy)_3^{2+}2Cl^-$ was synthesized by the method of Palmer and Piper [16]. The structure of 2,2′-bipyridinium salts used are shown in Fig. 1, which were synthesized according to the literature [17]. These compounds were recrystallized in methanol–water after changing the counter ions to chloride ions [18]. FNR was obtained from spinach leaves by Shin's method [19]. Alcohol dehydrogenase from *Thermoanaerobium brokii* was obtained from SIGMA Co. Other reagents were of the highest grade commercially available.

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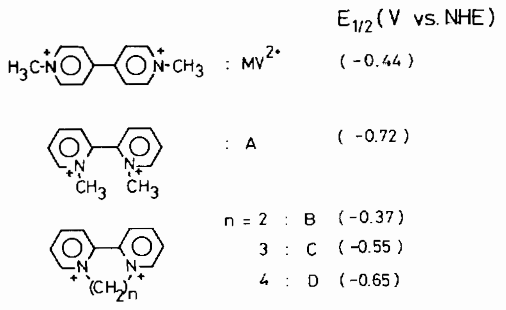


Fig. 1. The structures of electron carriers.

The activity of FNR was assayed in the presence of NADPH (5.7×10^{-4} M) by monitoring reduction of 2,6-dichlorophenolindophenol (DPIP) (1.4×10^{-4} M) in 0.1 M Tris-HCl buffer (pH 7.5), at 26 °C. The reduction of DPIP was monitored by the change in the absorbance of DPIP at 625 nm ($\epsilon = 2.1 \times 10^4$ M^{-1} cm^{-1}). One unit of enzyme activity is defined as the amount of catalyzing the reduction of 1.0×10^{-6} mol DPIP per minute.

Absorption spectra were recorded on a Shimadzu MPS-5000 spectrometer with a 0.3 cm path quartz cell.

Photolysis was carried out under anaerobic conditions at 30 °C. Sample solutions were deaerated by repeated freeze-pump-thaw cycles to remove dissolved oxygen. The pH of the sample solution was adjusted to be 7.3 ± 0.1 by using 0.1 M Tris-HCl buffer. In photolysis under steady irradiation, the sample in a cylindrical Pyrex cell equipped with an optical cell was irradiated with light from a 200 W tungsten lamp. The light of the wavelength less than 390 nm was cut off by a Toshiba L-39 cut-off filter.

The concentration of NADPH formed was determined by measuring the absorbance of sample solution at 340 nm.

GLC analysis was performed on a Hitachi 263-30 gas chromatograph with a PEG 6000 column to determine the concentration of 2-butanol. (Detection: FID, column length: 3 m, column temperature: 64 °C).

Results and Discussion

Photochemical Reduction of NADP with Ru(bpy)$_3^{2+}$

When the sample solution containing 2-mercaptoethanol (RSH) as an electron donor, Ru(bpy)$_3^{2+}$ as a photosensitizer, MV^{2+} as an electron carrier, FNR as a catalyst, and NADP was irradiated, the reduction of NADP to NADPH was observed as shown in Fig. 2. The absorbance at 340 nm which corresponds to NADPH increased with irradiation time. The dependence of the amount of NADPH formed on irradiation time is shown in Fig. 3(e). The amount of NADPH increased linearly at the initial stage of the reaction and then increased gradually. As the conversion of NADP after 1.5 h irradiation was 51.6%, the deviation from the linear increase of NADPH is caused by the decrease of the starting material, NADP, concentration.

