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LETTER

Medical Chemistry of Polyoxometalates Part 3. Electrochemical Study of a 1:1 Polyoxomolybdate–Flavin Mononucleotide Complex in Aqueous Solutions

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Certain polyoxomolybdates have been recognized to be a new type of antitumor substance [1]; in particular, hexakis(isopropylammonium) heptamolybdate trihydrate, $[NH_3Pr^i]_6[Mo_7O_{24}]\cdot 3H_2O$ (PM 8), was found to exhibit potent antitumor activity on animal transplantable tumors and human cancer xenografts. Based on the fact that the d^1 electronic configuration at a MoO_6 octahedron in the Mo_7O_{24} framework exhibits strong cytotoxicity, in contrast with the d^0 configuration, the antitumor activity of $[Mo_7O_{24}]^{6-}$ has been explained in terms of the reduction of $[Mo_7O_{24}]^{6-}$ in tumor cells, which results in repeated cycles of the redox reaction of

$$[Mo_7O_{24}]^{6-} + e^- + H^+ \rightleftharpoons [Mo_7O_{23}(OH)]^{6-}$$

in the cells. Electrophoretic profiles of the PM 8-treated pBR322 DNA undergoing digestion by nucleases emphasize the non-specific binding of PM 8 to single- and double-stranded DNAs, which seems to be a factor in the low cytotoxicity of PM 8 [2]. In an attempt to propose a mechanism for the antitumor activity of the polyoxomolybdates, the interaction of PM 8 with flavin mononucleotide (FMN) has been investigated in conjunction with the mechanism of the flavin-sensitized photoreduction of $[Mo_7O_{24}]^{6-}$ [3], since FMN is the chemically active cofactor of the flavoproteins, which are indispensable components in cellular redox metabolism and in several photobiological processes [4]. In this paper it is described that a 1:1 $[Mo_7O_{24}]^{6-}$–FMN complex allows one-electron reduction of the $[Mo_7O_{24}]^{6-}$ component, followed by the formation of a blue species in a way similar to the photoreduction process of $[Mo_7O_{24}]^{6-}$ [5].

Experimental

$[NH_3Pr^i]_6[Mo_7O_{24}]\cdot 3H_2O$ (PM 8) was synthesized according to previous procedures [5, 6] and other chemicals (Tokyo Kasei analytical grade) were used without further purification. All sample solutions for electrochemical experiments contained 0.1 M $NaClO_4$ as supporting electrolyte and were degassed with N_2. The pH levels of the solutions were adjusted by $HClO_4$ or NaOH. All electric potentials (E) are with reference to a Ag/AgCl electrode. Polarograms were recorded on EG & G PAR 174 A and 303 instruments. Controlled-potential electrolysis of the solutions was carried out in the cell with separate anode and cathode compartments, using a Hg-pool working electrode and Pt-wire counter and Ag/AgCl reference electrodes. The cathode compartment was placed at the optical path in a Hitachi 330 spectrophotometer to enable us to measure the absorption spectrum of the cathode solution. X-band ESR spectra were recorded on a Varian E 12 spectrometer equipped with a home-made capillary sample tube, in which an amalgamated Pt-wire working electrode was positioned inside the ESR cavity, with the Pt-counter and Ag-reference wire electrodes placed in a portion of the assembly near the cavity. Such a sample tube allowed close control of the working electrode potential, and the ESR signal and the electrolysis current could be monitored simultaneously as functions of potential or time. ^{13}C (12.5 MHz) and ^{31}P (40 MHz) NMR spectra were obtained in a thermostatted 10-mm diameter NMR tube (at 300 K) using JEOL FX450 and FX100 spectrometers, respectively. The ^{13}C and ^{31}P chemical shifts were referenced to internal dioxane and external 85% H_3PO_4 aqueous solution, respectively.

