Inorganica Chimica Acta, *137* (1987) 135–136

Photoinduced Reduction of NADP Using Bipyridinium Salts as Electron Carriers

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(Received February 16, 1987)

NAD(P)H is a valuable compound which functions as a coenzyme in organisms and serves as a hydrogen source for asymmetric reduction with dehydrogenases. In this 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 [1]. Recently, the photochemical systems for the reduction of NAD(P) have been reported [2]. In this paper, an attempt to improve the activity in photochemical reduction of NADP was tried by using several kinds of bipyridinium salts as electron carriers. The structures of the bipyridinium salts used are shown in Fig. 1. For a catalyst for the reduction of NADP, ferredoxin-NADP reductase (FNR) was used. Ruthenium tris(2,2'-bipyridine)dichloride ($Ru(bpy)_3^{2+}$) and zinc(II) tetraphenylporphyrintrisulfonate ($Zn\text{-}TPPS_3^{3-}$) were used as photosensitizers (PS in Scheme 1). These compounds are well known to be good photosensitizers for the reduction of methylviologen. 2-Mercaptoethanol (RSH) was used as an electron donor.

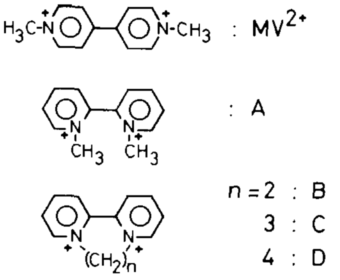


Fig. 1. The structures of the bipyridinium salts used.

Hydrogenation of the ketone was carried out by coupling the photochemical system with alcohol dehydrogenase (ALDH). This system is summarized in Scheme 1.

Experimental

The bipyridinium salts [3] and $Ru(bpy)_3^{2+}$ [4] were synthesized according to the literature. FNR was purified from spinach leaves by Shin's method [5]. Alcohol dehydrogenase from *Thermoanaerobium brokii* was obtained from Sigma Chemicals. The concentration of NADPH formed was determined by the absorbance at 340 nm. The sample solution (6.0 ml) in a cylindrical Pyrex cell equipped with an optical quartz cell was irradiated with visible light. As a light source a 200 W tungsten lamp was used. Light of wavelength less than 390 nm was cut off by a Toshiba L-39 filter. The reaction was carried out under anaerobic conditions. Gas liquid chromatography (GLC) was used to determine the concentration of alcohol.

Results and Discussion

When $Ru(bpy)_3^{2+}$ was used as a photosensitizer, MV^{2+} and the 2,2'-bipyridinium salts shown in Fig. 1 were effective for the reduction of NADP with FNR, as shown in Fig. 2 (a)–(e). In the case of the

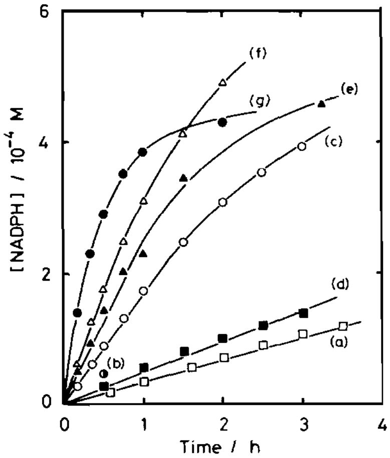
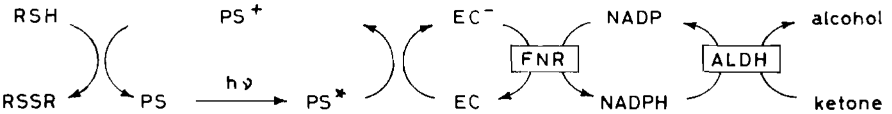


Fig. 2. The dependence of the amount of NADPH formed on the irradiation time: (a) $RSH-Ru(bpy)_3^{2+}-A-NADP-FNR$; (b) $RSH-Ru(bpy)_3^{2+}-B-NADP-FNR$; (c) $RSH-Ru(bpy)_3^{2+}-C-NADP-FNR$; (d) $RSH-Ru(bpy)_3^{2+}-D-NADP-FNR$; (e) $RSH-Ru(bpy)_3^{2+}-MV^{2+}-NADP-FNR$; (f) $RSH-Ru(bpy)_3^{2+}-C-NADP-FNR$; (g) $RSH-Zn\text{-}TPPS_3^{3-}-MV^{2+}-NADP-FNR$. RSH ($2.40 \times 10^{-1}$ M), $Ru(bpy)_3^{2+}$ (7.40×10^{-6} M), $ZnTPPS_3^{3-}$ (1.90×10^{-6} M), NADP (6.67×10^{-4} M), A, B, C, D, MV^{2+} (a–f = 1.08×10^{-3} M; g = 1.70×10^{-4} M), FNR (a–e: 0.22, f: 0.43, g: 0.18 units ml^{-1}), pH 7.2.