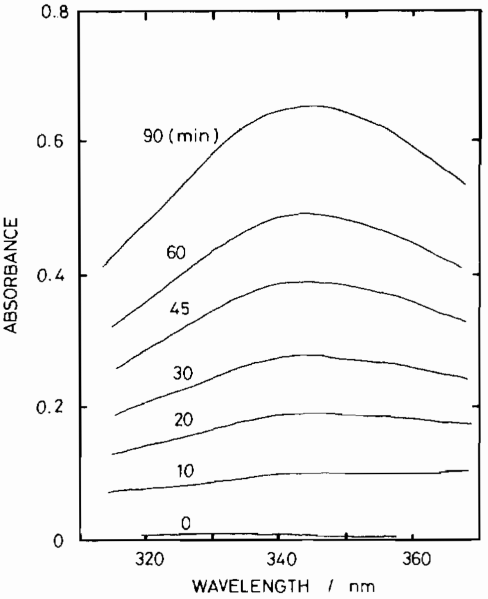


Fig. 2. Spectra change during steady irradiation: RSH (2.4×10^{-1} M), Ru(bpy)$_3^{2+}$ (7.4×10^{-6} M), MV^{2+} (1.1×10^{-3} M), FNR (0.22 units ml^{-1}), NADP (6.7×10^{-4} M).

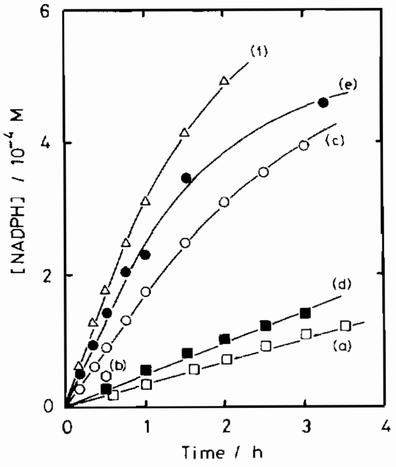


Fig. 3. The dependence of the amount of NADPH formed on the irradiation time in Ru(bpy)$_3^{2+}$ system: RSH (2.4×10^{-1} M), Ru(bpy)$_3^{2+}$ (7.4×10^{-6} M), NADP (6.7×10^{-4} M), **A** (curve a), **B** (curve b), **C** (curve c and f), **D** (curve d), MV^{2+} (curve e) (1.1×10^{-3} M), FNR (a–e: 0.22, f: 0.43 units ml^{-1}).

In the above system, the initial reaction rate for the reduction of NADP was proportional to the concentration of Ru(bpy)$_3^{2+}$, and was independent of the concentration of FNR.

At the initial stage of the reaction, the accumulation of MV^+ was not observed. These results show that the rate limiting step of the reaction is not the reduction of NADP by FNR but the photoreduction of MV^{2+}.

It has been reported that some 2,2′-bipyridinium salts shown in Fig. 1 are effective as electron carriers in the photoinduced hydrogen evolution system containing RSH, Zn–$TPPS_3^{3-}$, and hydrogenase [20]. These electron carriers have different redox potentials indicated in the parentheses in Fig. 1 [21]. Compounds **A**, **C**, and **D** were more effective than MV^{2+} as electron carrier for photoinduced hydrogen evolution under the conditions where the rate limiting step was the photoreduction of electron carriers [20]. It is expected that compounds **A**, **C**, and **D** are more efficient electron carriers than MV^{2+} also in the NADP photoreduction system. So, these electron carriers were applied to the photoreduction system of NADP.

The dependence of the amount of NADPH formed on irradiation time is shown in Fig. 3(a)–(d) when 2,2′-bipyridinium salts were used as electron carriers. All of these compounds were effective as electron carriers in the photoreduction systems of NADP. In all cases, the amount of NADPH increased linearly with irradiation time during the region of the low conversion of NADP. Under these reaction conditions, the order of the initial reduction rate of NADP was as follows: $MV^{2+} > \mathbf{C} > \mathbf{B} > \mathbf{D} > \mathbf{A}$. This order is different from that in the case of the photoinduced hydrogen evolution. The reasons of this difference are discussed later.