Results and Discussion

The electronic spectrum of FMN showing λ_{max} = 372 ($\epsilon = 1.136 \times 10^4$ M^{-1} cm^{-1}) and 445 nm ($\epsilon = 1.286 \times 10^4$ M^{-1} cm^{-1}) at the range of 360–490 nm is hardly changed by the coexistence of PM 8 [3], but the electrochemical properties are significantly affected by PM 8. Figure 1 shows differential pulse polarograms of 2 mM FMN, 2 mM PM 8 and their mixture in aqueous solutions at pH 4.6. As shown in Fig. 1, the differential pulse polarographic peak potential of FMN at −0.42 V is shifted to the more positive value of −0.30 V by PM 8, giving the peak

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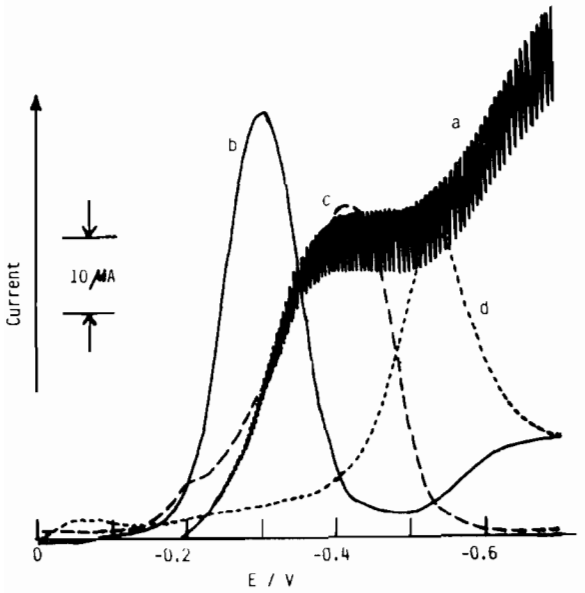

Fig. 1. Electrochemical reduction of a mixture of 2 mM PM 8 and 2 mM FMN in aqueous solutions at pH 4.6: d.c. polarogram of the mixture (a); differential pulse polarograms of the mixture (b), 2 mM FMN (c) and 2 mM PM 8 (d).

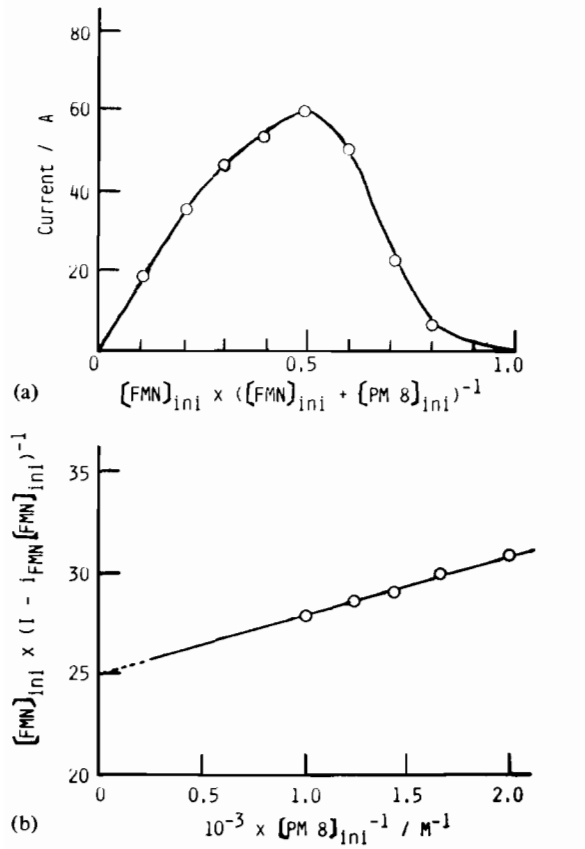

Fig. 2. Formation of a 1:1 PM 8–FMN complex by: (a) the method of continuous variation; (b) determination of the formation constant.

potential at –0.53 V. Addition of 2 mM $[NH_4]_6[Mo_7O_{24}]\cdot 2H_2O$ or 14 mM $Na_2MoO_4\cdot 2H_2O$ to the FMN system at pH 4.6 provided the same shift of the differential pulse polarographic peak potential as found in PM 8. Therefore, it is reasonable to assume that the peak potential at –0.30 V results from the interaction of FMN with $[Mo_7O_{24}]^{6-}$. The peak current at –0.30 V due to the interaction between FMN and $[Mo_7O_{24}]^{6-}$ was analyzed by a continuous variation method ($[FMN]_{ini} + [PM\ 8]_{ini}$ = constant, where $[FMN]_{ini}$ and $[PM\ 8]_{ini}$ denote initial concentrations of FMN and PM 8, respectively).