Scheme 1.

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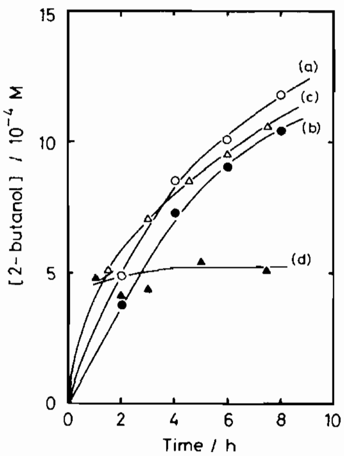

Fig. 3. The dependence of the amount of 2-butanol formed on the irradiation time: (a) RSH–$Ru(bpy)_3^{2+}$–MV^{2+}–NADP–FNR–ALDH–2-butanone; (b) RSH–$Ru(bpy)_3^{2+}$–C–NADP–FNR–ALDH–2-butanone; (c) RSH–Zn-$TPPS_3^{3-}$–MV^{2+}–NADP–FNR–ALDH–2-butanone; (d) RSH–$ZnTPPS_3^{3-}$–C–NADP–FNR–ALDH–2-butanone. R-SH (2.40×10^{-1} M), $Ru(bpy)_3^{2+}$ (7.40×10^{-6} M), Zn-$TPPS_3^{3-}$ (9.50×10^{-7} M), MV^{2+}, C (a, b = 1.08×10^{-3} M; c, d = 1.70×10^{-4} M), 2-butanone (1.86×10^{-1} M), FNR (a, b: 0.22; c, d: 0.18 units ml^{-1}), ALDH (0.83 units ml^{-1}), NADP (6.67×10^{-4} M).

MV^{2+} system, the reaction rate was independent of the concentration of FNR, and was proportional to that of $Ru(bpy)_3^{2+}$, showing that the rate-limiting step of the reaction is the photoreduction of MV^{2+}. In the case of the 2,2′-bipyridinium salt systems, however, the reaction rate was proportional to the concentration of FNR, showing that the rate-limiting step of the reaction is the reduction of NADP with FNR. The kinetic difference between MV^{2+} and the 2,2′-bipyridinium salts may be caused by the difference in the nature of FNR as a substrate. When a large amount of FNR was used, compound **C** (curve (f) in Fig. 2) was more effective than MV^{2+} (curve (e) in Fig. 2) for the reduction of NADP.

$ZnTPPS_3^{3-}$ was also an effective photosensitizer for the reduction of NADP, as shown in Fig. 2 (g). When $ZnTPPS_3^{3-}$ was used as a photosensitizer, the reaction proceeded with a higher reaction rate compared to the $Ru(bpy)_3^{2+}$ system at a lower concentration of photosensitizer and electron carrier.

When compounds **A**, **B**, **C** and **D** were used as electron carriers with $ZnTPPS_3^{3-}$, results similar to the $Ru(bpy)_3^{2+}$ system were obtained. The rate-limiting step was the same as that in the $Ru(bpy)_3^{2+}$ system: *i.e.*, the photoreduction of MV^{2+} in the MV^{2+} system; and the reduction of NADP with FNR in the 2,2′-bipyridinium salts systems.

When compound **C** was used as an electron carrier, the initial reaction rate was higher than in the case of MV^{2+}. In the case of compound **C**, however, bleaching of porphyrin was observed under steady state irradiation. In the case of MV^{2+}, such bleaching was not observed.

The reduction of 2-butanone to 2-butanol proceeded in the above photochemical system by adding ALDH, as shown in Fig. 3. When $Ru(bpy)_3^{2+}$ was used as a photosensitizer, alcohol formed steadily proportional to irradiation time in the cases of both MV^{2+} (curve a) and compound **C** (curve b). When $ZnTPPS_3^{3-}$ was used, the formation of alcohol proceeded steadily in the case of MV^{2+} (curve c); in the case of compound **C**, however, the formation of 2-butanol stopped after 1 h of irradiation (curve d). This is caused by the decomposition of Zn-$TPPS_3^{3-}$, as described above. In these photochemical systems, the turnover number of NADP ([2-butanol]/[NADP]) after 8 h irradiation was 1.5–1.8, showing that NADP was recycled catalytically.

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