The dependence of the initial reduction rate on the concentration of FNR is shown in Fig. 3(c) and (f) in the case of compound **C**. The initial rate was proportional to the concentration of FNR. The same tendencies were obtained in the case of other 2,2′-bipyridinium salts as shown in Table I. In the cases of 2,2′-bipyridinium salts, the initial rates were proportional to the concentration of FNR. This shows that the rate limiting step of the reaction is the reduction of NADP by FNR not the photoreduction of electron carriers in the cases of the systems containing 2,2′-bipyridinium salts as electron carriers. As the rate limiting step is different from that in the photoinduced hydrogen evolution system, it is obvious that the order of the initial reaction rate is different between the hydrogen evolution system and NADP reduction system.

TABLE I. The Dependence of the Initial Rate on the Concentration of FNR

Electron carrier	MV^{2+}	A	B	C	D
$r_1{}^a/10^{-8}$ M s^{-1}	8.2	0.99	2.6	4.9	1.5
$r_2{}^b/10^{-8}$ M s^{-1}	7.7	2.6	4.4	10.0	3.3

RSH (2.4×10^{-1} M), $Ru(bpy)_3^{2+}$ (7.4×10^{-6} M), Electron carriers (1.1×10^{-3} M), NADP (6.7×10^{-4} M), FNR (a: 0.22, b: 0.43 units ml^{-1}).

The rate difference between MV^{2+} and 2,2′-bipyridinium may be caused by the difference of the nature as substrates of FNR. The nature as substrates of FNR may be affected by the redox potentials and the structural factors, but the details are not clear yet.

In all cases shown in Fig. 3, $Ru(bpy)_3^{2+}$ recycled catalytically. In the cases of MV^{2+} and A, for example, the turnover number of $Ru(bpy)_3^{2+}$ after 90 min irradiation were 46 and 8, respectively.

Photochemical Reduction of NADP with Zn–$TPPS_3^{3-}$

Zn–$TPPS_3^{3-}$ is a good photosensitizer for the photoreduction of MV^{2+} as reported previously. So, Zn–$TPPS_3^{3-}$ was applied to the photoreduction system of NADP as a photosensitizer. When the sample solution containing RSH, Zn–$TPPS_3^{3-}$, MV^{2+}, FNR, and NADP was irradiated by visible light, the formation of NADPH was observed. The dependence of the amount of NADPH on irradiation time is shown in Fig. 4. The amount of NADPH increased linearly with irradiation time during the conversion of NADP was low, then the reaction rate decreased gradually. In the case of the system shown in Fig. 4(d), the conversion of NADP was 64% after 2 h irradiation.

As shown in Fig. 4(a)–(d), the initial reduction rate of NADP increased with the increase of the concentration of Zn–$TPPS_3^{3-}$. The dependence of the initial rate on the concentration of Zn–$TPPS_3^{3-}$

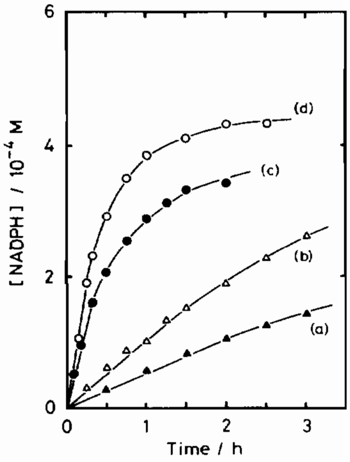


Fig. 4. The dependence of the amount of NADPH formed on the irradiation time in Zn–$TPPS_3^{3-}$–MV^{2+} system: RSH (2.4×10^{-1} M), MV^{2+} (1.7×10^{-4} M), NADP (6.7×10^{-4} M), FNR (0.18 units ml^{-1}), Zn–$TPPS_3^{3-}$ (a: 9.5×10^{-8}, b: 1.9×10^{-7}, c: 9.5×10^{-7}, d: 1.9×10^{-6} M).

TABLE II. The Dependence of the Initial Rate on the Concentration of FNR in $\text{Zn-TPPS}_3{}^{3-}$–MV^{2+} System[a]

[FNR] (units ml^{-1})	Initial rate (M s^{-1})
0.18	2.9×10^{-7}
0.37	3.1×10^{-7}

[a] RSH (2.4×10^{-1} M), $\text{Zn-TPPS}_3{}^{3-}$ (2.9×10^{-6} M), MV^{2+} (1.7×10^{-4} M), NADP (6.7×10^{-4} M).