The result indicates the formation of a 1:1 PM 8–FMN complex, as shown in Fig. 2(a). Then, the formation constant (K_f) of the complex for $[Mo_7O_{24}]^{6-} + FMN \rightarrow [Mo_7O_{24}]^{6-}...FMN$ (complex) was evaluated from the analysis of the current (I) at –0.30 V, as given by eqn. (1)

$$I = i_{FMN}([FMN]_{ini} - [complex]) + i_{PM\,8}([PM\ 8]_{ini} - [complex]) + i_{complex}[complex] \quad (1)$$

where i_{FMN} ($=1.02 \times 10^{-2}$), $i_{PM\,8}$ ($=0.20 \times 10^{-2}$), and $i_{complex}$ denote the molar current at –0.30 V (in AM^{-1} unit) of the differential pulse polarograms for FMN, PM 8 and the complex, respectively.

Under the experimental conditions of $[PM\ 8]_{ini} - [complex] \rightarrow [PM\ 8]_{ini}$ (or $[PM\ 8]_{ini} \gg [FMN]_{ini}$), one obtains eqn. (2)

$$\frac{[FMN]_{ini}}{I - i_{FMN}[FMN]_{ini}} = \frac{1}{K_f[PM\ 8]_{ini}(i_{complex} - i_{FMN})} + \frac{1}{i_{complex} - i_{FMN}} \quad (2)$$

Equation (2) predicts a line with a slope of $1/K_f(i_{complex} - i_{FMN})$ and an intercept of $1/(i_{complex} - i_{FMN})$, when $[FMN]_{ini}/(I - i_{FMN}[FMN]_{ini})$ is plotted versus $1/[PM\ 8]_{ini}$. The line drawn through the experimental points provides a slope of 2.8×10^{-3} A^{-1} M^2 and an extrapolation to the intercept at 25 A^{-1} M, as shown in Fig. 2(b). Then, the values $i_{complex} = 5.0 \times 10^{-2}$ AM^{-1} and $K_f = 8.9 \times 10^3$ M^{-1} can be evaluated.

The d.c. polarogram of the complex (Fig. 1) showed a linear plot of electrode potential (E) versus $\log[(i_d - i)/i]$ with a slope of 65 mV at 20 °C for the wave at the half-wave potential ($E_{1/2}$) of –0.30 V, where i_d denotes the diffusion-limited current. This indicates that the reduction wave at $E_{1/2} = -0.30$ V for the complex is approximately a reversible one-electron step. When mixtures of 2 mM FMN and 2 mM PM 8, which would produce about 2 mM $[Mo_7O_{24}]^{6-}...FMN$ complex, were electrolyzed at $E \leqslant -0.20$ V, the ESR signal of $\langle g \rangle = 1.92$ due to the Mo^V site with a ^{95}Mo and ^{97}Mo hyperfine splitting constant ($\langle A_{Mo} \rangle$) of about 57 G was developed and

accelerated by an increase of the charge passed up to −0.50 V, to yield a blue solution with λ_{max} = 710 and 1020 nm in the absorption spectrum of the electrolyte. An additional 12-line ESR signal at $\langle g \rangle$ = 2.00 due to the semiquinone radical of FMN ($FMNH^{\cdot}$) [7] increases at E = −0.30 V up to −0.60 V. The absorption band of $FMNH^{\cdot}$ showing λ_{max} = 570 nm [8] is covered by broad absorption bands of the Mo-blue species at the range of $500 < \lambda < 1300$ nm. Signal intensities at $\langle g \rangle$ = 1.92 and 2.00 decrease with increasing charge at $E < -0.50$ and < -0.60 V, respectively. The decrease in the signal intensities at highly negative E values is due to the formation of a diamagnetic Mo^{IV} site and leuco-FMN ($FMNH_2$) by an additional electrochemical reduction. The electrolysis of PM 8 in the absence of FMN yielded neither ESR-active Mo^{V} species nor blue species. The ESR spectrum and the dependence of the ESR signal intensities on E are shown in Fig. 3, where each intensity was recorded after some equivalent increments of charge.

It should be recalled that similar Mo^{V} species were obtained from the photolysis of PM 8 in aqueous

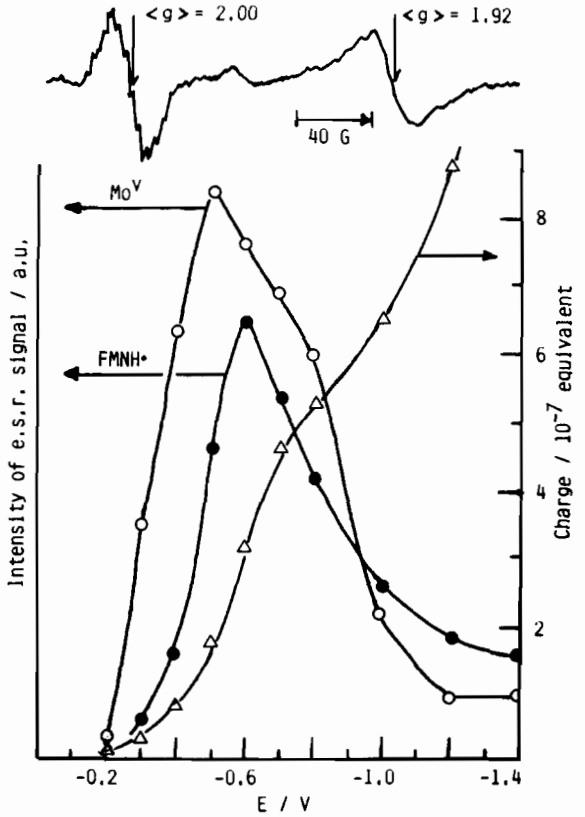

Fig. 3. ESR spectra of the electrolyte of the mixture of 2 mM PM8 and 2 mM FMN and dependence of the ESR signal intensities on the electrode potential. Signal intensities were recorded after indicated equivalents of charge had been passed.

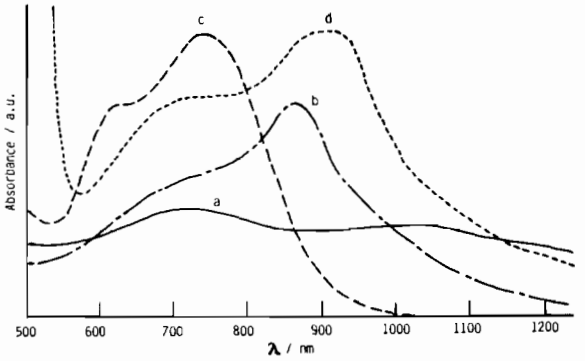

Fig. 4. Absorption spectra of the blue species: (a) electrolysis of the mixture of 2 mM PM 8 and 2 mM FMN at -0.60 V $\leqslant E \leqslant -0.20$ V; (b) electrolysis of the same mixture at $E \leqslant -0.70$ V; (c) photolysis of 10 mM PM 8; (d) after addition of 10 mM FMN into the photolyte of 10 mM PM 8.

solutions [5]: at high concentration ($\geqslant$10 mM) of PM 8, the photoexcitation of the O → Mo charge-transfer band induces a well-defined signal at $\langle g \rangle$ = 1.921 with six weak sateline lines of $\langle A_{Mo} \rangle$ = 51 G, resulting in the fomration of a blue species with λ_{sh} = 610 and λ_{max} = 730 nm. The absorption spectrum of the photolyte was changed to the one with λ_{sh} = 694 and λ_{max} = 905 nm by the addition of 10 mM FMN into the photolyte. This is not surprising, since the absorption spectra of the photolyte are affected by variations in temperature, pH and molybdate concentration [5, 6]. Electrolysis of the complex (about 2 mM) at $E \leqslant -0.70$ V where the reduction of Mo^{VI} to Mo^{IV} occurs [9] gave another peak which developed at 863 nm. Figure 4 shows the absorption spectra of the blue solutions obtained on reduction.