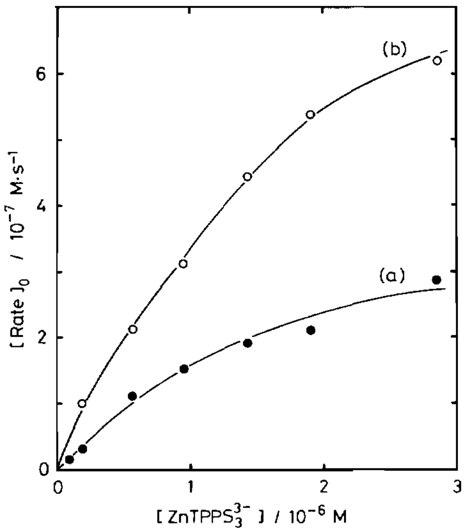


Fig. 5. The dependence of the initial rate on the concentration of $\text{Zn-TPPS}_3{}^{3-}$: RSH (2.4×10^{-1} M), MV^{2+} (curve a), C (curve b) (1.7×10^{-4} M), NADP (6.7×10^{-4} M), FNR (0.18 units ml^{-1}).

is shown in Fig. 5(a). The initial rate increased linearly with the increase of the concentration of $\text{Zn-TPPS}_3{}^{3-}$. The deviation, however, from the initial slope was observed at higher concentration of $\text{Zn-TPPS}_3{}^{3-}$. This is caused by the saturation of the absorption of $\text{Zn-TPPS}_3{}^{3-}$ for the Soret band.

The initial reduction rate of NADP was independent of the concentration of FNR as shown in Table II even at higher concentration of $\text{Zn-TPPS}_3{}^{3-}$. This shows that the rate limiting step of the reaction is the photoreduction of MV^{2+}. In both cases of $\text{Ru(bpy)}_3{}^{2+}$ and $\text{Zn-TPPS}_3{}^{3-}$, the rate limiting step of the reaction was photoreduction of MV^{2+} when MV^{2+} was used as electron carrier.

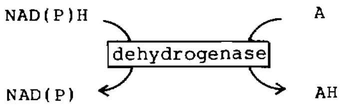
When compound **C** was used in place of MV^{2+}, the reduction of NADP to NADPH was also observed. The dependence of the initial reduction rate on the concentration of $\text{Zn-TPPS}_3{}^{3-}$ is shown in Fig. 5(b). The initial reduction rate in the case of **C** was greater than that in the case of MV^{2+}.

In the case of **C**, however, abnormal phenomena were observed. At lower concentration of $\text{Zn-TPPS}_3{}^{3-}$, the absorption of NADPH increased gradually and the stopped. At higher concentration of $\text{Zn-TPPS}_3{}^{3-}$, though the initial reduction rate was high, the absorption of NADPH disappeared and a new absorption band appeared below 320 nm by prolonged irradiation. When higher concentration of $\text{Zn-TPPS}_3{}^{3-}$ was used, the more rapid disappearance of the absorption of NADPH was observed.

When the disappearance of the absorption of NADPH was observed, the bleaching of the Soret band of $\text{Zn-TPPS}_3{}^{3-}$ was also observed. This bleaching of the absorption of $\text{Zn-TPPS}_3{}^{3-}$ may be caused by the reductive decomposition of $\text{Zn-TPPS}_3{}^{3-}$. The similar bleaching of porphyrin has been reported previously, *i.e.*, when Zn-TMPyP^{4+} is irradiated in the presence of EDTA (a reducing agent), irreversible reduction of the porphyrin occurs and the bleaching of the absorption of the porphyrin is observed [22]. The bleaching mechanism, however, is not clarified yet.