Electrolysis of the complex at $E \geqslant -0.60$ V, representing the one-electron reduction process, results in a one-electron reduction of the $[Mo_7O_{24}]^{6-}$ component and subsequent one-electron reduction of the FMN component (Fig. 3). This indicates that the two-electron reduction of the complex occurs under the electrochemical one-electron reduction of the complex at $E \geqslant -0.60$ V. Therefore, the delayed formation of $FMNH^{\cdot}$ can be attributed to another chemical reaction, such as disproportionation of the one-electron reduced complex. In connection with the similarity in the $\langle g \rangle$ value of the Mo^{V} site between the electrolyte of the complex and the UV-irradiated crystal of PM 8, the Mo^{V} center in the $[Mo_7O_{24}]^{6-}$ component in the complex can be assigned to $[Mo_7O_{23}(OH)]^{6-}$, which is the paramagnetic center in the UV-irradiated crystal of PM 8 and is converted partially to the blue species with a condensed framework in the aqueous solution in a way similar to the photolysis of PM 8 [5]. Thus, the steps outlined in eqns. (3) to (7) are proposed for the electrochemical one-electron reduction of the complex.

$$[Mo_7O_{24}]^{6-} + FMN \longrightarrow [Mo_7O_{24}]^{6-}...FMN \quad (K_f = 8.9 \times 10^3\ M^{-1}) \quad (3)$$

$$[Mo_7O_{24}]^{6-}...FMN \xrightarrow{e^-, H^+} [Mo_7O_{23}(OH)]^{6-}...FMN \quad (4)$$

$$2[Mo_7O_{23}(OH)]^{6-}...FMN \longrightarrow [Mo_7O_{23}(OH)]^{6-}...FMNH^{\cdot} + [Mo_7O_{24}]^{6-}...FMN \quad (5)$$

$$[Mo_7O_{23}(OH)]^{6-}...FMN \text{ or } [Mo_7O_{23}OH)]^{6-}...FMNH^{\cdot} \longrightarrow \text{blue species} \quad (6)$$

FMN contained as a prosthetic group in a flavoprotein is an electron carrier for the electron transfer from NADH to coenzyme Q [10]. Therefore, eqn. (4) reflects the occurrence of the reaction

$$[Mo_7O_{24}]^{6-} + e^- + H^+ \longrightarrow [Mo_7O_{23}(OH)]^{6-}$$

in the biological system which is involved in the mechanism proposed for the anticancer activity of PM 8 [1]. This implies that the activation of the O → Mo charge-transfer band in $[Mo_7O_{24}]^{6-}$ can be attained biologically without UV-photoexcitation by the interaction with FMN, since the electrolysis of $[Mo_7O_{24}]^{6-}$ in the absence of FMN resulted in no formation of the paramagnetic Mo^V site. Since the FMN-photo-sensitized reduction of $[Mo_7O_{24}]^{6-}$ represented the photochemical formation of $FMNH^{\cdot}$ by electron transfer from the ribitol residue linked to N9 with a resultant degradation of FMN [3], the one-electron reduction of $[Mo_7O_{24}]^{6-}$ may be explained in terms of the occurrence of an intra-electron transfer from FMN to the $[Mo_7O_{24}]^{6-}$ component in the complex, if the FMN component is reduced to $FMNH^{\cdot}$. Thus, as an alternative step it is proposed that the one-electron reduction of the $[Mo_7O_{24}]^{6-}$ component in the complex occurs via $[Mo_7O_{24}]^{6-}...FMNH^{\cdot}$, as denoted by eqn. (7) instead of eqn. (4)

$$[Mo_7O_{24}]^{6-}...FMN \xrightarrow{e^-, H^+} [Mo_7O_{24}]^{6-}...FMNH^{\cdot} \longrightarrow [Mo_7O_{23}(OH)]^{6-}...FMN \quad (7)$$

In ^{13}C and ^{31}P NMR spectra of the complex, the downfield (within 0.5 ppm) contribution to the chemical shift [11] for C4, C5 and several ribitol carbon and P atoms suggests the preferential interaction of $[Mo_7O_{24}]^{6-}$ with the isoalloxazine N5 atom and/or the ribitol P atom.

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