Photochemical Hydrogenation of 2-Butanone to 2-Butanol

The NAD(P)H-dependent dehydrogenases are potentially useful catalysts in chiral synthesis. This reaction can be written as follows,



where A and AH are the oxidized form of substrate and the hydrogenated substrate, respectively. In the system as shown in eqn. (3), the stoichiometric amount of NAD(P)H is needed to reduce the substrates. If the reduction of NAD(P) to NAD(P)H is accomplished, the asymmetric reduction of the substrates can be performed with the catalytic amount of NAD(P).

As described in the above section, photochemical reduction of NADP to NADPH was performed. So, in this section, the photochemical hydrogenation of 2-butanone to 2-butanol was tried by the combination of the photochemical reduction system of NADP and alcohol dehydrogenase (ALDH).

The dependence of the amount of 2-butanol on irradiation time is shown in Fig. 6(a). The system consists of RSH, $\text{Ru(bpy)}_3{}^{2+}$, MV^{2+}, FNR, NADP, ALDH, and 2-butanone. The amount of 2-butanol increased with irradiation time. A similar result was obtained in the system containing compound **C** in place of MV^{2+} as shown in Fig. 6(b).

As described in the above section, $\text{Zn-TPPS}_3{}^{3-}$ was also effective for the photochemical reduction of NADP to NADPH. So, the photochemical hydrogenation of 2-butanone was also tried in the system containing $\text{Zn-TPPS}_3{}^{3-}$ and MV^{2+}. The dependence of the amount of 2-butanol on irradiation time is shown in Fig. 7(a). In this system also, the amount of 2-butanol increased with irradiation time. In the system containing Zn–TPPS$_3^{3-}$ and compound **C**, however, the formation of 2-butanol stopped even after 1 h irradiation as shown in Fig. 7(b). This is caused by the decomposition of Zn–TPPS$_3^{3-}$ during irradiation.

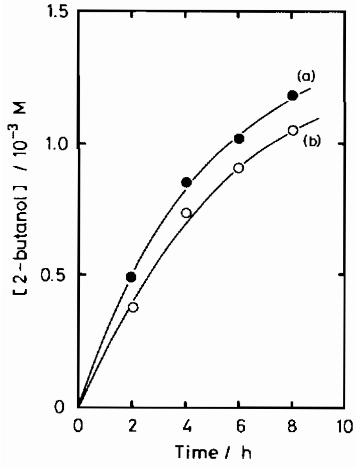


Fig. 6. The dependence of the amount of 2-butanol in Ru(bpy)$_3^{2+}$ system: RSH (2.4 × 10^{-1} M), Ru(bpy)$_3^{2+}$ (7.4 × 10^{-6} M), MV^{2+} (curve a), C (curve b) (1.1 × 10^{-3} M), FNR (0.22 units ml^{-1}), NADP (6.7 × 10^{-4} M), 2-butanone (1.9 × 10^{-1} M), ALDH (0.83 units ml^{-1}).

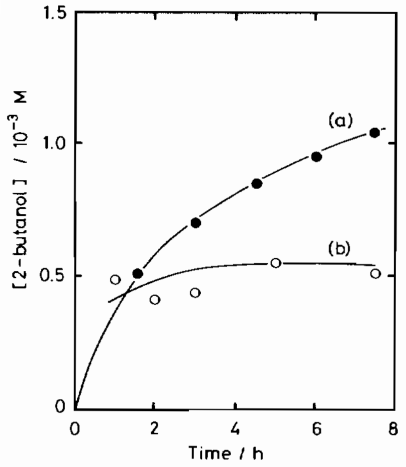


Fig. 7. The dependence of the amount of 2-butanol in Zn-TPPS$_3^{3-}$ system: RSH (2.4 × 10^{-1} M), Zn–TPPS$_3^{3-}$ (9.5 × 10^{-7} M), FNR (0.18 units ml^{-1}). NADP (6.7 × 10^{-4} M), MV^{2+} (curve a), C (curve b) (1.7 × 10^{-4} M), 2-butanone (1.9 × 10^{-1} M), ALDH (0.83 units ml^{-1}).

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